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Trait Anxiety and Economic Risk Avoidance Are Not Necessarily Associated: Evidence from the Framing Effect

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According to previous literature, trait anxiety is related to the tendency to choose safety options during risk decision-making, that is, risk avoidance. In our opinion, anxious people's risk preference might actually reflect their hypersensitivity to emotional information. To examine this hypothesis, a decision-making task that could elicit the framing effect was employed. The framing effect indicates that risk preference could be modulated by emotional messages contained in the description (i.e., frame) of options. The behavioral results have showed the classic framing effect. In addition, individual level of trait anxiety was positively correlated with the framing effect size. However, trait anxiety was not correlated with risk-avoidance ratio in any condition. Finally, the relationship between anxiety and the framing effect remained significant after the level of depression was also taken into account. The theoretical significance and the major limitations of this study are discussed.

Keywords: decision-making, framing effect, trait anxiety, depression, risk avoidance

INTRODUCTION

Anxiety is a negative emotion characterized by anticipatory affective, cognitive and behavioral responses towards a possible threat (Grupe and Nitschke, 2013). The concept of anxiety is multifaceted including state anxiety and trait anxiety (Endler and Kocovski, 2001). State anxiety refers to a transient level of physiological arousal and feelings of vigilance, dread, and tension; on the other hand, trait anxiety reflects an individual's disposition to experience anxiety-relevant feelings or thoughts or to show anxiety-related behaviors (Spielberger et al., 1983; Bekker et al., 2003). Both state and trait anxiety are linked with abnormal decision-making behavior. Eisenberg et al. (1995) first discovered that the participants with higher level of trait anxiety were overwhelmingly prone to choose safety options over risky options (i.e., risk avoidance). Later, Raghunathan and Pham (1999) found that the manipulation of increasing participants' state anxiety level also resulted in a stronger tendency to avoid risk. The idea of linking anxiety with risk avoidance has been confirmed by many follow-up studies (e.g., Wray and Stone, 2005;

Maner and Schmidt, 2006; Giorgetta et al., 2012; for reviews, see Hartley and Phelps, 2012; Paulus and Yu, 2012). Hartley and Phelps (2012) summarized previous findings and concluded that: "either heightened arousal to risky choice options or increased interoceptive awareness of arousal responses (or an interaction of the two) may lead anxious individuals to be more risk averse."

Nevertheless, we would like to point out that "risk" is a broad term that across different domains and may have caused misunderstandings in the literature. Most notably, Schonberg et al. (2011) remind their readers to be aware of the "gap" between naturalistic risk (e.g., drug abuse and skydiving) and economic risk. From the perspective of evolutionary psychology, anxiety is an adaptive emotion that protects people from potential dangers by guiding the attention toward threat-relevant information (Stein and Bouwer, 1997; Nesse, 1999). Therefore, it is not surprising that anxiety is inherently associated with more conservative behavior when facing naturalistic risk. Regarding economic risk, however, previous findings are more heterogeneous (e.g., Mano, 1992; Hockey et al., 2000; Miu et al., 2008; Tang et al., 2012; Zhang et al., 2015). For instance, in a series of experiments, Mitte (2007) discovered that the relationship between anxiety and risk-avoidance is unstable, depending on the way of response format (verbal vs. numerical). In our opinion, during economic decision-making, anxiety influences risk preference by raising the sensitivity to negative emotion (see also Browning et al., 2015). More specifically, anxious people may not avoid the economic risk *per se*, but the anticipatory negative emotion associated with the possibility of a larger loss (Engelmann et al., 2015). That is to say, anxiety and economic risk avoidance are not necessarily connected.

In most decision-making tasks, the risky options are related to stronger emotional reactions (Loewenstein et al., 2001), thus it is very difficult to distinguish the role of risk and that of emotion. We suggest that investigating the framing effect could shed light on this issue. The term "framing effect" refers to a phenomenon that people are more likely to choose the option framed (e.g., worded) in an emotionally positive way, but are less likely to do so when the same option is framed in a negative way (Tversky and Kahneman, 1981). Positive or negative emotion generated by the "frame" elicits approach or avoidance behaviors, respectively (Fagley et al., 2010). The framing effect was first discovered in the naturalistic rather than the economic domain, that is, the Asian disease problem (Tversky and Kahneman, 1981). Peng et al. (2014) have found that high trait-anxious participants were more likely to avoid risk when dealing with the Asian disease problem. However, Peng et al. (2014) investigated the effect of the self-frame (i.e., subjectively constructing the information in an ambiguous situation). Therefore, their study is unsuitable to test the hypothesis that trait anxiety is associated with heightened sensitivity to external emotional information. In the context of economic decision-making, De Martino et al. (2006) found that people prefer choosing the safety option when it is framed as a potential gain compared to when it is framed as a potential loss. In our opinion, the paradigm developed by De Martino et al. (2006) provides an opportunity to modulate the emotional effect independent of economic risk.

In one of our recent studies, Xu et al. (2013) reported that the level of trait anxiety [measured by Spielberger's Trait Anxiety Inventory (STAI-T); see Spielberger et al., 1983] was correlated with the framing effect size. Anxious people are more likely to choose the safety option when it is described as a gain, but less likely to do so when it is described as a loss, indicating that they are more susceptible to emotional information when making decisions. This idea is supported by brain-imaging findings that trait anxiety was correlated with activations of the amygdala, which is a key region in the emotional circuit (Xu et al., 2013). However, it remains unclear if anxiety level is consistently related to economic risk avoidance regardless of how the options are framed. In addition, the reliability of the findings of Xu et al. (2013) is harmed by its relatively small sample size (20 participants in total). Regarding the importance of reproducibility in psychological research (e.g., Open Science Collaboration, 2015), the experiment should be replicated in a larger sample.

Finally, the close relationship between anxiety and depression should be taken into account (Stavrakaki and Vargo, 1986). Depression is characterized by feeling of low mood, sadness, and loss of interest, and can be recognized as either a state or a cluster of symptoms (Zung, 1965; Rottenberg, 2005). Anxiety and depression overlap with each other in many aspects such as the component of negative affect (Lonigan et al., 1994; Joiner et al., 1996). Also, in the field of clinical psychology, anxiety and depressive disorders are highly comorbid (Domschke and Dannlowski, 2010). Therefore, it is recommended that the research on anxiety should control the effect of depression as a confounding variable (Beuke et al., 2003). Seeing that the current study investigates trait anxiety rather than state anxiety, we used Zung's self-rating depression scale (SDS) to measure the depression level, because the SDS estimates depressive symptoms in a prolonged period (Zung, 1965). According to Xu et al. (2013), the SDS score was not significantly correlated with the framing effect size. However, considering the high correlation between anxiety and depression ($r = 0.3{\sim}0.7$ in many studies, e.g., Knight et al., 1983; Bjelland et al., 2002; $r = 0.53$ in the sample of Xu et al., 2013), more rigorous statistical methods are needed to examine the potential influence of depression on the data.

This study employed a new sample to complete the task designed by De Martino et al. (2006), which could reliably elicit the classic framing effect during risk decision-making (Roiser et al., 2009; Xu et al., 2013). Individual levels of trait anxiety and depression were measured and entered into behavioral analyses. Our hypotheses were: (a) the level of trait anxiety would be positively correlated with the framing effect size, but not the tendency of risk avoidance; (b) taking depression into account would not affect the relationship between anxiety and the framing effect.

MATERIALS AND METHODS

Participants

Sixty-nine students from Beijing Normal University participated in the study. A total of six participants were excluded from data analysis due to failure to complete all the questionnaires or participation discontinuation. As a result, the final sample consisted of data from 63 participants (34 female). Informed consents were obtained from all participants. The experimental protocol was approved by the local Ethics Committee at Beijing Normal University.

The Chinese version of STAI-T was used to assess the level of trait anxiety. The Chinese version of SDS was used to assess self-reported symptoms of depression. Both scales have demonstrated internal consistency, convergent validity, and discriminate validity (STAI-T: Spielberger et al., 1983; Shek, 1993; SDS: Zung et al., 1965; Shu, 1993).

Procedure

Before the experiment, participants received the instruction about the formal task and were given 12 practice trials. They were also informed that their task performance (i.e., total points) would contribute to final payment. The relationship between point thresholds and corresponding participant payment was shown on a table, which indicated that the range of possible earning was 20–100 Chinese Yuan.

Figure 1 provides schematic illustration of a single trial. In the beginning of each trial, participants were shown a message screen (2 s) indicating an initial amount of reward (starting points: for instance, “You receive 100 points” in Chinese). There were four different starting point amounts (25, 50, 75, and 100 points), which were counterbalanced across conditions. However, participants would not actually get the reward before they make a decision between a “sure” and a “gamble” option, which appeared following the starting point presentation. The sure (safety) option indicates the amount of points that could be kept for certain if participants choose this option, while the gamble (risky) option indicates a win-or-nothing choice. Within each trial, the expected values of the sure and gamble options were identical and mathematically equivalent between conditions. The only difference between conditions was the description of the sure option; this option was described as money retained in the gain (positive) frame condition (e.g., “keep 80 points” of 100 points) but was described as money lost in the loss (negative) frame condition (e.g., “lose 20 points” of 100 points). In both conditions, the gamble option was presented identically as a pie chart depicting the probability of winning and losing in green and red color, respectively. There were four kinds of winning probabilities (20, 40, 60, and 80%) for the gamble option. Participants chose between the two options by pressing the F and J buttons on the keyboard (“F” for the option on the left side and “J” for the right side). The decision screen them disappeared immediately, which was followed by the next trial. No feedback was provided during the task (see De Martino et al., 2006; Xu et al., 2013, for more details). All experimental variables (including starting point amounts, positions of the two options, and winning probabilities) were fully counterbalanced between conditions.

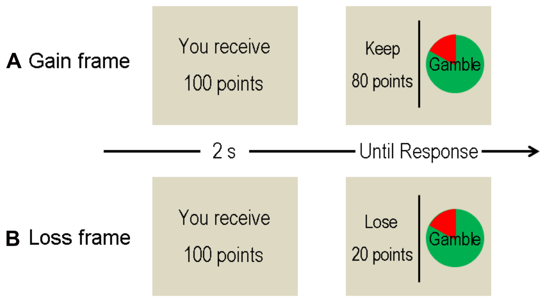

FIGURE 1 | Schematic diagram of a trial. (A) The gain frame condition. **(B)** The loss frame condition.

The task was divided into two identical sessions comprised of 64 trials (32 gain frame and 32 loss frame; ordered pseudorandomly). At the end of the task, total earned points were displayed to participants. Stimulus display and behavioral data acquisition were conducted using E-Prime software 1.1 (Psychology Software Tools).

Data Analysis

The framing effect in the current study was quantitatively defined as the difference between trials in which participants’ decisions were consistent with the frame (chose the sure option in the gain frame condition or the gamble option in the loss frame condition) and trials in which participants’ decisions ran counter to the frame (chose the gamble option in the gain frame condition or the sure option in the loss frame condition). Accordingly, the framing effect size was calculated as follows: $(\text{Gain}_{\text{sure}} + \text{Loss}_{\text{gamble}}) - (\text{Gain}_{\text{gamble}} + \text{Loss}_{\text{sure}})$ (De Martino et al., 2006).

Two-tailed one-sample *t*-test was used to examine the significance of the framing effect. Pearson correlation analysis (two-tailed) was used to determine the relationship between the framing effect and both anxiety and depression. Finally, linear regression analysis was performed to assess the unique contribution of anxiety.

For all the analyses, the results of descriptive statistics were reported as mean ± SD. The significance level was set at $p = 0.05$. In addition, the bias-corrected and accelerated (BCa) bootstrap 95% confidence intervals (CIs) were estimated based on bootstrapping with 5000 simulations (Efron, 1987). Statistical analysis was performed using IBM SPSS 19.0 (IBM Corporation).

RESULTS

Self-Report Measures

In the whole sample, the STAI-T score was 38.25 ± 7.06 (range: 26–55) and the SDS score was 37.13 ± 9.37 (range: 23–54). The Pearson correlation (two-tailed) between two scales was significant ($r = 0.292$, $p = 0.021$, 95% CI [0.081,0.496]).

Behavioral Results

The one-sample *t*-test revealed that the framing effect was significantly larger than zero (21.87 ± 20.20, $t(62) = 8.594$, $p < 0.001$); participants were more likely to make decisions in accordance with the frame ($\text{Gain}_{\text{sure}} = 53.37\%$,

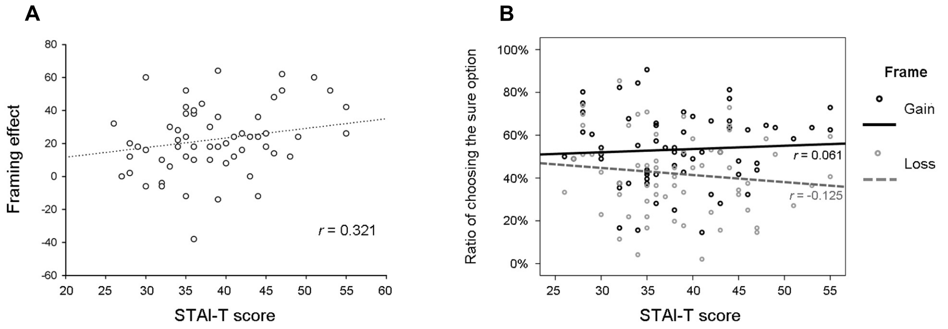

FIGURE 2 | (A) A scatter plot of the correlation between STAI-T score and the framing effect size ($p = 0.010$). **(B)** A scatter plot of the correlations between STAI-T score and the ratio of choosing the sure option in the gain frame and the loss frame conditions ($ps > 0.327$).

$\text{Loss}_{\text{gamble}} = 58.02\%$) rather than counter to the frame ($\text{Gain}_{\text{gamble}} = 46.63\%$, $\text{Loss}_{\text{sure}} = 41.98\%$).

Pearson correlation analysis revealed a positive correlation between the STAI-T score and the framing effect ($r = 0.321$, $p = 0.010$, 95% CI [0.095,0.520]) (**Figure 2A**). In contrast, the STAI-T score was not correlated with the risk-avoidance tendency (i.e., the ratio of choosing the sure option) in either the gain frame condition ($r = 0.061$, $p = 0.637$, 95% CI [–0.163,0.274]) or the loss frame condition ($r = –0.125$, $p = 0.327$, 95% CI [–0.347,0.107]) (**Figure 2B**). In addition, these effects were not sensitive to the amount of starting points or winning probabilities (results not showed for brevity). The SDS score was not significantly correlated with the framing effect ($r = 0.212$, $p = 0.098$, 95% CI [–0.005,0.414]), or the risk-avoidance tendency in the gain frame condition ($r = –0.089$, $p = 0.492$, 95% CI [–0.349,0.175]) or that in the loss frame condition ($r = –0.199$, $p = 0.121$, 95% CI [–0.428,0.041]).

Finally, a linear regression analysis was used to explain the framing effect based on the STAI-T and SDS scores (entered method). The regression model was significant [$F(2,59) = 4.136$, $p = 0.021$, $R^2 = 0.123$]. Whereas the STAI-T score was a significant predictor of the framing effect ($\beta = 0.805$, $p = 0.018$, 95% CI [0.129,1.504]), the SDS score was not ($\beta = 0.266$, $p = 0.309$, 95% CI [–0.268, 0.752]).

DISCUSSION

Consistent with Xu et al. (2013), the current study has found a positive correlation between the level of trait anxiety (measured by STAI-T) and the framing effect size. That is, the influence of the description of the safety option on individual risk preference increased as a function of trait anxiety. These results indicate that people with high trait anxiety are more sensitive to the influence of contextual emotional information during risk decision-making. Consequently, their decisions are more likely to be in accord with the framing effect compared to those with low trait anxiety (Xu et al., 2013). In addition, the results of linear regression analysis have confirmed the independent role of trait anxiety after the depression factor (measured by SDS) was considered.

In contrast, the current study showed no evidence of the relationship between trait anxiety and economic risk avoidance, regardless of whether the safety option was framed as a potential gain or a potential loss. We suggest that trait anxiety and economic risk avoidance are not necessarily associated in certain circumstances, especially when incidental emotions generated by external information affect anxious people's judgment. This idea is supported by one of our recent studies which discovered that during risk decision-making, the neural responses to feedback presentation were stronger under the influence of emotional facial expression in anxious participants compared to their non-anxious counterparts (Wang et al., 2016). As described in the Introduction, many studies have reported that the level of trait anxiety is consistently related to risk avoidance (Hartley and Phelps, 2012; Paulus and Yu, 2012). One should be very cautious if he/she would like to re-interpret the previous findings according to our theory, seeing that the current study has only examined one specific decision-making task. In our opinion, the key point of this study is that the relationship between trait anxiety and risk avoidance could be manipulated by the emotional context.

The possible mechanisms that connect trait anxiety with the framing effect should be discussed. At the physiological level, one of the major characteristics of anxiety is physiological hyperarousal (Joiner et al., 1996). It is possible that anxious individuals are more likely to be driven by emotional arousal during decision-making (Mano, 1992). As a result, their decision tends to be in accordance with the valence of emotional information, that is, making approach or avoidance responses under the influence of positive or negative emotion, respectively. At the brain level, trait anxiety magnitude is associated with structural and functional differences in the brain that may affect cognitive performance (Kuhn et al., 2011). Among the brain areas that are sensitive to trait anxiety level, the amygdala and the prefrontal cortex are most often highlighted (Kim and Whalen, 2009; Comte et al., 2015; Greening and Mitchell, 2015). According to Xu et al. (2013), trait anxiety level was positively correlated with amygdala-based "emotional" system activation when decisions were consistent with the framing effect, but negatively correlated with the anterior cingulate cortex (ACC)-based "analytic" system activation when decisions ran counter to the framing effect. It is thus possible that trait anxiety affects risk decision-making by modulating the activity of the brain networks being involved in the task. However, we should keep in mind that the major findings of this study were derived from correlation analyses. Strictly speaking, the direction of the causal relationship between trait anxiety and the framing effect remains undetermined.

The final part of this paper is about the limitations of the current study. First, regarding the high correlation between STAI-T and STAI-S scores ($r \approx 0.8$ in many studies; e.g., Wu et al., 2013), this study only collected STAI-T data. Seeing that trait anxiety and state anxiety are qualitatively distinct, follow-up research is necessary to examine the impact of state anxiety on the framing effect. Second, consistent with the task design in De Martino et al. (2006), only the description of the safety option, but not that of the risky option, was different between frame conditions. We did not change the original task because it reliably elicits the framing effect (see Introduction). Nevertheless, it would be interesting to check whether individual preference to the risky option would also be affected by the frame, and whether this effect would be sensitive to anxiety. Third, we predict that in naturalistic scenarios (e.g., the Asian disease problem), the relationship between anxiety and risk avoidance would not be overshadowed by the framing effect, but this idea was untested in the current study.

In a word, the current study indicates that the relationship between trait anxiety and risk decision-making is more complicated than what previous literature suggested, and further research is still needed to explore this issue.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of American Psychological Association (2010) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the local Ethics Committee at Beijing Normal University.

AUTHOR CONTRIBUTIONS

RG, RW, and PX conceived and designed the experiments. RW and PX performed the experiment. RG, YJ, and PX analyzed the data. RG, LB, YJ, and PX wrote the manuscript. RX, QY, and Y-JL contributed to manuscript revision.

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NEUROLINGUISTICS

Distinct distributed patterns of neural activity are associated with two languages in the bilingual brain

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A large body of previous neuroimaging studies suggests that multiple languages are processed and organized in a single neuroanatomical system in the bilingual brain, although differential activation may be seen in some studies because of different proficiency levels and/or age of acquisition of the two languages. However, one important possibility is that the two languages may involve interleaved but functionally independent neural populations within a given cortical region, and thus, distinct patterns of neural computations may be pivotal for the processing of the two languages. Using functional magnetic resonance imaging (fMRI) and multivariate pattern analyses, we tested this possibility in Chinese-English bilinguals when they performed an implicit reading task. We found a broad network of regions wherein the two languages evoked different patterns of activity, with only partially overlapping patterns of voxels in a given region. These regions, including the middle occipital cortices, fusiform gyri, and lateral temporal, temporoparietal, and prefrontal cortices, are associated with multiple aspects of language processing. The results suggest the functional independence of neural computations underlying the representations of different languages in bilinguals.

INTRODUCTION

The human brain has been equipped with a marked ability to acquire more than one language, as in bilingual individuals. However, it remains unsolved as to how different languages are represented in the bilingual brain (*1*–*4*). A large body of neuroimaging studies has suggested that multiple languages are processed and organized in a single brain system (*5*–*7*) [but see related studies (*8*–*10*)]. Brain areas are activated to a comparable degree when bilinguals performed tasks in the first (L1) and second (L2) languages at both the word level and the sentence level (*11*–*19*), although differential activation may be seen in some studies because of different proficiency levels and/or age of acquisition of the two languages (*8*, *15*, *20*, *21*).

The traditional single cortical mechanism hypothesis assumes that shared regions are recruited for processing L1 and L2, but one important possibility is that the two languages may involve interleaved but functionally independent neural populations, and thus, distinct patterns of neural computations may be pivotal for the processing of the two languages. Bilingual speakers are able to use each of their languages appropriately and swiftly switch from one language to the other. It is therefore reasonable to hypothesize that there might be some degree of segregation in the neural representations of each language to avoid cross-talk (*22*, *23*). In the typically used univariate analysis of functional neuroimaging data, images are spatially smoothed, and each voxel is treated independently, which leads to loss of fine-grained pattern information (*24*, *25*). Multivariate pattern analysis (MVPA) extracts the signal that is present in the pattern of response across multiple voxels, and it could therefore resolve this problem by operating on patterns of neural activation and by directly linking activation patterns to experimental conditions (*24*, *26*, *27*). It is suited for detecting fine-grained pattern differences even if they occur in the absence of regional-average differences (*28*).

Here, we used MVPA and investigated whether the neural representations of L1 were distinguishable from those of L2 by analyzing the pattern of functional magnetic resonance imaging (fMRI) blood-oxygen-level dependent (BOLD) signals in Chinese-English bilinguals. We used an implicit word-processing task in which the subjects pressed a key when two consecutive words were the same (Fig. 1). The implicit reading task does not ask for explicit reading, but reading occurs obligatorily, and it provides comparable processing demands for different groups of subjects or conditions (*29*, *30*). By using region of interest (ROI)–based and searchlight-based MVPA, we found a broad network of regions wherein the two languages evoked different patterns of activity.

RESULTS

Behavioral results

Reaction time and accuracy data were submitted to repeated-measures analysis of variance (ANOVA). The results showed that there was no significant effect for reaction time [$F(3, 33) = 0.61$, $P = 0.613$], but there was a significant effect for accuracy [$F(3,33) = 6.328$, $P < 0.01$]. Followed-up paired t tests revealed greater accuracy for Chinese real words than Chinese false fonts [$t(11) = 3.3$, $P < 0.01$)] and English false fonts [$t(11) = 3.4$, $P < 0.01$] and greater accuracy for English real words than English false fonts [$t(11) = 2.9$, $P < 0.05$]. There were no significant differences in accuracy between Chinese real words and English real words [$t(11) = 1.33$, $P = 0.21$], between Chinese false fonts and English false fonts [$t(11) = 0.54$, $P = 0.60$], and between Chinese false fonts and English real words [$t(11) = 2.13$, $P = 0.06$] (as shown in Fig. 1B).

Multivoxel classification of L1 and L2

We first conducted MVPA to discriminate between L1 real words and L2 real words on the basis of ROIs that were consistently reported to be involved in reading and language processing according to previous studies (*31*–*36*), including the lateral occipital cortex (LOC; inferior/middle occipital cortex), fusiform gyrus (FusiG), lateral temporal cortex (LTC; superior/middle temporal gyri), temporoparietal cortex (TPC; supramarginal/angular gyri/inferior parietal lobule), and lateral prefrontal

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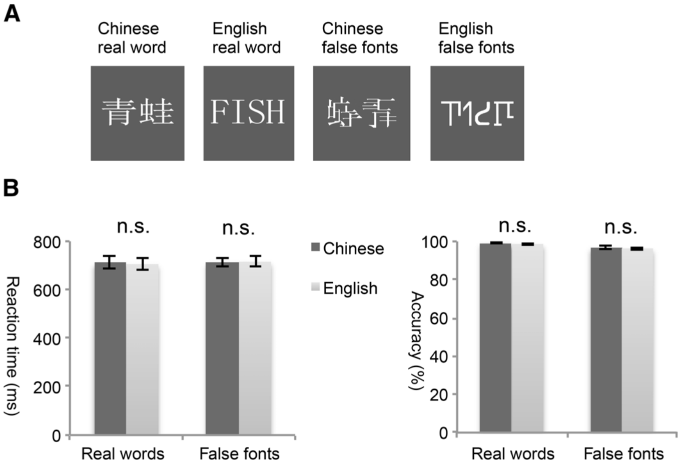

Fig. 1. Examples of experimental stimuli and behavioral performance. (**A**) Examples of Chinese real words, English real words, Chinese false fonts, and English false fonts used in the task. (**B**) Mean reaction time and accuracy rate for the four conditions. Error bars depict SEM. n.s., not significant.

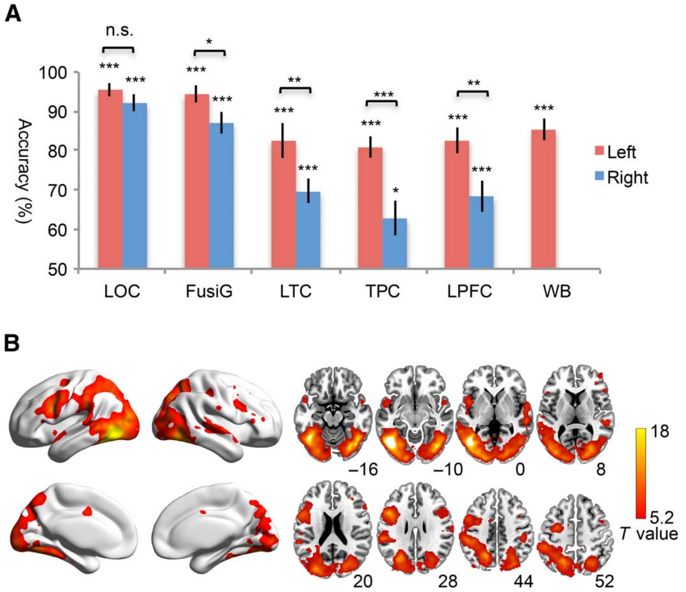

Fig. 2. MVPA results for classification of L1 real words versus L2 real words. (**A**) Classification accuracies for the left (red bar) and right (blue bar) hemispheric ROIs. Error bars depict SEM. Significance markings for individual bars indicate above-chance (50%) classification accuracy; significance marking between bars indicates significant difference between left and right hemispheric ROIs. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (**B**) Searchlight MVPA results presented as a *T*-map indicating the statistical significance of voxel-wise classification accuracies against the chance level [$P < 0.001$, false discovery rate (FDR)–corrected, equivalent to $t = 5.2$].

cortex (LPFC; inferior/middle frontal gyri) (fig. S1). Both the left and right hemispheric ROIs were defined. The results showed that the classification accuracies based on individual ROIs and the whole brain (WB) were all significantly greater than chance level (50%) after corrections for multiple comparisons (Fig. 2A): LOC [left: $t(13) = 29.3$, $P < 0.0001$; right: $t(13) = 19.4$, $P < 0.0001$]; FusiG [left: $t(13) = 21.6$, $P < 0.0001$; right: $t(13) = 13.4$, $P < 0.0001$]; LTC [left: $t(13) = 10.2$, $P < 0.0001$; right: $t(13) = 5.4$, $P < 0.001$]; TPC [left: $t(13) = 11.0$, $P < 0.0001$; right: $t(13) = 3.0$, $P < 0.05$]; LPFC [left: $t(13) = 9.7$, $P < 0.0001$; right, $t(13) = 4.5$, $P < 0.001$]; and WB [$t(13) = 13.4$, $P < 0.0001$]. We next performed a repeated-measures ANOVA with ROIs and the hemispheres as within-subject factors to explore hemispheric differences. Results revealed a significant main effect of hemisphere [$F(1,13) = 43.7$, $P < 0.0001$] and region [$F(4, 52) = 22.3$, $P < 0.001$]. There was a significant hemisphere × region interaction [$F(4, 52) = 3.7$, $P < 0.05$], indicating that the hemispheric effect was not the same across ROIs. To explore this, we performed post hoc paired *t* tests in each ROI separately and found that four ROIs showed significantly greater accuracies in the left than the right hemisphere: FusiG [$t(13) = 2.6$, $P < 0.05$]; LTC [$t(13) = 3.8$, $P < 0.01$]; TPC [$t(13) = 5.1$, $P < 0.001$]; and LPFC [$t(13) = 3.6$, $P < 0.01$], indicating that the left hemispheric ROIs carried more discriminative information than their right hemispheric counterparts. In contrast, there was no hemispheric difference in classification accuracy in LOC [$t(13) = 1.4$, $P = 0.19$]. To further examine whether classification of L1 versus L2 was mainly determined by positive BOLD response or negative BOLD response, we extracted the β values of the most informative voxels in the left hemispheric ROIs (see Materials and Methods). The analysis revealed that the classification accuracies were determined both by voxels with positive BOLD responses (36 to 74% of voxels across ROIs) and by voxels with negative BOLD responses (26 to 64% of voxels across ROIs) (fig. S2).

Searchlight MVPA

To ensure that we did not overlook any anatomical regions that were sensitive to L1 and L2, we used a "searchlight" approach to identify regions of high classification accuracy throughout the brain (*37*). As shown in Fig. 2B, analyses with a 4-mm-radius searchlight revealed left-lateralized discriminative patterns, with significant searchlight centers located in the bilateral occipital cortex and fusiform gyri {peak at Montreal Neurological Institute (MNI) coordinates [−44 −64 −8] and [40 −70 −14]}, bilateral inferior and middle frontal gyri ([−46 12 28] and [54 28 8]), left TPC ([−58 −34 28]), and bilateral superior and middle temporal gyri ([−46 4 −4] and [58 −28 2]). This analysis also revealed sensitivity of the bilateral superior parietal lobule/precuneus (peak at [−24 −66 34] and [30−74 38]) in distinguishing between L1 and L2. Therefore, in the following analyses, left and right superior parietal cortices (SPCs; superior parietal lobule/precuneus) were also included as ROIs.

Univariate analyses for L1 versus L2

Univariate analyses were conducted to facilitate comparisons between pattern-based and voxel-based analyses. We found several small clusters that showed greater activity for L2 real words than L1 real words in the left inferior frontal gyrus, left occipitotemporal cortex, and left precuneus (Fig. 3 and table S1), whereas no greater activity was found for L1 than L2, indicating that brain activation elicited by L1 and L2 overlapped substantially. Relative to univariate analyses, MVPA revealed markedly widespread brain regions that could successfully discriminate between L1 and L2 by incorporating the signal from multiple voxels.

Discriminative information in the ROIs

First, to examine whether the discriminative information concerns only low-level visual complexities that are distinct for the two languages, we applied the classifiers trained to distinguish between L1 and L2 real words to classify L1 and L2 false fonts (see Materials and Methods). Because the false fonts have similar structure and complexity to the real words but contain no linguistic features, the generation of activation

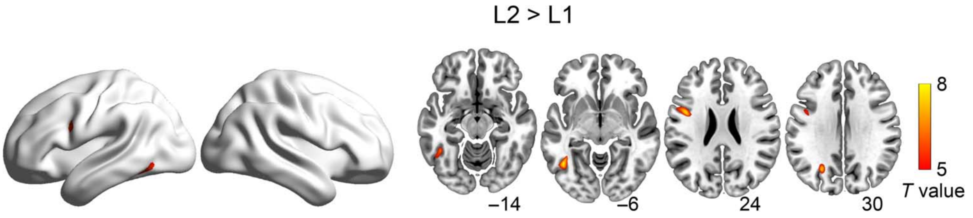

Fig. 3. Cortical activation associated with L2 real words minus L1 real words revealed by the univariate analysis ($P < 0.05$, FDR-corrected, equivalent to $t = 5.0$). No significant activation was found for L1 real words minus L2 real words.

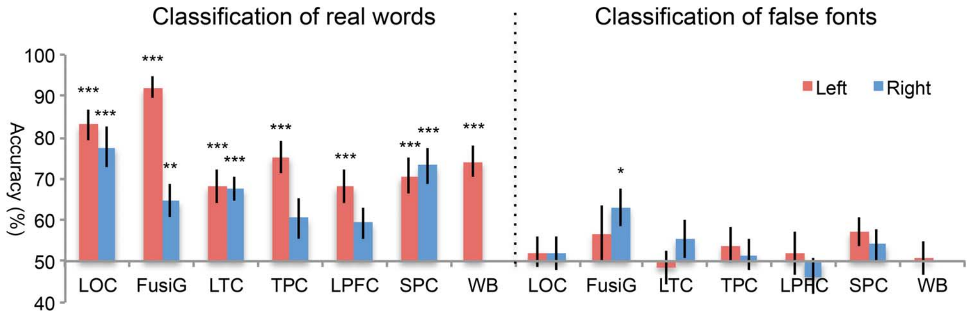

Fig. 4. Accuracies for classifying real words (left) and false fonts (right) between different languages using SVM classifier trained to discriminate between L1 and L2 real words. Error bars depict SEM. Significant above-chance (50%) classification accuracy is indicated by asterisks. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

patterns between real words and false fonts may indicate that L1 and L2 real words are distinguished on the basis of their visual complexity rather than differences in linguistic features of the two languages. As shown in Fig. 4, accuracies for classifying L1 and L2 real words were significantly greater than chance for most of the ROIs except in the right TPC and right LPFC. The accuracies in this step were lower than those obtained in the above ROI analyses, as shown in Fig. 2A, which may be due to less training data used (*24*). Accuracies for classifying L1 false fonts and L2 false fonts were statistically significant only in the right FusiG [$t(13) = 2.9$, $P < 0.05$] but not in any other ROI. We then performed a correlation analysis between the accuracies for classifying L1 and L2 real words and the accuracies for classifying L1 and L2 false fonts across ROIs. The result shows that the correlation is not significant ($r = 0.238$, $P = 0.434$) (fig. S3), suggesting that the regions containing the most discriminative information for L1 real words versus L2 real words do not necessarily contain the most discriminative information for L1 false fonts versus L2 false fonts. Correlation analyses were also performed within each ROI to examine whether the accuracies for classifying real words correlated with the accuracies for classifying false fonts of the two languages across subjects. We found significant correlation only in the right FusiG ($r = 0.576$, $P = 0.031$) but not in any other ROI. These results suggested that in most of the ROIs, activity patterns distinguishing between L1 real words and L2 real words were not based on low-level visual complexity.

Second, the frontal and parietal regions that we defined as language ROIs also belong to the domain-general frontoparietal executive network, which has been shown to decode different aspects of task demands and materials [see the study of Woolgar *et al.* (*38*) for a recent review]. To examine whether the voxels within these ROIs responded more strongly to words than false fonts, we performed a univariate analysis on (Chinese real words + English real words) versus (Chinese false fonts + English false fonts). The activation maps (threshold set at $P < 0.05$, FDR-corrected at voxel level) were masked by the anatomical ROIs of LPFC, TPC, and SPCs, respectively. We then compared the number of voxels that activated for real words − false fonts (language-responsive) and the number of voxels that activated for false fonts − real words (cognitive demand–sensitive) [for a similar rationale, see the study of Fedorenko *et al.* (*39*)]. Results showed that there were much more language-responsive than cognitive demand–sensitive voxels in the LPFC (1940 versus 338) and TPC (1319 versus 599), suggesting that these two ROIs may distinguish between L1 and L2 more on the basis of the language-related information. In contrast, there were much fewer language-responsive voxels in the SPC (555 versus 1718), indicating that this region may rely more on the domain-general information to distinguish between the two languages.

Spatial distribution of the most informative voxels coding for L1 and L2

To examine the extent to which the most informative voxels coding for L1 were separated from those for L2, we performed classifiers in the left hemispheric ROIs for the two languages separately: L1 real words versus L1 false fonts (L1 discrimination) and L2 real words versus L2 false fonts (L2 discrimination). We found high classification accuracies for both L1 discrimination (90.6 to 97.6%) and L2 discrimination (84.4 to 96.6%) in all ROIs (fig. S4). We then generated maps for the best-coding voxels by including voxels whose weights exceeded 2 SD in the group analysis. The most informative voxels coding for L1 were spatially separated from those for L2 mainly in the left FusiG, LTC, TPC, LPFC, and SPC (Fig. 5, B to F), with separation percentages as follows: FusiG, 96.5%; LTC, 79.7%; TPC, 74.8%; LPFC, 67.3%; and SPC, 72.5% (the number of best-coding voxels surviving threshold is reported in table S2). In addition, we found considerable overlap of informative voxels between the two languages mainly in the left LOC (Fig. 5A; separation percentage, 50.1%). To facilitate a comparison between the univariate approach and MVPA, we followed a similar procedure to calculate the separation percentages for the univariate method in the left hemispheric ROIs. The separation percentages for individual ROIs yielded from the univariate analysis are as follows: LOC, 9.6%; FusiG,

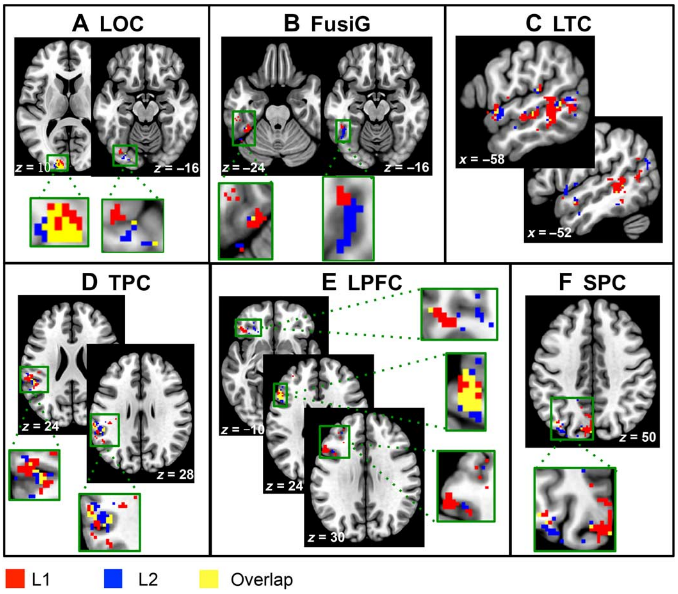

Fig. 5. Spatial distribution of the most informative voxels coding for L1 and L2. The maps show the best-coding voxels for L1 discrimination (red), L2 discrimination (blue), and their overlap (yellow) in the left hemispheric LOC (**A**), FusiG (**B**), LTC (**C**), TPC (**D**), LPFC (**E**), and SPC (**F**).

32.3%; LTC, 30.0%; TPC, 22.5%; LPFC, 28.5%; and SPC, 28.3% (fig. S5 and table S3), which are much less than those from MVPA.

DISCUSSION

Previous neuroimaging studies using the traditional univariate analyses have consistently found overlapping regions for L1 and L2 [for example, (*11*–*19*)]. Here, by using a more fine-grained MVPA technique, our study has demonstrated that different languages were processed in common regions but in distinguishable patterns of response, implicating that functionally independent neural populations are involved (*28*). Segregation in the neural representations of each language might be crucial for bilingual speakers to appropriately use each language and avoid cross-talk.

Previous aphasic and neurosurgical studies have supported distinct brain systems for different languages in bilinguals. Observations of brain-damaged patients showed that brain injuries may produce selective impairments of only one language in bilinguals and that patients may recover one language but not the other (*40*–*45*). Neurosurgical studies have also identified language-specific areas in dominant frontal, parietal, and temporal regions because electrical stimulation of these sites produces interferences in only one language and not in any other (*46*–*50*). Our findings may provide some implications for the evidence from neuroimaging and aphasic/neurosurgical studies. The MVPA that we used here discriminates neural representations of different languages by taking into account the relationships across voxels, whereas the univariate method previously used examines only changes in the gross neural activity and may therefore fail to detect the important differential patterns. Meanwhile, our results suggest that L1 and L2 are represented in specific patterns that involve neuronal populations in the critical regions to different degrees, such that electrical stimulation or inhibition of the neuron populations crucial for one language could cause interference of that language but not the other.

One question to address is what sort of information decoded by the brain distinguishes between L1 and L2. The implicit reading task that we used here is not designed to address any specific aspect of language processing, and thus, different brain regions that show distinct neural patterns may be associated with different levels of language processing. Written Chinese presents sharp contrast to English in terms of orthographic features and how the written symbols map onto sounds and semantics (*51*, *52*). Therefore, information about the visual appearance, orthography, phonology, and semantics of the word stimuli could be used to classify the two languages. Chinese characters are formed with intricate strokes filled in square configurations, as opposed to the linear structure of alphabetic words. This difference may lead to the neural separations in the LOCs and the fusiform gyri for the representations of visual features and orthographic properties (*30*, *34*, *36*, *53*) and in the SPCs for fine-grained visuospatial representations of written words in the two languages (*54*, *55*). Moreover, Chinese characters map directly to monosyllables in an arbitrary way with tonal information that convey different meanings of words, whereas English is a nontonal language, and words are predominantly read out by assembling the phonemic components (*32*, *56*). Distinct neural patterns in the temporal and lateral prefrontal cortices for the two languages may thus reflect their differences in phonological representations and in the mapping from written symbols to sound and meaning (*31*, *33*, *35*, *36*). We also found that the distinct patterns for L1 and L2 in most regions are unlikely to reflect different visual complexities of the two languages because the support vector machine (SVM) classifier trained to discriminate between response patterns of Chinese real words and English real words failed to classify their corresponding false-font stimuli in all ROIs, except in the right FusiG, which may be recruited for holistic and configural analysis of the stimulus (*57*). Therefore, the differences in linguistic features might contribute to the discrimination between the two languages. Further research will need to more specifically examine the nature of the discriminative information, including comparing the auditory versus visual representations of words, to know whether the mapping between written forms and sound/meaning contributes to the classification.

Previous studies using MVPA to classify L1 and L2 have focused on specific sets of regions and produced inconsistent results (*58*–*60*). For example, Willms *et al.* (*58*) found no difference in multivoxel patterns for L1 and L2 in Spanish-English bilinguals, but they performed MVPA on ROIs restricted to these regions that showed greater activity for verb than noun processing in both L1 and L2. There was little overlap between the voxels in the ROIs and those in the regions that showed an interaction effect of language and grammatical class. Thus, the brain areas that were likely to distinguish between L1 and L2 were not included in the MVPA in the study. In another study, Bai *et al.* (*60*) found similar spatial patterns of response in visual word form areas to different languages in Korean-Chinese bilinguals, which is inconsistent with our results. One possibility is that written words of Korean and Chinese are both in square shape, and it might therefore be more difficult to discriminate them in the fusiform areas. An alternative (but not mutually exclusive) explanation is that Bai *et al.* (*60*) performed MVPA based on very restricted ROIs (6-mm radius centered at the peak of Chinese + Korean − fixation), whereas we used the whole FusiG as a priori anatomical ROIs. A recent study by Hsu *et al.* (*59*) investigated the emotional aspect of language processing and found distinguishable patterns for L1 and L2 in German-English bilinguals in emotion-related regions. Because these regions are not closely associated with language processing, the study does not necessarily address the question of pattern differences between L1 and L2 per se.

Note that our findings do not imply that there are no shared representations for different languages in the bilingual brain. On the contrary, we identified a number of subsets of informative voxels overlapping for L1 and L2. This was consistent with previous MVPA findings that representations of semantic information can be generalized between L1 and L2 (*61*, *62*) and with results from studies using fMRI adaptation paradigms that found language-independent semantic representations in the left lateral prefrontal (*63*, *64*) and temporal regions (*18*, *63*).

Together, we demonstrated here that L1 and L2 could be neuroanatomically separated by widely distributed patterns of activity using the pattern analysis technique. The finding may provide new leverage points for examining the underlying neural processes for different languages and thus offers an insight into the links between brain representations and language disorders in bilinguals. We note that the present study has limitations that should be addressed in the future. For example, although the subjects' behavioral performance on the task was comparable for L1 and L2, nonequivalent proficiency of L1 and L2 might have confounded the MVPA results. Further research is needed to more specifically examine the nature of these signals related to L1 and L2 in various brain areas, including whether they are modulated by proficiency and age of acquisition.

MATERIALS AND METHODS

Subjects

Fourteen bilingual subjects (12 females) were recruited in our experiment (aged 23 to 33 years; mean = 26.5, SD = 3.3). They were native Mandarin Chinese speakers from China and were studying or working in the Boston area during the time of scanning. The subjects had started to learn English as their L2 between 6 and 15 years of age. They completed a language-background questionnaire (*65*) and were measured of L2 proficiency using Peabody Picture Vocabulary Test (PPVT4) (mean score = 149.2, SD = 28.5). The subjects were physically healthy and free of neurological disease, head injury, and psychiatric disorder. They were right-handed, as assessed by the handedness inventory. The study was approved by the ethics committee at the Massachusetts Institute of Technology (MIT), and all subjects gave informed consent before the experiments.

Design and materials

The subjects performed an implicit word-processing task, in which they were presented with a sequence of words and required to press a key when two consecutive words were the same. The task was simple, providing comparable processing demands for L1 and L2. Animal words and action words were used for both languages. To match Chinese words and English words in length, we chose Chinese words containing two characters (average number of phonemes = 4.9, SD = 0.9; mean frequency = 11.5 per million) and English words containing three to six letters (average number of syllables = 1.1, SD = 0.3; average number of phonemes = 3.3, SD = 0.7; mean frequency = 26.5 per million). Chinese words were the closest possible translation of the English words, and thus, they were matched for meaning. The subjects also performed the task with false fonts, which were constructed by scrambling the strokes of the words used in the real-word conditions. With this method, the false fonts have a similar complexity to the real words but contain no linguistic features (Fig. 1A). The subjects underwent six to eight functional runs. There were four blocks for each condition within each run, and condition order was counterbalanced. Each block consisted of 16 stimuli, among which 2 would be the same as the last one. On each trial, a white stimulus was displayed on the center of a gray background for 700 ms, followed by a 300-ms blank interval. To reduce practice effects, we used two different sets of stimulus items alternatively among different runs. All subjects had some practice before scanning, and they were instructed to perform as quickly and accurately as possible. We failed to record two subjects' behavioral responses, and thus, the behavioral results were based on data from the remaining 12 subjects. Their behavioral performance under each condition is illustrated in Fig. 1B.

MRI acquisition

Functional images were acquired using a 3T Siemens MRI scanner with a 32-channel head coil at the Athinoula A. Martinos Imaging Center at MIT. A gradient–echo echo planar imaging (EPI) sequence was used (TR (repetition time)/TE (echo time) = 2000/30 ms; flip angle = 90°; voxel size of 3.1 × 3.1 × 3, with a 0.3-mm gap). Visual stimuli were presented through a projector onto a translucent screen, and subjects viewed the screen through a mirror attached to the head coil.

fMRI data preprocessing and univariate analysis

We used the Statistical Parametric Mapping software package (SPM8) (www.fil.ion.ucl.ac.uk/spm/) for preprocessing. Functional images were realigned to the first volume of the first functional scan to remove movement artifact. They were then spatially normalized to an EPI template based on the International Consortium for Brain Mapping (ICBM) 152 stereotactic space. Voxels were resampled at a voxel size of $2 \times 2 \times 2$ mm^3. For the MVPA, functional images were not spatially smoothed. For the univariate analyses, an isotropic Gaussian kernel of 8 mm full width at half maximum (FWHM) was applied for spatial smoothing. The preprocessed images were passed to a general linear modeling (GLM), which was used to obtain parameter estimate (β) images associated with each stimulus condition. GLM was constructed with experimental regressors modeled as boxcar function and convolved with a canonical hemodynamic response function. Realignment parameters were included in the model to regress out movement-related variance. Each time series was high-pass–filtered with a cutoff period set at 128 s to remove low-frequency drifts. For the univariate analysis, contrast images were generated for each subject and were then used to create group contrast images at the second level.

Multivoxel pattern analyses

A linear support vector machine (LibSVM; regularization parameter $C = 1$) was performed using PRoNTo (for ROI approach) (*66*) and the Decoding Toolbox (for searchlight approach) (*67*). The β images were extracted for each run separately and used as input for the classifiers. We calculated the accuracy for classification using a leave-one-session-out cross-validation procedure. For MVPA based on ROIs, an SVM was trained and tested separately on WB and each ROI. Anatomical ROIs were generated using the Wake Forest University PickAtlas. For the statistical tests of classification accuracies across ROIs, results were corrected for multiple comparisons using FDR at $P = 0.05$ following the Benjamini-Hochberg procedure. To examine whether the classification accuracies of L1 and L2 were determined by positive BOLD response, negative BOLD response, or a combination of the two, we extracted the β values of the most informative voxels. Linear SVM assigns a weight to each voxel that indicates its importance in the classification. The most informative voxels were defined as those voxels whose weights exceeded ±2 SD in the group analysis. We calculated the percentages of voxels with positive BOLD response (positive β value) and negative BOLD response (negative β value), as well as the averaged β values for each left hemispheric ROI.

In searchlight-based MVPA, a spherical searchlight with a radius of 4 mm was moved across the entire brain by taking each voxel in the volume as the searchlight center. For each sphere, a linear SVM was trained and tested as described above, and the classification accuracy score was assigned to the central voxel. The WB classification accuracy maps of individual subjects were spatially smoothed at 6-mm FWHM and were then subjected to random-effect group analysis. The resulted T-map indicated the statistical significance of voxel-wise accuracies against a chance-level accuracy of 50%. The T-map was thresholded at $P < 0.001$ and FDR-corrected.

To test whether the discriminative information concerns only low-level visual complexities that are distinct for L1 words and L2 words, we trained SVM classifiers to discriminate between response patterns of L1 real words and L2 real words and tested their predictive capacity for discriminating between L1 false fonts and L2 false fonts. We split the data set into two parts, with the first half as training data and the second half as testing data. The SVM classifiers were trained with the first half of real words, and the trained classifiers were then applied to predict the second half of real words and false fonts separately.

Finally, to examine spatial distributions of the most informative voxels coding for L1 and L2, we performed SVM classifiers using leave-one-session-out cross-validation for the two languages separately: L1 real words versus L1 false fonts (L1 discrimination) and L2 real words versus L2 false fonts (L2 discrimination). We generated maps for the left hemispheric ROIs to include the most informative voxels whose weights exceeded 2 SD in the group analysis. To quantify the different spatial distributions of informative voxels between L1 and L2, percentages of unique voxels were calculated for each language by dividing the number of unique voxels (that is, voxels for one language that do not show overlap with the other language) by the total number of voxels surviving threshold. Separation percentages were then calculated by averaging percentages of unique voxels for L1 and L2, that is, $(\mathrm{L1}_{\mathrm{unique}}/\mathrm{L1} + \mathrm{L2}_{\mathrm{unique}}/\mathrm{L2})/2$. To compare the degrees of spatial separation of L1 versus L2 using different approaches, we followed a similar procedure as in the MVPA to calculate the separation percentages in the univariate analysis. Contrast images of L1 real words minus L1 false fonts and L2 real words minus L2 false fonts were generated for each subject, and at the second-level analysis, anatomical ROIs were used as masks to create group contrast images. The most activated voxels with activation levels exceeding 2 SD in the group analysis were used to calculate separation percentages.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/7/e1603309/DC1

fig. S1. Axial view of ROIs overlaid on standard brain.

fig. S2. Percentages and the averaged β values of the voxels showing positive (red) and negative (blue) BOLD responses among the voxels contributing the most to the classification of L1 real words versus L2 real words in each of the left hemispheric ROIs.

fig. S3. Correlation between the accuracies for classifying L1 and L2 real words and the accuracies for classifying L1 and L2 false fonts across ROIs.

fig. S4. Accuracies for classifying L1 real words versus L1 false fonts (L1 discrimination, left panel) and classifying L2 real words and L2 false fonts (L2 discrimination, right panel).

fig. S5. Spatial distribution of the activated voxels for L1 and L2 in the univariate analysis.

table S1. Coordinates of activation peaks for the contrast of English words minus Chinese words in the univariate analysis.

table S2. Number of best-coding voxels surviving threshold ($z > 2$) and percentages of unique voxels for L1 and L2 for each ROI in MVPA.

table S3. Number of voxels surviving the threshold ($z > 2$) and percentages of unique voxels for L1 and L2 for each ROI in the univariate analysis.

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Distinct distributed patterns of neural activity are associated with two languages in the bilingual brain

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A Meta-Analytic Study of the Neural Systems for Auditory Processing of Lexical Tones

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The neural systems of lexical tone processing have been studied for many years. However, previous findings have been mixed with regard to the hemispheric specialization for the perception of linguistic pitch patterns in native speakers of tonal language. In this study, we performed two activation likelihood estimation (ALE) meta-analyses, one on neuroimaging studies of auditory processing of lexical tones in tonal languages (17 studies), and the other on auditory processing of lexical information in non-tonal languages as a control analysis for comparison (15 studies). The lexical tone ALE analysis showed significant brain activations in bilateral inferior prefrontal regions, bilateral superior temporal regions and the right caudate, while the control ALE analysis showed significant cortical activity in the left inferior frontal gyrus and left temporo-parietal regions. However, we failed to obtain significant differences from the contrast analysis between two auditory conditions, which might be caused by the limited number of studies available for comparison. Although the current study lacks evidence to argue for a lexical tone specific activation pattern, our results provide clues and directions for future investigations on this topic, more sophisticated methods are needed to explore this question in more depth as well.

Keywords: meta-analysis, lexical tones, auditory processing, neuroimaging, activation likelihood estimation (ALE) meta-analysis

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INTRODUCTION

The functional anatomy of speech perception has been intensively investigated for over a century in the neuropsychology literature, and more recently in the neuroimaging literature. Speech processing is known to preferentially rely on cortical regions in the left hemisphere (Hickok and Poeppel, 2007), but neural specialization of different aspects (e.g., tone, rhyme, stress and other spectral and temporal properties) of speech remains controversial. For tonal language speakers, lexical tone plays a critical role in spoken word recognition, which involves complex acoustic and phonological processes. While a large number of studies have been designed to uncover the perceptual and cognitive mechanisms in tone processing, it is only until recently that researchers have begun to focus on the neural substrates underlying tone perception. Since around half of the world's languages are tonal (Maddieson, 2013), understanding how lexical tone is processed and represented in the brain could provide significant insights into mechanisms of speech perception.

Lexical tone in tonal languages is characterized by pitch variations at the syllable level, and it is used to distinguish lexical or grammatical meanings. While it is known that the processing of non-linguistic pitches such as music and melodies are associated with right hemispheric regions (e.g., Zatorre et al., 1992, 1994), researchers have started to ask whether the neural specialization for linguistic pitch patterns (i.e., lexical tones) would be different from that of non-linguistic pitch patterns. Two prominent models have been proposed regarding the hemispheric dominance of human pitch perception, the domain-specific model and the cue-specific model. The domain-specific model (or the functional hypothesis) assumes lateralization depends on the function of pitch patterns: when tones are processed as acoustic units (i.e., pure variation in pitch) their processing is right lateralized, but when they are processed as phonological units (i.e., as linguistic or semantic information) their processing is left lateralized (Whalen and Liberman, 1987; Liberman and Whalen, 2000). By contrast, the cue-specific model (or the acoustic hypothesis) assumes that pitch patterns are processed according to their acoustic structures, regardless of their functions, and are therefore lateralized only to the right hemisphere (Van Lancker, 1980; Zatorre and Belin, 2001).

The brain basis of lexical tone processing has been examined in neuroimaging studies for over a decade. East Asian tone languages such as Mandarin and Thai have been frequently studied in order to investigate neural correlates underlying lexical tone perception in native speakers of tonal language. In the brain imaging literature, the cortical representation of lexical tone perception has been examined with several approaches. Early studies have focused on the cross-linguistic comparisons of the neural basis for lexical tone perception in tonal vs. non-tonal language speakers (Gandour et al., 1998, 2000, 2002, 2003; Hsieh et al., 2001; Klein et al., 2001; Wong et al., 2004). These cross-linguistic studies have consistently indicated left hemisphere specialization for lexical tone processing in native speakers of tonal languages, contrasting the right hemisphere specialization in native speakers of non-tonal languages. Since non-tonal language speakers who have had no prior experience with a tonal language failed to show brain activity in the left hemisphere in lexical tone perception, Wang et al. (2003) and Wong et al. (2007) conducted lexical tone training studies to test whether American learners could process lexical tones in ways similar to native speakers after learning. They found that successful lexical tone learners showed enhanced cortical activations in left superior temporal regions (BA22, BA42), whereas less successful learners showed greater activation in the right hemispheric regions relative to the successful learners, such as the superior temporal region and inferior frontal gyrus. More recent studies have focused on examining the neural system of lexical tone perception in native tonal language speakers, with two commonly used experimental paradigms: explicit lexical tone perception (Li et al., 2003, 2010; Xu et al., 2006; Nan and Friederici, 2013; Yu et al., 2014) and lexical tone production (Liu et al., 2006; Chang et al., 2014). These investigations with native speakers have indicated contributions of numerous brain regions in the processing of lexical tones, including: (1) bilateral frontal language areas (i.e., posterior prefrontal gyrus, middle frontal gyrus); (2) bilateral fronto-parietal network; (3) bilateral superior temporal and surrounding regions; and (4) the left insular cortex. Moreover, structural imaging studies have reported increased gray matter volume in brain regions of tonal language speakers relative to non-tonal language speakers, such as the left Heschl's gyrus (Wong et al., 2008), left insula/transverse temporal gyrus (BA42) and right superior temporal gyrus (Crinion et al., 2009).

Although previous studies have enhanced our understanding of the neural basis of auditory tone perception, these studies have generated markedly different patterns of findings and failed to show consistent patterns of hemispheric laterality of linguistic pitch processing in native speakers of tonal language. These divergent results are likely due to inter-subject variability and differences in experimental tasks, among other variables that characterize different studies. The present study is designed to analyze such variables across the existing neuroimaging studies of tone perception, aiming at providing a clearer picture of the brain networks that are most consistently involved in auditory perception of lexical tones. This study is exempt from ethics approval. In particular, we utilized a meta-analytic technique, activation likelihood estimation (ALE) method, to quantitatively synthesize results across published data from healthy adult participants in the relevant literature, and to reveal patterns of convergence among the brain regions associated with lexical tone perception. We did not recruit more human subjects for further analysis. Meta-analysis has proven to be an important statistical method to combine results from independently published brain imaging studies that may involve different task designs and scanner equipment. Integration of data from multiple studies could increase statistical power of findings, thereby providing a stronger conclusion than arguments gained from individual studies (Turkeltaub et al., 2012). However, a single meta-analysis might not be sufficient to explain the commonalities and specificities in the brain processing of lexical tones when compared to regular speech processing in general. Thus, we also conducted a meta-analysis that involved studies with similar language conditions in non-tonal languages, in which participants were engaged in lexico-semantic processing when auditory stimuli were presented. We sought to compare neural mechanisms engaged in both processes in tonal and non-tonal langauges, and to investigate the neural substrates specifically mediating lexical tone processing in tonal languages.

MATERIALS AND METHODS

Literature Selection

We utilized the PubMed database[1] to search for articles relevant to the meta-analysis. All selected lexical tone articles fulfilled the following selection criteria: (1) all involved normal, healthy, right-handed adults; (2) lexical tone related tasks were used in

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[1] https://www.ncbi.nlm.nih.gov/pubmed

the studies (all involved task-activation paradigms); and (3) all reported imaging data on 3D coordinates (*x*, *y*, *z*) in stereotactic space. With these selection criteria, we found 24 neuroimaging studies of auditory lexical tone processing in the PubMed database (as of March 27, 2017). Among these 24 studies, we excluded three studies that measured and contrasted subjects' structural brain volume (Wong et al., 2008; Crinion et al., 2009; Qi et al., 2015), because brain structural differences may not be directly associated with lexical tone processing, and in this meta-analysis we therefore focus only on the cortical activation evoked by functional tasks. One functional MRI study (Zhang et al., 2011) was also excluded, because it was based on region-of-interest (ROI) analyses while the ALE meta-analysis computes whole-brain analysis data only. We further excluded three studies that used silence/rest as baseline (Klein et al., 2001; Xu et al., 2006; Kwok et al., 2016), since these contrasts do not provide activation specific to language/auditory stimuli processing.

All selected articles for the control meta-analysis involved: (1) healthy, right-handed adults; (2) auditory lexical decision task was used in the studies (all involved task-activation paradigms); and (3) all reported imaging data on 3D coordinates (*x*, *y*, *z*) in stereotactic space. With these selection criteria, we found 19 neuroimaging studies involved in access to lexical information through audition in non-tonal languages in the PubMed database (as of March 27, 2017). Among these 19 studies, we excluded one study that did not state whether the 3D coordinates were in MNI or Talairach space (Roxbury et al., 2014), two more studies were excluded because they did not report the activation contrasts that we were interested in (Prabhakaran et al., 2006; Minicucci et al., 2013). We further excluded one study that used silence/rest as baseline (Zhuang et al., 2011), since these contrasts do not provide activation specific to language/auditory stimuli processing.

According to these inclusion and exclusion criteria, a set of 17 studies with Mandarin and Thai tone perception was entered into our meta-analysis: 11 used an explicit tone perception task (Gandour et al., 1998, 2000, 2002, 2003; Hsieh et al., 2001; Li et al., 2003, 2010; Wong et al., 2004; Nan and Friederici, 2013; Zhang et al., 2016, 2017), three used Mandarin tone production (Liu et al., 2006; Chang et al., 2014; Chang and Kuo, 2016), and three used Mandarin tone training (Wang et al., 2003; Wong et al., 2007; Yang et al., 2015). Among the 17 studies, 13 utilized

TABLE 1 | Summary of selected literature for lexical tone meta-analysis.

Study	Language	*N*	Experimental task	Baseline
Gandour et al. (1998)	Thai	5	Tonal discrimination	Nonspeech pitch discrimination
Gandour et al. (2000)	Thai	5	Tonal discrimination	Nonspeech pitch discrimination
Hsieh et al. (2001)	Mandarin	20	Tonal discrimination	Passive listening
Gandour et al. (2002)	Mandarin, Thai	20	Tonal discrimination	Nonspeech pitch discrmination
Gandour et al. (2003)	Mandarin	20	Tonal discrimination	Passive listening
Li et al. (2003)	Mandarin	12	Tonal discrimination	Syllable discrimination
Wang et al. (2003)	Mandarin	6	Tone identification	Visual, auditory and motor control tasks
Wong et al. (2004)	Mandarin	7	Tonal discrimination	Passive listening
Liu et al. (2006)	Mandarin	10	Pinyin-naming; character-naming	Fixation
Wong et al. (2007)	English	17	Tonal discrimination	Sinewave discrimination
Li et al. (2010)	Mandarin	12	Tonal discrimination	Consonant and vowel discrimination
Nan and Friederici (2013)	Mandarin	18	Tone congruity judgment of Chinese phrases	Tone congruity judgment of musical phrases
Chang et al. (2014)	Mandarin	15	Production of mixed tone sequences	Production of repeated tone sequences
Yang et al. (2015)	English	39	Tonal discrimination	Fixation
Chang and Kuo (2016)	Mandarin	30	Production of mixed tone sequences	Production of repeated tone sequences
Zhang et al. (2016)	Cantonese	19	Lexical tone discrimination	Talker's voice discrimination
Zhang et al. (2017)	Cantonese	11	Lexical tone discrimination	Musical notes discrimination

TABLE 2 | Summary of selected literature for auditory lexical judgment meta-analysis.

Study	Language	*N*	Experimental task	Baseline
Dapretto and Bookheimer (1999)	German	8	Semantic judgment	Syntactic judgment
Kotz et al. (2002)	German	13	Semantic task	Semantic judgment of nonwords
Rissman et al. (2003)	English	15	Semantic task	Semantic judgment of nonwords; tone decision
Poeppel et al. (2004)	English	10	Semantic task	Categorical perception; FM sweeps
Orfanidou et al. (2006)	English	13	Lexical decision of real words	Lexical decision of nonwords
Palti et al. (2007)	Hebrew	14	Semantic judgment	Semantic judgment of reversed words
Binder et al. (2008)	English	26	Semantic task	Non-speech tone decision; phoneme decision
Bilenko et al. (2009)	English	16	Semantic judgment of ambiguous words	Semantic judgment of unambiguous words
Raettig and Kotz (2008)	German	16	Semantic judgment of real words	Semantic judgment of nonwords
Ruff et al. (2008)	English	15	Semantic judgment	Lexical decision
Balthasar et al. (2011)	German	18	Homonym decision	Target word decision
Wright et al. (2011)	English	14	Lesical decision task of real words	Non-speech tone decision
Méndez Orellana et al. (2014)	Dutch	20	Semantic task	Presentation of nonwords
Lopes et al. (2016)	Brazilian	24	Semantic decision	Non-speech tone decision
Ludersdorfer et al. (2016)	German	29	Semantic task	Tone decision

TABLE 3 | Activation likelihood estimation (ALE) meta-analysis of auditory processing of lexical tones*.

Anatomical region	BA	Coordinates			ALE ($\times 10^{-2}$)	Volume (mm^3)
		x	*y*	*z*		
L inferior frontal gyrus	44	−44	12	24	1.84	760
	9	−40	4	30	1.49	
R medial frontal gyrus	8	2	18	44	1.84	552
L posterior transverse temporal gyrus	42	−58	−18	8	2.21	544
R superior temporal gurys	22	58	−6	2	1.84	320
R anterior cerebellum	-	2	−64	−26	1.88	312
R posterior transverse temporal gyrus	41	56	−22	4	1.82	280
R caudate	-	12	6	8	1.66	136

**BA, Brodmann area; L, left; R, right. Coordinates were based on Talairach space.*

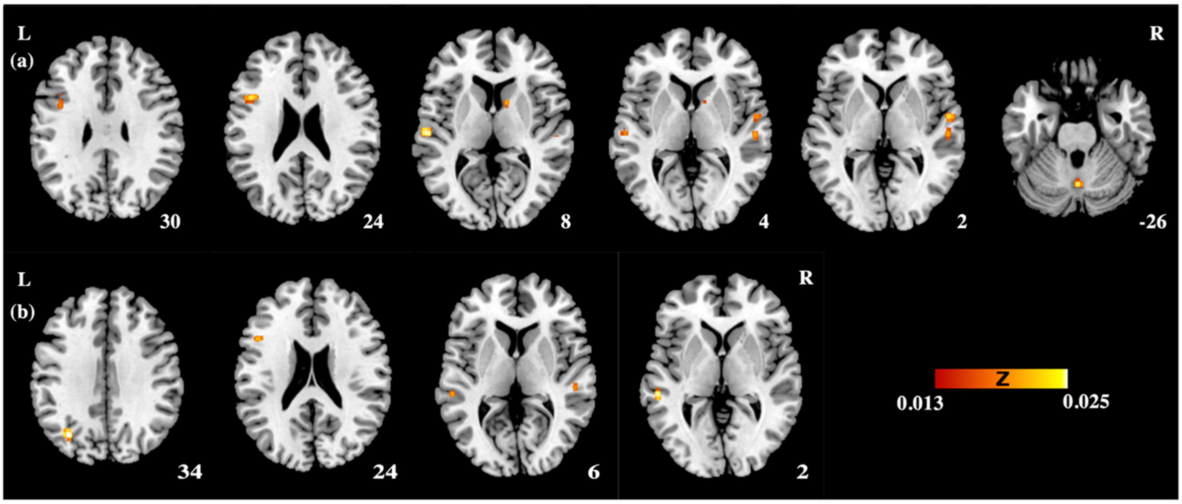

FIGURE 1 | The activation likelihood estimation (ALE) maps showing significant activation likelihood across studies of **(A)** auditory processing of lexical tones ($P < 0.05$ false discovery rate (FDR) corrected); **(B)** auditory lexico-semantic processing in non-tonal languages ($P < 0.05$ FDR corrected). L, left hemisphere; R, right hemisphere.

fMRI, and four utilized positron emission tomography (PET) to acquire brain images. Among the set of 15 studies that involved subjects to engage in the access of lexical information through audition in non-tonal languages, all of them used auditory semantic/lexical decision task, 14 acquired the imaging data through fMRI and one used PET. **Tables 1** and **2** present the full details of the selected studies. Although the studies had different baseline conditions due to the tasks used and issues addressed, the ALE meta-analysis of these data should allow us to determine the neural mechanisms subserving auditory lexical tone processing in tonal languages and auditory lexical processing in non-tonal languages.

Activation Likelihood Estimation (ALE)

The GingerALE software package is available on BrainMap website[2]. ALE is a coordinate-based meta-analysis technique that assesses the convergence of activation foci from different neuroimaging studies, modeled as probability distributions of activation at given coordinates against null distributions of random spatial associations between studies (Turkeltaub et al., 2012; Laird et al., 2005; see Wager et al., 2007 for review). The method has been used widely in recent years as an effective meta-analysis tool for functional imaging data.

The reported tasks involved auditory lexical tone judgment (266 subjects, 160 activation foci) and auditory lexical semantic judgment (251 subjects, 259 activation foci), in which participants were instructed to make discrimination judgments to the presented lexical tones. The activation foci generated in the contrasts of lexical tone tasks relative to baseline tasks (i.e., passive listening, non-speech pitch discrimination) and the contrasts of auditory lexical decision relative to baseline tasks (i.e., non-word judgment, non-speech tone decision) were included in the analysis, all foci data was imported to a text file and entered into the ALE software.

ALE maps were computed for 17 auditory lexical tone studies and 15 auditory lexical decision studies respectively. Prior to the analysis, all coordinates were transformed into a single stereotactic space: all MNI coordinates were converted into Talairach space using the icbm2tal transform tool (Lancaster et al., 2007) implemented in GingerALE software package

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[2] www.brainmap.org/ale

TABLE 4 | ALE meta-analysis of auditory lexical decision in non-tonal languages*.

Anatomical region	BA	Coordinates			ALE ($\times 10^{-2}$)	Volume (mm^3)
		x	*y*	*z*		
L precuneus	19	−34	−68	34	2.52	496
L middle temporal gyrus	22	−56	−34	2	2.29	400
L inferior frontal gyrus	9	−46	16	22	1.99	168
R transverse temporal gyrus	41	52	−26	6	1.78	112

**BA, Brodmann area; L, left; R, right. Coordinates were based on Talairach space.*

(Eickhoff et al., 2009, 2011). ALE maps were generated by the ALE method (Turkeltaub et al., 2012), using a full-width half-maximum (FWHM) of 10 mm. Each reported coordinate was treated as the center for the 3D Gaussian probability distribution. Statistical significance was determined by a permutation test of randomly distributed foci. The test was corrected for multiple comparisons using the false discovery rate (FDR) with a threshold at $P < 0.05$ corrected, with 100 mm^3 minimum volume size.

RESULTS

Table 3 and **Figures 1A**, **2A** illustrate the results of our ALE meta-analysis of the selected literature on auditory lexical tone processing. Eight clusters of activation likelihood are identified. First, the left inferior frontal cortex with two submaxima located in left inferior frontal gyrus (BA44; $x = -44$, $y = 12$, $z = 24$; BA9; $x = -40$, $y = 4$, $z = 30$), and the right medial frontal gyrus (BA8; $x = 2$, $y = 18$, $z = 44$) play central roles in auditory lexical tone processing. The left prefrontal cortex shows the highest convergence, with cluster sizes of 760 and 552 mm^3 respectively. Second, several other brain regions are consistently involved in mediating lexical tone perception, including the left posterior transverse temporal gyrus (BA42; $x = -58$, $y = -18$, $z = 8$), right superior temporal gyrus (BA 22; $x = 58$, $y = -6$, $z = 2$). Third, although their activation clusters are around 300 mm^3 or below, the right anterior cerebellum ($x = 2$, $y = -64$, $z = -26$), right transverse temporal gyrus (BA41; $x = 56$, $y = -22$, $z = 4$), and right caudate ($x = 12$, $y = 6$, $z = 8$) may also be implicated in lexical tone processing.

Table 4 and **Figures 1B**, **2B** illustrate the results of our ALE meta-analysis of the selected literature on auditory lexical processing in non-tonal languages. Four clusters of activation likelihood are identified. The left precuneus (BA19; $x = -34$, $y = -68$, $z = 34$) shows the largest convergence with a cluster size of 496 mm^3, followed by the left middle temporal gyrus (BA22; $x = -56$, $y = -34$, $z = 2$; cluster size: 400 mm^3). The left inferior frontal gyrus (BA9; $x = -46$, $y = 16$, $z = 22$) and right superior temporal gyrus (BA41; $x = 52$, $y = -26$, $z = 6$) have relative small cluster sizes below 200 mm^3, but these brain regions are activated when subjects were accessing lexical semantics through an auditory paradigm in non-tonal languages.

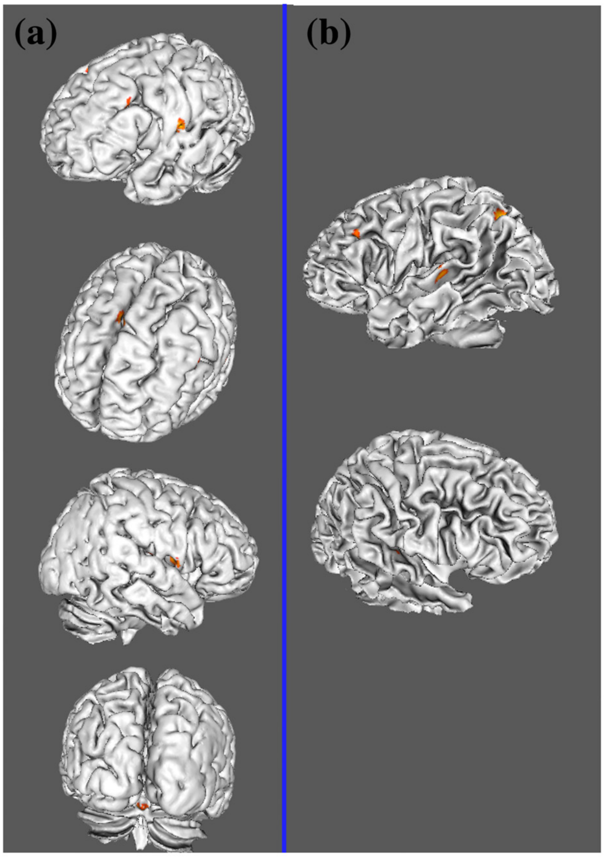

FIGURE 2 | Lateral view of two meta-analyses. **(A)** ALE results of auditory lexical tone processing in tonal languages; **(B)** ALE results of auditory lexico-semantic processing in non-tonal languages.

Contrast Analysis

Next, we performed a contrast analysis to investigate neural correlates, which were more specific to lexical tone processing in tonal languages relative to the processing of lexical semantics through audition in non-tonal languages. Yet, no significant differences were found. The opposite contrast (i.e., non-tonal relative to tonal) also showed no significant differences as well. The only significant result was obtained from the conjunction analysis. The cortical activation in the left inferior frontal gyrus (BA9; $x = -46$, $y = 14$, $z = 24$; cluster size = 16 mm^3) showed significant similarity between both datasets. According to the GingerALE software, at least 15 studies in each dataset are required in order to have enough statistical power (Cortese et al., 2012; Wagner et al., 2014). In this study, we only have marginally sufficient number of studies in each dataset (17 and 15 articles, respectively) and thus, the failure to reach significance

TABLE 5 | ALE contrast analysis of non-tonal vs. tonal auditory processing*.

Anatomical region	BA	Coordinates			ALE	Volume (mm^3)
		x	*y*	*z*		
At $p < 0.001$ uncorrected						
L middle temporal gyrus	19	−43	−73	26	3.24	952
	39	−40	−68	27	3.09	
At $p < 0.005$ uncorrected						
L middle temporal gyrus	19	−43	−73	26	3.04	2384
	39	−38	−71	28	2.88	
L angular gyrus	39	−48	−60	34	2.75	
	39	−52	−65	35	2.73	
L superior occipital gyrus	19	−40	−78	32	2.71	
L middle temporal gyrus	21	−54	−32	−8	3.04	1416
	21	−58	−35	3	2.99	
	21	−57	35	−3	2.95	
	21	−59	−40	0	2.88	
L superior temporal gyrus	38	−50	6	−10	3.16	208
	21	−51	4	−14	3.04	
L middle temporal gyrus	21	−52	2	−18	2.77	

**BA, Brodmann area; L, left. Coordinates were based on Talairach space.*

might be due to the lack of statistical power. Since both datasets contained only limited number of studies, we further analyzed the data in a more lenient approach, at $P < 0.001$ and $P < 0.005$ uncorrected threshold (see **Table 5**). No cluster survived in the tonal vs. non-tonal contrast at both thresholds, but we found activations in the opposite contrast (i.e., non-tonal vs. tonal). At uncorrected $P < 0.001$, one cluster of activation likelihood is identified at the left middle temporal gyrus (BA19; $x = -43$, $y = -73$, $z = 26$; BA39; $x = -40$, $y = -68$, $z = 27$) with a cluster size of 952 mm^3. When the threshold further dropped to uncorrected $P < 0.005$ for non-tonal vs. tonal contrast, three clusters of activation likelihood are identified with cluster sizes of 2384, 1416 and 208 mm^3, respectively. All clusters are located in the left temporo-parietal area, including left middle temporal gyrus (BA19; $x = -43$, $y = -73$, $z = 26$; BA39; $x = -38$, $y = -71$, $z = 28$; BA21; $x = -54$, $y = -32$, $z = -8$), left angular gyrus (BA39; $x = -48$, $y = -60$, $z = 34$), left superior occipital gyrus (BA19; $x = -40$, $y = -78$, $z = 32$) and the left superior temporal gyrus (BA38; $x = -50$, $y = 6$, $z = -10$).

DISCUSSION

There has been a growing literature in the auditory processing of lexical tones from a neurocognitive perspective, as seen in the number of publications devoted to this subject in the last decade (for a review, see Gandour, 2006). Given the importance of tones in the speech perception of languages such as Chinese and Thai, it is important for us to understand the neurocognitive mechanisms underlying lexical tone perception. However, there has been no consensus on the specific brain regions that support this perception or the overall lateralization pattern that subserves the process. In this study, we performed an ALE meta-analysis on the growing literature in the neuroimaging study of tone perception. In order to gain in-depth understanding on the neural systems specific to the processing of lexical tones, we performed a control ALE meta-analysis on a similar language condition in non-tonal languages for the sake of comparison. The results of the present ALE meta-analyses shed light on the neural basis of lexical tone processing in speech. Both analyses reveal that activation clusters are centered at the frontal and temporal regions, highlighting the importance of the inferior prefrontal and superior temporal regions for speech processing (Hickok and Poeppel, 2007).

The ALE results of auditory lexical tone processing showed that the largest cluster with the highest convergence was located in the left inferior prefrontal gyrus (i.e., BA44 and BA9). The left PFC has been consistently associated with the extraction of phonetic information, such as extraction of consonant structure (Zatorre et al., 1992, 1996; Binder et al., 1997; Burton, 2001). Apart from pitch processing, the inferior frontal gyrus has also been implicated in lexical-semantic processing (Petersen et al., 1988; Rumsey et al., 1997; Mummery et al., 1999; Tan et al., 2001; Chan et al., 2004). Thus, this region is also activated in our control meta-analysis on non-tonal languages. The major function of lexical tones in tonal languages such as Chinese and Thai serves to distinguish meanings, and therefore we assume that the prefrontal cortex is responsible for processing both the lexical pitch and lexical semantics of auditory linguistic stimuli.

The second largest activation cluster was located in the right medial frontal gyrus (BA8). Previous studies have revealed several functions of this brain region. The right medial frontal cortex is responsible for maintaining memory and attention, and is highly important for executive function tasks (Simons and Spiers, 2003; Baird et al., 2006; Euston et al., 2012). This region is also associated with pitch perception, such as tonality processing (Janata et al., 2002) and pitch identification among non-musicians (Schwenzer and Mathiak, 2011). Moreover, it is involved in the spectral processing of acoustic signals (Pedersen et al., 2000; Reiterer et al., 2005). Since we did not found any significant activation in the medial frontal gyrus in our control meta-analysis, we assume that this region plays a crucial role in processing lexical tone information.

The left posterior transverse temporal area (BA42) was the third largest activation cluster. Our analysis shows that the bilateral transverse temporal gyrus (BA41, 42) are consistently involved in lexical tone processing, although the level of convergence in the right transverse temporal gyrus was relatively lower than that in the left hemisphere. However, BA41 and 42 were not involved in the auditory lexical processing in non-tonal languages. Previous studies have indicated that the basic processing of simple acoustic stimuli, such as frequency-modulated tones and sound with discontinuous acoustic patterns, activate BA42 (Mirz et al., 1999; Binder et al., 2000). Thus, we hypothesize that the transverse temporal region is involved in the initial processing of auditory stimuli that may not be speech-related. The right superior temporal gyrus (STG; BA22) was also involved in mediating auditory processing of lexical tones according to our analysis. The right STG has been repeatedly shown to be critical to perceptual pitch processing, vocal pitch error detection and voice control in the literature (Robin et al., 1990; Johnsrude et al., 2000; Zatorre and Belin, 2001; Zatorre et al., 2002; Flagmeier et al., 2014), and in the case of Chinese tones, shows more sensitivity to acoustic than phonological variations (Zhang et al., 2010, 2011).

A final region showing significant activation in our analysis was the right caudate. The caudate is involved in various motor and non-motor processes (Seger and Cincotta, 2005; Grahn et al., 2008). Some suggested that the caudate might be the center of language control (Friederici, 2006), and involved in selection or inhibition of language (Robles et al., 2005; Wang et al., 2007). The lexical tone discrimination tasks mostly required subjects to distinguish lexical tone information only, while subjects actually processed both phonology and meaning of the presented syllable as a whole at the same time. Thus, the caudate seems to participate in suppressing further analysis on the vowel, consonant or the lexico-semantics but focus on the extraction of lexical tone information.

Since both meta-analyses examined the processing of lexical information through audition, some common cortical regions are activated, such as the left inferior frontal gyrus (BA9) and bilateral superior temporal regions. When looking at the results of two individual ALEs, relative to lexico-semantic processing of non-tonal languages, lexical tone processing in tonal languages appeared to recruit more right hemispheric regions such as the medial frontal gyrus, right transverse temporal gyrus, right caudate and right anterior cerebellum. However, our results in the contrast analysis could not support this argument. Moreover, it is apparent that most lesion evidence suggests left hemispheric dominance in lexical tone processing in tonal languages (Naeser and Chan, 1980; Gandour and Dardarananda, 1983; Hughes et al., 1983; Packard, 1986).

One major limitation of this study is the insufficient number of studies available for a powerful contrast analysis between datasets that we are interested in. Thus, we lack evidence to make a strong and convincing claim on the lexical tone specific neural network, if there is any. More sophisticated methods are required to investigate the specificity in lexical tone processing. Despite our failure to obtain significant results from the contrast analysis, we tried to visualize the trend of the potential differences between tonal and non-tonal auditory processing at less stringent statistical thresholds ($P < 0.001$, $P < 0.005$ uncorrected for multiple comparisons). When compared to tonal processing, the left temporo-parietal regions are more activated in non-tonal processing, mainly in the left middle temporal gyrus and the left angular gyrus (BA19 and 39). The left middle temporal gyrus and left angular gyrus have been implicated in lexical semantic processing, and according to the dual-stream model of speech processing (Hickok and Poeppel, 2000, 2004, 2007), these two regions are reliably activated across a range of semantic tasks (Démonet et al., 1992; Vigneau et al., 2006; Mashal et al., 2008; Binder et al., 2009; Seghier et al., 2010; Zhao et al., 2013). This finding showed that the temporo-parietal region is tended to be less involved in semantic processing in tonal languages. Future studies could focus on the investigation of semantic processing in lexical tones.

Apart from providing the overall picture across the 17 lexical tone studies, our lexical tone meta-analysis also offers insights into language processing across modalities. A recently published study on the neural basis of lexical tone reading has shown that lexical tone processing in reading Chinese characters involved a distributed network in both hemispheres including bilateral frontal regions, left inferior parietal lobule, left posterior middle/medial temporal gyrus, left inferior temporal region, bilateral visual systems and cerebellum (Kwok et al., 2015). In contrast to our analysis here that shows the crucial role of both the left and right STG, Kwok et al.'s (2015) lexical tone reading task involved no bilateral STG activation. Thus, our ALE results along with Kwok et al.'s (2015) data suggest that the bilateral STG are modality-specific regions for lexical tone perception in the spoken language only (see also Zhang et al., 2011).

In sum, our ALE results give a picture of the crucial brain regions processing non-tonal auditory lexicon and lexical tones respectively. Although we failed to uncover any lexical tone specific pattern at the moment, our findings provide valuable insights and directions to future investigations on tonal and non-tonal auditory processing, and more sophisticated methods are needed to explore this question in more depth.

AUTHOR CONTRIBUTIONS

VPYK, GD, PTF and L-HT designed and performed the research; VPYK, GD, KY, SM and L-HT analyzed the data; and VPYK, GD, PL and L-HT wrote the article.

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THE ROLE OF ANXIETY IN STUTTERING: EVIDENCE FROM FUNCTIONAL CONNECTIVITY

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Abstract—Persistent developmental stuttering is a neurologically based speech disorder associated with cognitive-linguistic, motor and emotional abnormalities. Previous studies investigating the relationship between anxiety and stuttering have yielded mixed results, but it has not yet been examined whether anxiety influences brain activity underlying stuttering. Here, using functional magnetic resonance imaging (fMRI), we investigated the functional connectivity associated with state anxiety in a syllable repetition task, and trait anxiety during rest in adults who stutter ($N = 19$) and fluent controls ($N = 19$). During the speech task, people who stutter (PWS) showed increased functional connectivity of the right amygdala with the prefrontal gyrus (the left ventromedial frontal gyrus and right middle frontal gyrus) and the left insula compared to controls. During rest, PWS showed stronger functional connectivity between the right hippocampus and the left orbital frontal gyrus, and between the left hippocampus and left motor areas than controls. Taken together, our results suggest aberrant bottom-up and/or top-down interactions for anxiety regulation, which might be responsible for the higher level of state anxiety during speech and for the anxiety-prone trait in PWS. To our knowledge, this is the first study to examine the neural underpinnings of anxiety in PWS, thus yielding new insight into the causes of stuttering which might aid strategies for the diagnosis and treatment of stuttering. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: persistent developmental stuttering, trait anxiety, state anxiety, functional connectivity.

INTRODUCTION

Persistent developmental stuttering is a speech disorder that is characterized by involuntary repetitions, prolongations, blocks or hesitations of speech sounds and affects approximately 5% preschool children and 1% adult population (Bloodstein and Ratner, 1995). Using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), previous neuroimaging studies have identified several cortical and subcortical brain regions with aberrant function in people who stutter (PWS) including the motor cortex (Fox et al., 1996; Braun et al., 1997; Brown et al., 2005; Watkins et al., 2008; Chang et al., 2009; Budde et al., 2014), anterior cingulate gyrus (De Nil et al., 2001; Neumann et al., 2003), auditory areas (Fox et al., 1996; Toyomura et al., 2011), inferior frontal gyrus (Kell et al., 2009; Lu et al., 2010a), frontal operculum/insula (Preibisch et al., 2003; Brown et al., 2005), basal ganglia (Wu et al., 1997; Giraud et al., 2008) and cerebellum (Fox et al., 2000; Brown et al., 2005). In addition, functional connectivity studies demonstrated how the irregular integration and interaction between speech-related regions contribute to stuttering. For example, task-induced functional connectivity studies have shown altered functional connectivity in the inferior frontal gyrus-motor circuit (Lu et al., 2009; Chang et al., 2011) and the basal ganglia-thalamocortical circuit (Lu et al., 2010b) during speech production in PWS relative to fluent controls. Other studies examining resting-state functional connectivity have shown atypical functional connectivity within the auditory-motor loop (Chang and Zhu, 2013), the inferior frontal gyrus-motor loop (Xuan et al., 2012) and the basal ganglia-thalamocortical loop (Chang and Zhu, 2013) in PWS compared to fluent controls. These findings have been interpreted as the neural basis for atypical speech planning and execution in PWS (Lu et al., 2009; Lu et al., 2010a). In summary, hyperactivation in the motor cortex (most frequently in the right hemisphere) (Fox et al., 1996; Braun et al., 1997; Watkins et al., 2008; Chang et al., 2009) and hypoactivation in the auditory cortex (Fox et al., 1996; Brown et al., 2005; Toyomura et al., 2011) are the most replicable findings of functional imaging studies of stuttering, which may underlie the motor control and auditory feedback abnormalities during speech in PWS. Additionally, functional disruption in the basal ganglia network in PWS, revealed by both functional activation and connectivity studies (Wu et al., 1997; Giraud et al., 2008; Lu et al., 2010b; Chang and Zhu, 2013), has been suggested to be associated with timing deficits (Alm, 2004a).

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Abbreviations: BOLD, blood oxygen level-dependent; FDR, false discovery rate; fMRI, functional magnetic resonance imaging; MNI, Montreal Neurological Institute; PWS, people who stutter; ROI, region of interest; STAI, Spielberger's State-Trait Anxiety Inventory.

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A symptom that stuttering occurs more frequently during speaking in public than during speaking when alone (Ingham, 1984; Bloodstein and Ratner, 1995) leads to a hypothesis that PWS are more likely to be nervous and anxious than others. Anxiety, one of the most widely studied emotional concomitants of stuttering, is conceptualized as an adaptive response for coping with threatening or challenging stimuli involving cognitive, behavioral and physiological components (Tovote et al., 2015). Over the past several decades, the investigation of the relationship between anxiety and stuttering has yielded mixed results. For example, previous questionnaire studies have demonstrated abnormally high levels of anxiety in children (Blood and Blood, 2007), adolescents (Blood et al., 2007; Mulcahy et al., 2008) and adults who stutter (Craig, 1990; Blumgart et al., 2010). In addition, psychophysiological measures showed that the mean heart rate was significantly lower in PWS than in controls during speech, and the group differences became more evident as task became more stressful (Caruso et al., 1994), implying the anticipatory anxiety for speech in PWS (Alm, 2004b). On the contrary, in another study Blood et al. (1994) examined anxiety levels during baseline, low-stress and high-stress situations in adults who stutter and fluent controls, but they did not find significant group differences in anxiety levels in either states. The inconsistency in results of the association between stuttering and anxiety is, at least partially, due to the methodological confounds (Menzies et al., 1999; Iverach et al., 2011). For example, some questionnaires used by previous studies may fail to provide a comprehensive measure of anxiety, resulting in a low sensitivity to tap the tangled relationship between anxiety and stuttering (Menzies et al., 1999). To clarify these inconsistent findings, a meta-analysis study was conducted to compare the distribution of anxiety levels between PWS and non-stuttering controls which showed a moderate to large differences in anxiety between the two groups (Craig and Tran, 2014). Given the evidence for the relationship between anxiety and stuttering, an important question is how anxiety affects the frequency of stuttering. A hypothesis is proposed that elevated anxiety may overload the capacity of speech motor system, which in turn disrupts speech processing (Attanasio, 2000). But, due to an effect of inadequate methods, the nature of influence of anxiety to stuttering is still elusive (Alm, 2014c).

Neuroimaging methods have been extensively applied to advance our understanding of anxiety. Functional neuroimaging studies have identified several brain regions that are associated with anxiety and anxiety disorders including the amygdala, insula, hippocampus and medial/lateral prefrontal gyrus (Bannerman et al., 2004; Bishop et al., 2004; Etkin and Wager, 2007; Stein et al., 2007; Alvarez et al., 2008; Fullana et al., 2015). In addition, resting-state functional connectivity studies showed that the amygdala-prefrontal connectivity (Kim et al., 2011a; Burghy et al., 2012) and the amygdala-insula connectivity (Baur et al., 2013) are significantly correlated with anxiety levels which are thought to support bottom-up and/or top-down modulation of anxiety. Another essential neural pathway for anxiety is the hippocampus-prefrontal connectivity that has been proposed to mediate the recall of fear extinction (Milad et al., 2007). Functional connectivity analysis has been proposed to be a better way to predict the outcomes of anxious response than the examination of activity of either region alone (Kim et al., 2011b). However, to date, no previous studies have examined whether anxiety influences the brain activity underlying stuttering, which is crucial for the stuttering treatment via targeting anxiety.

In the current study, we sought to investigate the role of alternations of anxiety-related functional connectivity in stuttering. According to the viewpoint of Spielberger et al. (1983), there are two types of anxiety: state anxiety and trait anxiety. State anxiety refers to an unpleasant emotional arousal in response to transient or acute threatening events, while trait anxiety refers to stable individual differences in the tendency in response to threatening events. Hypothetically, as a speech disorder, the contribution of state anxiety to stuttering could be better detected under speech conditions. Hence, we employed a syllable repetition task to examine the role of state anxiety-associated functional connectivity in stuttering. As trait anxiety represents a stable individual difference across various situations, functional connectivity associated with trait anxiety would be better untangled in a task-independent context. Resting-state functional connectivity measures the temporal coherence of the spontaneous blood oxygen level-dependent (BOLD) fluctuations under task-free conditions (Greicius et al., 2009) which has been evidenced as a reliable and stable neural index in prediction of individual differences in behavior (Fox and Raichle, 2007; Seeley et al., 2007). Consequently, resting-state functional connectivity has great potentials in tapping the group differences in trait anxiety-related functional connectivity between PWS and fluent speakers. We hypothesized that PWS would exhibit atypical functional connectivity within anxiety-related neural networks during both speech task and rest.

EXPERIMENTAL PROCEDURES

Participants

Thirty-eight participants were scanned using fMRI: 19 PWS (16 male and three female; mean age = 26 years, range from 21 to 35) and 19 fluent controls (16 male and three female; mean age = 24 years, range from 22 to 31). The inclusion criteria for PWS included a reported history of stuttering since childhood, absence of treatments during the year prior to this study and at least 3% of syllables stuttered in speech samples. The severity of stuttering ranged from very mild to severe in PWS as judged by the Stuttering Severity Instrument-3 (SSI-3) (Riley, 1994) in which frequency and duration of stuttering occuring in the speech sample, as well as any physical consomitants related to stuttering were evaluated by two independent judges. The Inter-rater reliability for stuttering severity measurements was relatively high (Cronbach's Alpha = 0.96, $P < 0.001$). Another clinical assessment performed on PWS was the Overall Assessment of the Speaker's Experience of Stuttering (OASES) which evaluates the experience of stuttering from the perspective of PWS in terms of affective, behavioral and cognitive reactions to stuttering, general perspectives about stuttering and the impact of stuttering to their life (Yaruss and Quesal, 2006). The exclusion criteria for all the participants included non-native Chinese speakers, history of neurological disease or psychiatric disorder, left-handed people as assessed by the handedness inventory (Snyder and Harris, 1993) and other contraindications to safe MRI scanning, including metal fragments or implants. The study was approved by the Institutional Review Board of Beijing MRI Center for Brain Research. The methods were carried out in accordance with the approved guidelines. Prior to the experiment, informed consent was obtained from each subject.

Self-report measure of anxiety

A self-report measure of anxiety was conducted in the two groups of participants using the Spielberger's State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983) which contains 20 items for state anxiety and 20 items for trait anxiety. The Chinese version of STAI scale has been found to be reliable and valid (Shek, 1988). Moreover, speaking rate during reading a paragraph in front of two examiners was calculated as an index of general speaking efficiency. The Inter-rater reliability between the two examiners was very high (Cronbach's Alpha = 0.99, $P < 0.001$). Finally, to examine the influence of anxiety to stuttering, we performed correlation analyses between anxiety and the severity of stuttering as well as speaking rate. Detailed clinical and demographic characteristics of participants are presented in Table 1.

fMRI design and materials

Speech task. An overt syllable repetition task was used as the speech task. Stimuli were Chinese monosyllables which were digitally recorded by a female native speaker, e.g. 'du', 'dan', 'da'. Participants were asked to overtly repeat three syllables immediately in reverse order to that in which they were presented. The requirement of reversal served to increase task difficulty for better tapping anxiety. In the baseline condition, participants were required to fixate at a cross in the center of the screen.

A block design was used in which three blocks of syllable repetition and three blocks of baseline were alternately appeared. Each syllable repetition block consisted a 2-s instruction and eight trials. In each trial, three syllables were successively exposed for 1.7 s followed by a 2.3-s response period. Each baseline block lasted 12 s.

Resting state. Following the speech task scan and a 10-min structural scan, resting-state scans were acquired during which participants were required to close their eyes and to relax without intentional thinking for 8 min.

Imaging acquisition

MRI data were collected on a Siemens 3T Siemens MRI scanner at the Beijing MRI Center for Brain Research of the Chinese Academy of Science. Functional images during the speech task and resting state were acquired using a BOLD-sensitive gradient echo-plane-image (EPI) sequence (TR = 2000 ms, TE = 30 ms, slices thickness = 4 mm, in-plane resolution = 3.4 mm × 3.4 mm and flip angle = 90°). Thirty-three axial slices were collected. High spatial resolution anatomical images were acquired using a T1-weighted, magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (TR = 2600 ms, TE = 3.02 ms, slice thickness = 1 mm, in-plane resolution = 1.0 mm × 1.0 mm and flip angle = 8°).

IMAGING DATA ANALYSIS

Preprocessing

Image preprocessing and statistical analyses were processed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/, Wellcome Department of Cognitive Neurology, University College London, London).

Speech task. For the speech task data, fMRI images were corrected for head motion, coregistered to the native T1, spatially normalized into the Montreal Neurological Institute (MNI) stereotactic space and then resampled into 2 × 2 × 2-mm cubic voxels. Finally, normalized images were smoothed with an isotropic Gaussian kernel of 6-mm full-width at half-maximum.

Table 1. Demographic characteristics of the two groups

	PWS (n = 19) Mean (SD)	Controls (n = 19) Mean (SD)	*t*-tests *p*-Value
Age (in years)	26 (3.43)	24 (2.79)	0.17
Handedness	All right-handed	All right-handed	
Education (years)	14.16(3.16)	15.26(2.42)	0.235
SSI-3	25.74(7.01)	n/a	
OASES	58.76(11.33)	n/a	
Anxiety			
State	45.84(9.7)	36.84(11.16)	0.012
Trait	51.53(8.22)	38.74(8.8)	< 0.001
Speaking rate (character/s)	3.64(0.88)	4.52(0.66)	0.001

Note: SD = standard deviation; s = second; n/a = not applicable

Resting state. For the resting state data, the first two images were discarded for magnetization equilibrium. The remaining images were corrected for slice timing and head motion, coregistered to the native T1, spatially normalized into the MNI stereotactic space and then resampled into $2 \times 2 \times 2$-mm cubic voxels. Finally, normalized images were smoothed with an isotropic Gaussian kernel of 6-mm full-width at half-maximum. Three stuttering participants who showed extreme head motion (exceed 3-mm translation, 4-degree rotation) were excluded for the following resting-state functional connectivity analysis.

Functional connectivity analysis

Seed-to-voxel functional connectivity analysis was performed using the CONN-fMRI toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012) for SPM8 (http://www.nitrc.org/projects/conn).

Region of interest (ROI) definition. To identify the specific ROIs for state and trait anxiety, we conducted a correlation analysis between self-report anxiety scores and brain activation during speech. At the first level, activation maps contrasting syllable repetition versus baseline were generated using the general linear model (GLM) in which time series were convolved with the canonical hemodynamic response function for each subject. The data were high-pass-filtered at 128 s. At the second level, we conducted a whole-brain-based correlation analysis with trait and state anxiety scores as regressors respectively for the entire group of 38 participants (voxel-wise $P < 0.05$, corrected for multiple comparisons via a false discovery rate (FDR)). Spherical ROIs with a radium of 6 mm were defined based on the peaks of state or trait anxiety-related activation (see Results). Meanwhile, all the ROIs were restricted within brain regions known to be associated with anxiety (Bishop et al., 2004; Etkin and Wager, 2007; Milad et al., 2007; Baur et al., 2013; Fullana et al., 2015). Accordingly, ROIs for state anxiety included the insula (left: $x = -44$, $y = 6$, $z = -6$; right: $x = 40$, $y = -8$, $z = -10$, in MNI coordinates), amygdala (left: $x = -22$, $y = -8$, $z = -14$; right: $x = 28$, $y = -8$, $z = -14$) and hippocampus (left: $x = -28$, $y = -28$, $z = -6$; right: $x = 32$, $y = -38$, $z = -4$). ROIs for trait anxiety included the insula (left: $x = -32$, $y = 16$, $z = 10$; right: $x = 40$, $y = 24$, $z = -6$) and the hippocampus (left: $x = -26$, $y = -40$, $z = -2$; right: $x = 20$, $y = -24$, $z = -10$).

State anxiety-related functional connectivity during the speech task. For the speech task data, the effect of nuisance covariates including fluctuations in BOLD signal from CSF and white matter and their derivatives, task effects and their first temporal derivatives, as well as the realignment parameter noises were reduced using the CompCor strategy implemented in the CONN-fMRI. Data were band-pass filtered (0.008 HZ $< f <$ infinite). At the first level, Pearson's correlation coefficients between the time course of state anxiety-related ROIs and all rest voxels were computed in each group separately, and for syllable repetition and baseline respectively, which then were transformed to Fisher's *Z*-scores. At the second level, group differences in functional connectivity during speech (syllable repetition minus baseline) were examined using independent two-sample *t* tests with an uncorrected voxel-wise threshold of $P < 0.001$ and a FDR-corrected cluster-size threshold of $P < 0.05$.

Trait anxiety-related functional connectivity during rest. For the resting-state data, the effect of nuisance covariates including fluctuations in BOLD signal from CSF and white matter and their derivatives and the realignment parameter noises were reduced. After temporal bandpass (0.008 HZ $<$ f $<$ 0.09 HZ), correlation coefficients between the time course of trait anxiety-related ROIs and all rest voxels in the brain were calculated and were then converted to Fisher's *Z*-scores. The individual resting-state functional connectivity maps were entered into a random effects model for between-group comparison using an uncorrected voxel-wise threshold of $P < 0.001$ and a FDR-corrected cluster-size threshold of $P < 0.05$.

Furthermore, to verify the reliability of between-group differences in the case of possible violations of the assumptions of the general linear model, we used nonparametric permutation tests with 1000 permutations to confirm the results of parametric tests using an uncorrected voxel-wise threshold $P < 0.001$ and a FDR-corrected cluster-mass threshold of $P < 0.05$ (Nichols and Holmes, 2002).

Post-hoc analysis of correlation coefficients. For both speech task and resting-state data, one-sample *t* tests were employed to examine the connectivity coefficients within groups in order to explore the nature of group differences. Finally, to confirm the relationship between functional connectivity and anxiety, we conducted correlation analyses between connectivity coefficients and self-report anxiety scores across the two groups of participants. Statistic thresholds were set at $P < 0.05$, uncorrected for multiple comparisons.

RESULTS

Self-report measure of anxiety

The results of STAI measure indicated that PWS exhibited higher levels of state anxiety [$t(36) = 2.65$, $P = 0.012$] and trait anxiety [$t(36) = 4.63$, $P < 0.001$] than fluent controls. Additionally, PWS showed slower speaking rate than controls [$t(36) = -3.47$, $P = 0.001$] that was negatively correlated with anxiety (state anxiety: $r = -0.38$, $P = 0.018$; trait anxiety: $r = -0.44$, $P = 0.006$) (Fig. 1).

Imaging results

Anxiety-associated activation during the speech task. The whole-brain correlation analysis demonstrated that both state and trait anxiety were correlated with the

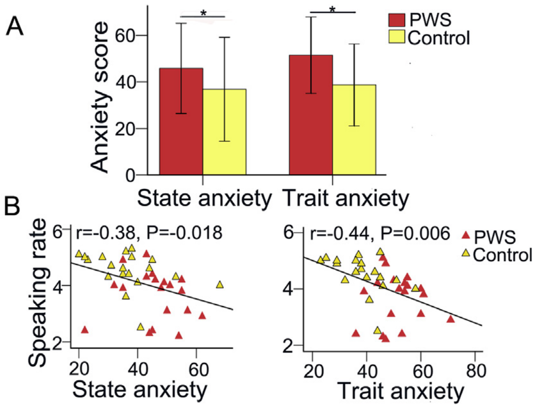

Fig. 1. Self-report anxiety levels and their relation to behavioral performance. (A) Group differences in state and trait anxiety between PWS and fluent controls. (B) Correlation between anxiety and speaking rate across the two groups of participants. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

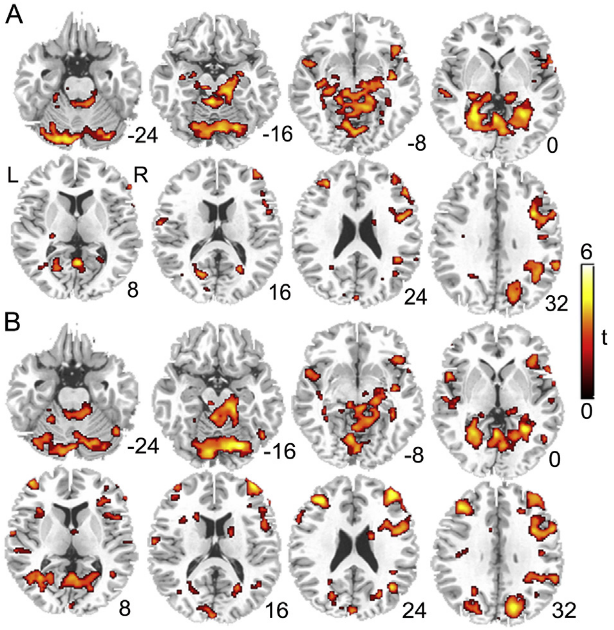

Fig. 2. Brain activation significantly correlated with anxiety during the speech task (voxel-wise $P < 0.05$, FDR corrected). (A) Brain activation associated with state anxiety. (B) Brain activation associated with trait anxiety.

activity in the bilateral superior, middle and inferior frontal gyrus, insula, amygdala, hippocampus (parahippocampus) and cerebellum during the syllable repetition task. The findings were in line with previous studies examining the anxiety-associated activation during speech (Lorberbaum et al., 2004), indicating anxiety-related brain activation in our speech task (Fig. 2).

State anxiety-related functional connectivity during the speech task

PWS showed altered functional connectivity within the amygdala-prefrontal network relative to fluent controls. Specifically, compared to controls, PWS showed greater functional connectivity of the right amygdala with the left medial frontal gyrus (PWS: $r = 0.19$, $P = 0.003$; controls: $r = -0.13$, $P = 0.017$; PWS *vs.*controls: $t(36) = 4.27$, $P < 0.001$), the right middle frontal gyrus (PWS: $r = 0.09$, $P = 0.043$; controls: $r = -0.21$, $P < 0.001$; PWS *vs.* controls: $t(36) = 5.61$, $P < 0.001$) and the left insula (PWS: $r = 0.16$, $P = 0.001$; controls: $r = -0.17$, $P = 0.004$; PWS *vs.* controls: $t(36) = 5.12$, $P < 0.001$). On the contrary, PWS showed decreased functional connectivity between the right insula and the left middle frontal gyrus relative to controls (PWS: $r = -0.19$, $P = 0.002$; controls: $r = 0.16$, $P = 0.005$; PWS *vs.* controls:$t(36) = -4.89$, $P < 0.001$) (Table 2, Fig. 3). Nonparametric tests confirmed the results of parametric tests (Fig. 4A).

However, we did not find significant correlations between the functional connectivity showing group differences and state anxiety scores across the two groups of participants.

Trait anxiety-related functional connectivity during rest

The group differences in functional connectivity associated with trait anxiety were identified during rest. The results showed that PWS showed stronger functional connectivity between the right hippocampus and the left orbital frontal gyrus (PWS: $r = 0.14$, $P < 0.001$; controls: $r = -0.05$, $P = 0.018$; PWS *vs.* controls: $t(33) = 5.76$, $P < 0.001$) as well as the left inferior frontal gyrus (PWS: $r = 0.16$, $P < 0.001$; controls: $r = -0.06$, $P = 0.032$; PWS *vs.* controls: $t(33) = 5.33$, $P < 0.001$). Likewise, PWS exhibited greater functional connectivity of the left hippocampus with the left precentral gyrus (PWS: $r = 0.16$, $P < 0.001$; controls: $r = -0.02$, $P = 0.388$; PWS *vs.* controls: $t(33) = 5.62$, $P < 0.001$), the left middle frontal gyrus (PWS: $r = 0.09$, $P < 0.001$; controls:$r = -0.08$, $P < 0.001$; PWS *vs.* controls: $t(33) = 6.85$, $P < 0.001$) and the left superior temporal gyrus (PWS: $r = 0.13$, $P < 0.001$; controls: $r = -0.04$, $P = 0.008$; PWS *vs.* controls: $t(33) = 8.88$, $P < 0.001$) than fluent controls. In contrast, PWS exhibited weaker functional connectivity between the left hippocampus and the right inferior parietal lobule than controls (PWS: $r = -0.18$, $P < 0.001$; controls: $r = -0.03$, $P = 0.133$; PWS *vs.* controls: $t(33) = -5.83$, $P < 0.001$) (Table 2, Fig. 5A). These results were confirmed by nonparametric tests (Fig. 4B).

Correlation analyses revealed that the score of trait anxiety was significantly correlated with functional connectivity between the right hippocampus and the left orbital frontal gyrus ($r = 0.45$, $P = 0.007$), between the left hippocampus and the left precentral gyrus ($r = 0.39$, $P = 0.021$) and between the left hippocampus and the left middle frontal gyrus ($r = 0.49$, $P = 0.003$) for the two groups of participants (Fig. 5B). To exclude the possibility that the relationship between self-report anxiety and functional connectivity was driven by the group differences in speech performance, we employed additional partial correlation analyses, partialing out the effect of speaking rate. It was found that trait anxiety was still significantly correlated with functional

Table 2. Group differences in functional connectivity associated state anxiety during speech and trait anxiety during rest (voxel-wise significance: $P < 0.001$, uncorrected; cluster significance: $P < 0.05$, FDR corrected). *Z*-score correspond to the actual maximum pixel value within the brain region from the SPM. Amy = amygdala, Ins = insula, MFG = middle frontal gyrus, IFG = inferior frontal gyrus, MedFG = medial frontal gyrus, PreG = precentral gyrus, OrbFG = orbital frontal gyrus, STG = superior temporal gyrus, MTG = middle temporal gyrus, IPL = inferior parietal lobule, MOG = middle occipital gyrus, Hip = hippocampus, parahip = parahippocampus, Cun = cuneus, Cereb = cerebellum, Thala = thalamus, Lin = lingual gyrus. L = left; R = right; BA = Brodmann's area

Seed regions	Target regions	BA	Talairach			Z score	Cluster size
			x	*y*	*z*		
State anxiety							
PWS > Controls							
R Amy	L MedFG	10	−8	53	3	3.96	129
	R MFG	9	24	40	16	5.03	158
	L Ins	13	−36	16	10	4.36	126
Controls > PWS							
R Ins	L MFG	9	−48	17	30	4.3	156
Trait anxiety							
PWS > controls							
L Hip	L PreG	4	−53	−12	25	4.87	360
	L MFG	6	−42	−1	52	5.09	358
	L PreG	4	−40	−13	49	4	
	L STG	38	−38	18	−21	4.87	703
	L IFG	46	−48	32	9	4.33	
		47	−46	24	−16	5.05	
	R MTG	21	63	3	−9	4.23	122
	L MOG	18	−30	−78	−6	4.25	4002
	L IOG	18	−32	−80	−3	4.84	
	L Lin	18	−26	−78	−8	4.7	
	L parahip	27	−14	−35	0	4.2	
	L Cun	17	−18	−89	6	5.09	
	L thala		−18	−29	−2	4.33	
	L cereb		−34	−63	−10	5.07	
R Hip	L orbFG	11	−6	42	−21	4.34	208
	L IFG	47	−16	27	−13	4.65	152
Controls > PWS							
L Hip	R IPL	40	46	−46	44	4.81	347

connectivity between the right hippocampus and the left orbital frontal gyrus ($r = 0.41$, $P = 0.015$) and between the left hippocampus and the left middle frontal gyrus ($r = 0.37$, $P = 0.032$), and marginally significantly correlated with the connectivity between the left hippocampus and the left precentral gyrus ($r = 0.29$, $P = 0.094$), confirming the contributions of altered functional connectivity to high trait anxiety in PWS.

DISCUSSION

To the best of our knowledge, this is the first study to examine the relationship between stuttering and anxiety using both questionnaire and neuroimaging methods. The questionnaire measure indicated that PWS exhibited higher levels of state and trait anxiety than fluent controls, consistent with previous studies using the same questionnaire (Craig, 1990; Craig and Tran, 2014). The significant negative correlation between the speaking rate and anxiety suggests an interferential impact of anxiety to speech. Critically, using task-based and resting state fMRI, we found hyper-functional connectivity between the limbic areas (amygdala, hippocampus) and the prefrontal gyrus in PWS, illuminating the neural mechanisms underlying the role of anxiety in stuttering. We discuss the results in detail in the following sections.

State anxiety-related functional connectivity during the speech task

First, the results indicated that PWS showed altered functional connectivity between the right amygdala and the bilateral prefrontal gyrus (the left ventromedial frontal gyrus and right middle frontal gyrus) in the speech task. Referring to the network perspective, the functional integration between the amygdala and the prefrontal gyrus was found to be essential for the top-down and bottom-up modulation for anxiety (Kim et al., 2011a). Specifically, the amygdala is known to be crucial for detection and production of fear response to threaten events in normal population (Etkin et al., 2004; Somerville et al., 2004) and individuals with anxiety disorders (Etkin and Wager, 2007; Tillfors et al., 2014). The bilateral prefrontal gyrus was thought to control and regulate amygdala output in response to anxiety (Bishop et al., 2004; Bishop, 2007). Thus, the greater functional connectivity between the amygdala and the prefrontal gyrus in PWS may reflect the deficit in consciously top-down regulation over the anxious response triggered by the speech task.

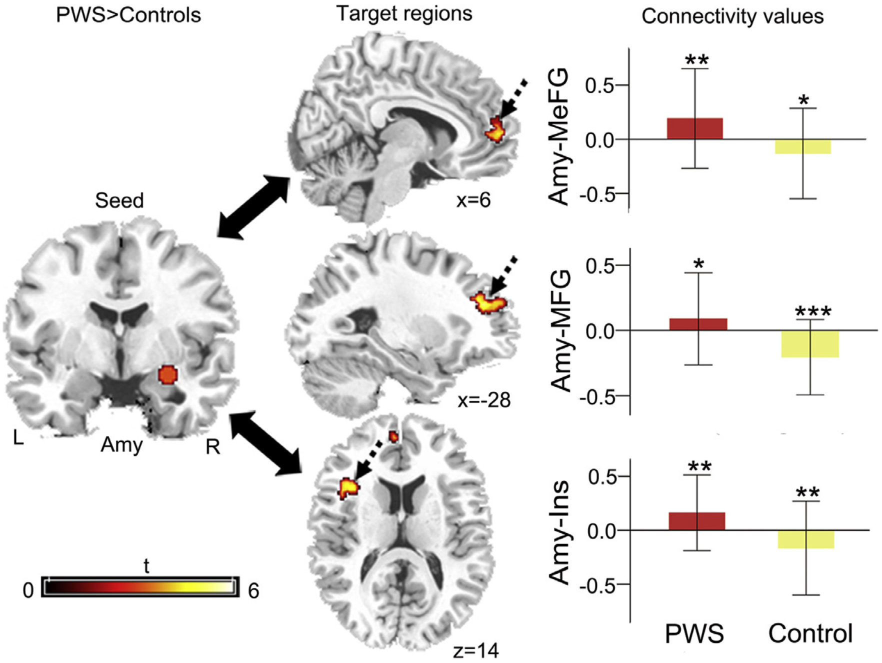

Fig. 3. Group differences in state anxiety-related functional connectivity during the speech task (voxel-wise significance: $P < 0.001$, uncorrected; cluster-size significance: $P < 0.05$, FDR corrected). Connectivity values correspond to standardized correlation coefficients. Amy = amygdala, MeFG = medial frontal gyrus, MFG = middle frontal gyrus, Ins = insula. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

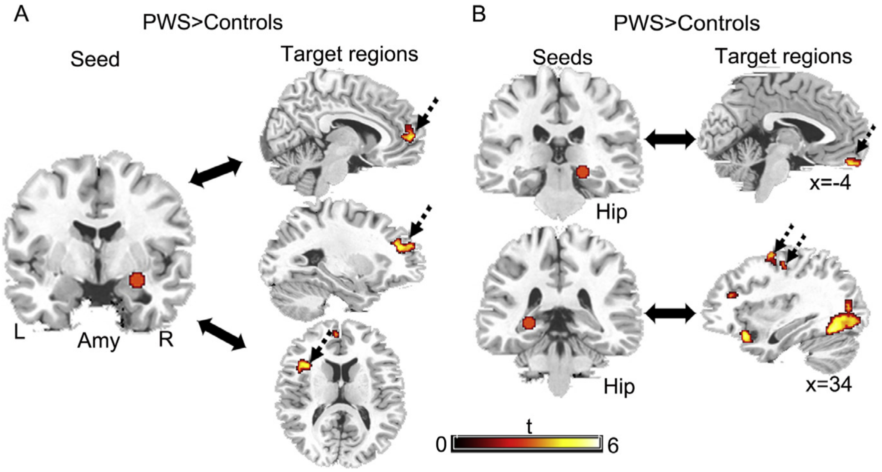

Fig. 4. The results of nonparametric permutation tests (voxel-wise significance: $P < 0.001$, uncorrected; cluster-mass significance: $P < 0.05$, FDR corrected). (A) Group differences in state anxiety-related functional connectivity during the speech task. (B) Group differences in trait anxiety-related functional connectivity during rest. MeFG = medial frontal gyrus, MFG = middle frontal gyrus, Ins = insula, OrbFG = orbital frontal gyrus, PreG = precentral gyrus, Hip = hippocampus, Amy = amygdala.

This assumption is coincident with the viewpoint that anxiety is linked to ineffective top-down inhibition (Kim and Whalen, 2009). Alternatively, such hyperconnectivity in PWS may result from an exaggerated initial bottom-up reaction to speech events which could not be controlled by normal top-down regulation.

In addition, we found increased functional connectivity between the right amygdala and the left anterior insula during speech in PWS relative to fluent controls. Our findings are consistent with a recent study showing that the functional connectivity between the anterior insula and amygdala was positively correlated with state anxiety, but not with the trait anxiety (Baur et al., 2013). Functionally, anterior insula was found to serve the detection of salient stimuli and the initialization of appropriate responses (Menon and Uddin, 2010), as well as the interoception of internal state (Paulus and Stein, 2006). The abnormally strong functional connectivity between the amygdala and insula in PWS suggests a high sensitivity or attentional bias for threatening stimuli, eventually leading to a high level of state anxiety.

To further confirm the contributions of altered functional connectivity to the high state anxiety in PWS, we conducted post hoc correlation analyses between state anxiety and functional connectivity showing group differences. Unexpectedly, we did not find significant correlation between the two variables. One possibility is that the off-line self-report measure probably be less sensitive to tap the group difference in state anxiety compared to the online measure of neural activity, as state anxiety is a transient and situational response. Another possibility is that owing to the small sample size, the correlation failed to reach statistical significance, despite we tried to increase the statistical power by combining the two groups of participants.

Finally, because previous studies (e.g. Kim et al., 2011a) and the present data demonstrated considerable overlap in brain regions associated with state and trait anxiety, we need to consider the contributions of trait anxiety to the group differences in functional connectivity during the speech task. Due to lack of measure of state anxiety during the speech task, we would be careful not to strongly claim that our results solely reflect state anxiety as opposed to trait anxiety, though the specific ROIs for state anxiety (i.e. amygdala) were adopted. The examination of distinct neural substrates for state and trait anxiety in further studies would be helpful for advancing our understanding on the unique role of state anxiety in stuttering.

Trait anxiety-related functional connectivity during rest

During rest, we found increased functional connectivity between the right hippocampus and the left orbital

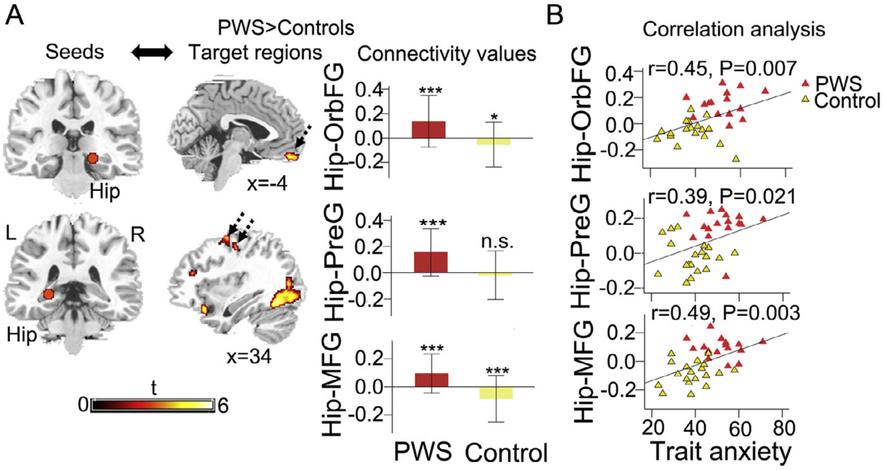

Fig. 5. Group differences in trait anxiety-related functional connectivity during rest (voxel-wise significance: $P < 0.001$, uncorrected; cluster-size significance: $P < 0.05$, FDR corrected). (A) PWS showed greater functional connectivity than fluent controls. (B) Correlation between connectivity coefficients and self-report score of trait anxiety. Hip = hippocampus, OrbFG = orbital frontal gyrus, PreG = precentral gyrus, MFG = middle frontal gyrus. n.s. = nonsignificant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

frontal gyrus as well as motor areas in PWS relative to controls. Apart from the memorial function, convergent evidence from animal and human studies suggested the involvement of hippocampus in anxiety (Milad et al., 2007; Alvarez et al., 2008; Satpute et al., 2012). Specifically, the anterior hippocampus was found to be associated with state anxiety, while the posterior hippocampus was associated with trait anxiety (Satpute et al., 2012). Consistently, we found that group differences in trait anxiety-related functional connectivity between PWS and controls was originated from the right posterior hippocampus. Previous studies reported that the orbital frontal gyrus is a key region for top-down regulation of peripheral physiological response for emotional experience (Bishop et al., 2004; Ohira et al., 2006; Kim et al., 2011a). With regard to the network perspective, the functional connectivity between the ventral medial prefrontal gyrus and the hippocampus has been suggested to be involved in the recall of fear extinction (Milad et al., 2007). As a result, we speculated that the increased functional connectivity between the right hippocampus and the left ventral medial frontal gyrus in PWS underlies the deficiency in mediation of fear extinction in daily speech which in turn shapes an anxiety-prone trait.

Moreover, PWS showed abnormally high functional connectivity between the left hippocampus and the left motor areas (BA4/6). A large body of evidence has demonstrated functional abnormalities in the motor areas in PWS, suggesting the vital role of motor dysfunction in stuttering (Fox et al., 1996; Brown et al., 2005; Watkins et al., 2008). In terms of the relation between anxiety and stuttering, there is a hypothesis proposing that the increased level of anxiety may worsen stuttering via overloading speech motor systems or disrupting maladaptive coping behavior (Attanasio, 2000). Consequently, our findings provide the neural evidence for the relation between anxiety and motor dysfunction in PWS, suggesting that the hippocampus-motor circuit is probably a unique neural signature of high trait anxiety in PWS.

As the resting-state session was conducted after the speech task session, the potential influence of cognitive processing to the resting-state functional connectivity should be taken into account. A prior study has addressed this question and has demonstrated that at the group level, resting-state functional connectivity maps are largely consistent between two resting-state sessions separated by a 5-min language task (Waites et al., 2005). In the present study, the resting-state scan was separated from the speech task scan by a 10-min structural scan, and as a result the impacts of speech processing to the resting-state results, if any, would be minimal.

Limitations

Firstly, the participants of the present study are adults who have been stuttering for a long time since onset in early childhood, and thus it is not clear whether the differences in anxiety-related functional connectivity between PWS and controls are the causes or the consequences of stuttering. At the genetic level, a prior study has identified stuttering-associated mutations in a gene that expresses in emotion-related brain circuit (Kang et al., 2010), suggesting an innate role of the high level of anxiety in stuttering. But, Alm et al. (2014) found that children who stutter did not show higher levels of anxiety-related traits than age-matched peers, suggesting that the high anxiety is more likely to be a consequence of stuttering. Further neuroimaging studies recruiting young children near stuttering onset are needed to address this question.

Secondly, due to the limited sample size of female participants, we could not examine sex difference in functional connectivity associated with anxiety in PWS that has been suggested by recent neuroimaging studies (Chang and Zhu, 2013; Chang et al., 2015).

Thirdly, we used a more liberal threshold for the post hoc analysis of connectivity coefficients within groups. The limited statistic power is a caveat of such analysis that may lead to overrepresentation of connectivity strength for each group. Further studies with large samples are needed to confirm the alternations of anxiety-related functional connectivity in PWS.

CONCLUSIONS

In this study, we found that PWS demonstrated increased functional connectivity between the limbic regions (amygdala, hippocampus) and the prefrontal gyrus during speech and rest relative to fluent controls. Our results for the first time reveal the neural signatures of high arouse of state anxiety during speech and anxiety-prone trait in PWS, thus having innovative implication for the causes, diagnosis and treatment strategies of stuttering.

AUTHOR CONTRIBUTIONS

Y.Y., F.J., W.T.S. and L.H.T. conceived and designed the experiment. Y.Y. and F.J. performed the experiment. Y.Y. performed the data analyses. Y.Y. and L.H.T. co-wrote the paper. Y.Y., F.J., W.T.S. and L.H.T. discussed the data and commented on the manuscript.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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A Genome-Wide Association Study Identifies Genetic Variants Associated with Mathematics Ability

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Mathematics ability is a complex cognitive trait with polygenic heritability. Genome-wide association study (GWAS) has been an effective approach to investigate genetic components underlying mathematic ability. Although previous studies reported several candidate genetic variants, none of them exceeded genome-wide significant threshold in general populations. Herein, we performed GWAS in Chinese elementary school students to identify potential genetic variants associated with mathematics ability. The discovery stage included 494 and 504 individuals from two independent cohorts respectively. The replication stage included another cohort of 599 individuals. In total, 28 of 81 candidate SNPs that met validation criteria were further replicated. Combined meta-analysis of three cohorts identified four SNPs (rs1012694, rs11743006, rs17778739 and rs17777541) of *SPOCK1* gene showing association with mathematics ability (minimum p value 5.67×10^{-10}, maximum β -2.43). The *SPOCK1* gene is located on chromosome 5q31.2 and encodes a highly conserved glycoprotein testican-1 which was associated with tumor progression and prognosis as well as neurogenesis. This is the first study to report genome-wide significant association of individual SNPs with mathematics ability in general populations. Our preliminary results further supported the role of *SPOCK1* during neurodevelopment. The genetic complexities underlying mathematics ability might contribute to explain the basis of human cognition and intelligence at genetic level.

Mathematics serves as a fundamental instrument in modern society as it plays an important role in many fields including science, engineering, and economics. It also used as a key index of human intelligence. Exceptional mathematics ability was frequently observed among genius from many domains. Meanwhile, dyscalculia, characterized by impaired number processing skills, is a specific developmental disorder of mathematics ability that affects approximately 3 to 6% of children[1]. Childhood mathematics ability was associated with adult socioeconomic status and quality of life[2]. Understanding mathematics ability is an essential step to improve children's numeracy skills and academic achievements and could also provide novel insights into human brain functions. Mathematics ability is a complex trait that involves neurological and cognitive development as well as postnatal education and training. In particular, it is estimated that considerable proportion of variation in mathematic ability could be explained by genetic factors.

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Variables	GWAS		Replication Cao (n = 599)
	Liangshan (n = 494)	Dongming (n = 504)	
Grade			
2	98 (19.84)	98 (19.44)	121 (20.20)
3	103 (20.85)	101 (20.04)	134 (22.37)
4	122 (24.70)	102 (20.24)	136 (22.70)
5	82 (16.60)	99 (19.64)	108 (18.03)
6	89 (18.02)	104 (20.63)	100 (16.69)
Sex			
Male	213 (43.12)	202 (40.08)	283 (47.25)
Female	281 (56.88)	302 (59.92)	316 (52.75)
Age, year	9.79 ± 1.39	9.51 ± 1.44	9.40 ± 1.45
Math score*	97 (93,99)	91 (83, 95)	95 (89,98)

Table 1. Basic characteristics of three populations. *Full score is 100. Data are expressed as number of participants (percentage), mean ± SD or median (Q1, Q3).

	Math score*			*P value*
	<80	80–90	≥90	
Grade				
2	12 (6.22)	35 (12.15)	275 (24.64)	<0.001
3	38 (19.69)	91 (31.60)	221 (19.80)	
4	44 (22.80)	47 (16.32)	261 (23.39)	
5	43 (22.28)	70 (24.31)	172 (15.41)	
6	56 (29.02)	45 (15.63)	187 (16.76)	
Sex				
Male	77 (39.90)	125 (43.40)	506 (45.34)	0.28
Female	116 (60.10)	163 (56.60)	610 (54.66)	
Region				
Liangshan	16 (8.29)	49 (17.01)	429 (38.44)	<0.001
Dongming	92 (47.67)	149 (51.74)	263 (23.57)	
Cao	85 (44.04)	90 (31.25)	424 (37.99)	
Raven score**	45.76 ± 5.63	45.13 ± 5.75	46.69 ± 5.67	<0.001

Table 2. Breakdown of math score in all participants. Data are expressed as number of participants (percentage) or mean ± SD. *Full math score is 100. **Full Raven score is 60.

Recent years, genome-wide association study (GWAS) has been widely applied to investigate genetic components underlying complex traits[3]. The first GWAS of mathematics ability was performed among children with high and low mathematics ability respectively and nominated top-performing SNPs for subsequent validation in a large sample of individuals spanning the entire distribution of mathematical ability[4]. The study did not observe any SNPs alone showing genome-wide significant association with mathematic ability but hypothesized that genetic contribution to mathematics ability might be explained by multiple quantitative trait locus (QTLs) of small effect[4]. Indeed, the top 10 candidate SNPs only accounted for 2.9% of phenotypic variance in mathematics ability[4]. The second GWAS of mathematics ability used children's verbal ability as control and then divided them into groups of high and low mathematic ability[5]. Candidate SNPs from the discovery stage were individually genotyped for validation but none of them exceeded threshold of genome-wide significance[5]. In the meantime, another GWAS in monozygotic and dizygotic twin pairs also observed a number of SNPs showing signals of associations with mathematic ability, but none of them achieved genome-wide significant level[6].

To date, none of studies has identified genome-wide significant association with mathematics ability in general populations due to small effects of common variants. However, research with specific populations might increase statistical power to detect significant association as it was reported that prevalence of mathematic disability was higher among children with neurodevelopmental disorders such as reading disability, attention-deficit/hyperactivity disorder (ADHD) and autism[7,8]. The GWAS performed in German dyslexic children identified rs133885 as a genome-wide significant SNP associated with mathematics ability[9]. This variant is a coding variant of *MYO18B* and is associated with intraparietal sulcus morphology[9]. However, a recent replication study of rs133885 failed to find its association with mathematics ability in either dyslexic or general populations[10].

Previous GWAS of mathematic ability is mainly performed in Western populations of which genetic backgrounds are substantially different to Chinese populations. In the present study, we performed GWAS of mathematic ability in the Han Chinese elementary school students through QTL-based approach. In total, we identified

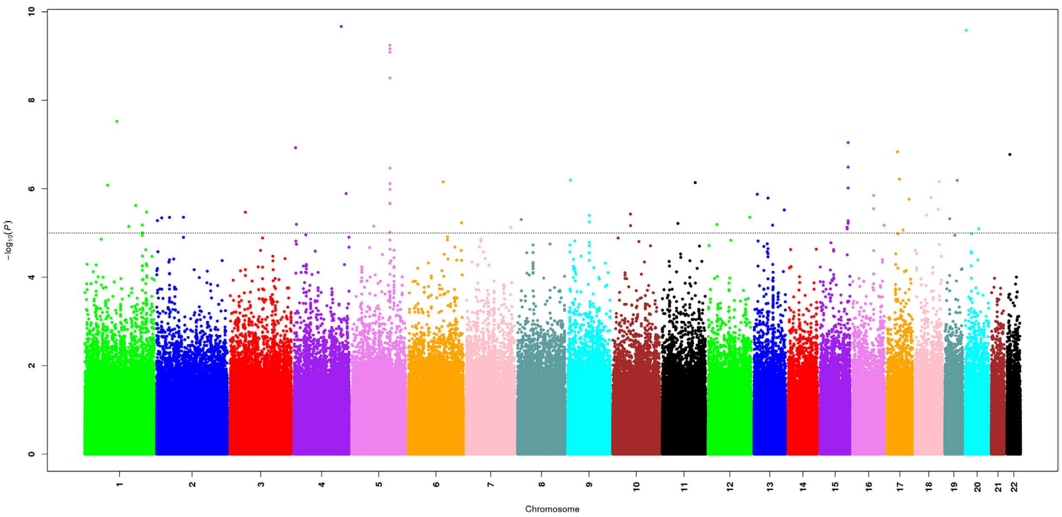

Figure 1. Manhattan plot of –log$_{10}$ (P values) of meta-analysis result from the additive model after adjustment for sex, age, school and nominal significant principal components in GWAS in Liangshan and Dongming population. The genome-wide threshold for significant ($P = 5 \times 10^{-8}$) and suggestive ($P = 1 \times 10^{-5}$) association are indicated by the horizontal blue and red lines, respectively. 81 SNPs in meta-analysis had P value $< 1 \times 10^{-5}$, of which 28 met the criteria for further replication. The symbol for the gene where the significant SNPs are in combined meta-analysis is shown in italics.

four SNPs exceeding genome-wide significant threshold which is the first time to report genome-wide significant association of individual SNPs with mathematics ability in general populations. Our results provide novel evidence to explain genetic complexities underlying mathematic ability and the basis of human intelligence at the genetic level.

Results

In the initial discovery phase, we performed a GWAS scan in two cohorts of Liangshan and Dongming (Supplementary Figure S1; Table 1). Breakdown of math scores according to grades, sex and regions of all participants were presented in Table 2. After quality control, about 1.1 million autosomal SNPs were analyzed (see Methods) in 998 samples (494 from the Liangshan cohort and 504 from the Dongming cohort). We performed linear regression in each cohort with adjustment for age, sex, school and top ten significant principal components of the corresponding cohort to test the additive effect of minor alleles of each SNP. In total, 13082 and 11170 SNPs with P-value less than 0.01 were identified in Liangshan and Dongming cohort respectively (Supplementary Figure S2). Results of the two discovery cohorts were combined by meta-analysis and 81 SNPs with P-value less than 1×10^{-5} were identified (Fig. 1). Finally, 28 SNPs met the criteria selection for subsequent replication stage (see Methods; Table 3).

The 28 SNPs met the replication criteria were evaluated in an independent cohort (Cao, Table 4). After meta-analysis of all data from discovery and replication stages, four SNPs (rs1012694, rs11743006, rs17778739 and rs17777541) mapping to *SPOCK1* gene exhibited association on genome-wide significant level for multiple testing ($P < 5 \times 10^{-8}$; Fig. 2; Table 4). However, rs17777541 did not show significant association with mathematics ability in the replication population. Results of these four significant SNPs were summarized in Table 5.

Discussion

Despite substantial heritability underlying mathematics ability, contribution of SNPs to this complex cognitive trait remained inconclusive. Previous GWAS of mathematics ability in general populations proposed candidate SNPs spanning chromosome 2, 3, 4, 5, 6, 7, 11, 12, 13, 20 and 21[4–6]. However, none of them showed consistent association during subsequent replication and therefore failed to exceed genome-wide significant level. Meanwhile, some studies reported sporadic association of copy number variations with mathematics ability but few of them been validated independently[11–13]. In the present study, we performed GWAS of mathematics ability in the Han Chinese general populations. Our genome-wide scan during discovery stage covered all previous nominated regions that might associate with mathematics ability. Although some SNPs located near previous reported region showed significant signal, only four SNPs (rs1012694, rs11743006, rs17778739 and rs17777541) were successfully replicated and achieved genome-wide significant level (minimum P value 5.67×10^{-10}). Each minor allele of these four SNPs was associated with decrease of math score ranges from 2.33 to 2.43 points approximately. To our knowledge, it is the first time to report association of a single SNP with mathematic ability at genome-wide significant level in general populations.

These SNPs are intron variants of *SPOCK1* and in highly linkage disequilibrium. The most significant SNP rs1012694 is located between exon 3 and 4 of *SPOCK1*. This gene is located on chromosome 5q31.2 and encodes sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1. Testican-1 is a highly conserved glycoprotein that involved in regulating proliferation, cell-cycle progression, apoptosis, adhesion, and cell-matrix

CHR	SNP	MAF	Position	Minor allele	Major allele	Liangshan			Dongming			Meta-analysis*			
						β	SE	P	β	SE	P	β	SE	P**	P_Q#
1	rs10779824	0.22	230231256	T	C	−1.95	0.53	2.35E-04	−1.91	0.76	1.28E-02	−1.94	0.43	7.79E-06	0.96
1	rs12733302	0.19	230259718	A	G	−2.12	0.55	1.49E-04	−2.19	0.78	5.38E-03	−2.14	0.45	2.18E-06	0.94
3	rs13096852	0.02	99909642	G	A	−5.50	1.58	5.42E-04	−5.52	1.85	2.98E-03	−5.51	1.20	4.50E-06	0.99
4	rs10007531	0.04	31909657	C	T	−4.11	1.07	1.31E-04	−4.67	1.60	3.64E-03	−4.28	0.89	1.37E-06	0.77
4	rs10014657	0.04	31909622	T	G	−4.12	1.07	1.27E-04	−4.96	1.65	2.83E-03	−4.36	0.89	1.09E-06	0.67
5	rs1012694	0.21	136535675	T	C	−2.90	0.56	3.13E-07	−2.36	0.81	3.82E-03	−2.73	0.46	3.17E-09	0.58
5	rs11743006	0.21	136550583	A	C	−2.90	0.56	3.13E-07	−2.27	0.80	4.99E-03	−2.69	0.46	4.33E-09	0.52
5	rs11746206	0.21	136483914	A	G	−2.14	0.56	1.58E-04	−2.47	0.80	2.29E-03	−2.25	0.46	1.08E-06	0.74
5	rs17720840	0.22	136513869	C	T	−2.09	0.57	2.44E-04	−2.27	0.81	5.13E-03	−2.15	0.46	3.46E-06	0.86
5	rs17777541	0.21	136522062	C	G	−2.89	0.56	3.89E-07	−2.28	0.80	4.89E-03	−2.69	0.46	5.23E-09	0.53
5	rs17778739	0.21	136551998	G	C	−2.90	0.56	3.13E-07	−2.28	0.80	4.89E-03	−2.70	0.46	4.22E-09	0.52
5	rs6890599	0.22	136458109	A	G	−1.88	0.55	7.10E-04	−2.29	0.80	4.34E-03	−2.01	0.45	9.31E-06	0.67
6	rs117368522	0.02	109576943	A	G	−6.91	1.80	1.40E-04	−9.86	2.70	2.86E-04	−7.82	1.50	1.77E-07	0.36
6	rs140472982	0.02	65729359	A	G	−5.34	1.63	1.16E-03	−9.76	2.57	1.67E-04	−6.61	1.38	1.63E-06	0.15
6	rs6927133	0.17	49543614	G	A	−1.65	0.59	5.37E-03	−3.56	0.85	3.10E-05	−2.27	0.48	2.61E-06	0.06
7	rs61109185	0.06	149025920	G	T	−4.00	1.05	1.59E-04	−4.37	1.35	1.30E-03	−4.14	0.83	6.04E-07	0.83
9	rs10746628	0.24	82952311	C	T	−1.49	0.53	5.01E-03	−3.26	0.75	1.81E-05	−2.07	0.43	1.60E-06	0.05
11	rs117620298	0.07	17960297	C	T	−3.10	0.90	6.55E-04	−4.17	1.28	1.17E-03	−3.46	0.74	2.77E-06	0.49
13	rs17076102	0.01	52492509	C	T	−6.60	1.81	2.90E-04	−7.27	3.02	1.66E-02	−6.77	1.55	1.26E-05	0.85
15	rs3751566	0.12	22940670	T	C	−2.55	0.68	1.85E-04	−2.52	1.09	2.14E-02	−2.54	0.58	9.94E-06	0.98
15	rs956120	0.13	22937838	G	A	−2.18	0.65	9.26E-04	−3.12	0.95	1.12E-03	−2.48	0.54	4.13E-06	0.42
16	rs1345872	0.18	73500800	G	A	−1.78	0.57	1.95E-03	−2.46	0.85	3.90E-03	−2.00	0.47	2.62E-05	0.51
17	rs11657625	0.16	21381802	C	T	−1.92	0.64	2.92E-03	−3.43	0.83	4.24E-05	−2.48	0.51	9.91E-07	0.15
18	rs12968137	0.08	33796927	T	C	−2.47	0.80	2.31E-03	−4.00	1.17	7.00E-04	−2.96	0.66	8.31E-06	0.28
19	rs74260502	0.04	45101336	T	C	−6.18	1.26	1.23E-06	−4.69	1.54	2.42E-03	−5.58	0.97	9.84E-09	0.45
20	rs11698429	0.30	13009926	T	C	−1.46	0.51	4.32E-03	−2.29	0.68	7.97E-04	−1.76	0.41	1.57E-05	0.32
20	rs3761896	0.32	12989901	C	T	−1.68	0.49	6.63E-04	−2.15	0.67	1.52E-03	−1.84	0.40	3.37E-06	0.57
20	rs3859619	0.32	12993893	C	A	−1.67	0.49	6.76E-04	−2.05	0.67	2.45E-03	−1.80	0.40	5.13E-06	0.65

Table 3. SNPs met the criteria in GWAS discovery phase for further replications. [*]Meta-analysis of discovery 1 and 2 association results. [**]P value from fixed model. [#]P_Q is the p value for Cochran's Q statistic.

interaction[14]. *SPOCK1* and its gene product testican-1 have been associated with tumor progression and prognosis of different cancer types. Expression of *SPOCK1* at mRNA and protein level was upregulated by the transcription factor CHD1L which could directly bound to promoter region of *SPOCK1*[15]. In hepatocellular carcinoma and gallbladder cancer, elevated expression of *SPOCK1* resulted in activation ofPI3K/AKT signaling which could block apoptosis and promote proliferation, invasiveness and metastasis of cancer cells[16,17]. In addition, increased expression of *SPOCK1* was implicated in epithelial-to-mesenchymal transition (EMT) which promoted migration and invasion in lung cancer and esophageal squamous cell carcinoma and conferred acquired drug resistance in gastric cancer[18–20]. Therefore, *SPOCK1* has been considered as a novel prognostic and therapeutic target for various cancer types.

Although the role of *SPOCK1* in cancers has been relatively well understood, its contribution to neurological and cognitive development remains elusive. Recently, novel de-novo *SPOCK1* mutation was reported in a female proband with developmental delay, microcephaly and agenesis of corpus callosum[21]. Her features were similar to previously reported microdeletions of 5q31 for intellectual disability[21]. As there were no mutations or variants of other genes identified in the proband showed potential relevance, *SPOCK1* located within 5q31 was suggested to be a candidate gene of observed developmental abnormalities. The identified de-novo mutation of *SPOCK1* might be protein-damaging which could potentially lead to developmental delay and microcephaly. Therefore, *SPOCK1* might play a critical role during neurogenesis. Indeed, testican-1, encoded by *SPOCK1*, was shown to inhibit attachment of Neuro-2a cells and their ability to form neurite extensions[22]. In addition, it also served as a strong competitive inhibitor of the lysosomal cysteine protease cathepsin L[23]. During early development of mice embryos, testican-1 was strongly expressed in developing brain and modulates neurogenesis and axonal growth[24]. At later developmental stage, testican-1 was particularly prevalent within developing synaptic fields[25]. Altered expression pattern of testican-1 mRNA was observed in reactive astrocytes after brain injury therefore suggested a role of testican-1 in regenerating axons[26].

Some potential limitations of the present study should be noted. The effect sizes might have been slightly overestimated due to lack of the adjustment for risk factors such as family socioeconomic status. Using nonverbal intelligence as an exclusion criteria might result in biased distribution of children's math scores as it was assumed that children with lower nonverbal intelligence seems more likely to have lower math scores as well. In addition,

CHR	SNP	Position	Minor allele	Major allele	Cao			Combined Meta*			
					β	SE	*P*	β	SE	*P***	P_Q#
1	rs10779824	230231256	T	C	0.64	0.79	4.16E-01	−1.34	0.38	4.26E-04	0.02
1	rs12733302	230259718	A	G	0.21	0.85	8.02E-01	−1.62	0.40	4.99E-05	0.05
3	rs13096852	99909642	G	A	0.73	1.80	6.87E-01	−3.59	1.00	3.27E-04	0.02
4	rs10007531	31909657	C	T	0.31	1.48	8.33E-01	−3.06	0.76	5.59E-05	0.03
4	rs10014657	31909622	T	G	−0.55	1.55	7.22E-01	−3.41	0.78	1.10E-05	0.09
5	**rs1012694**	**136535675**	**T**	**C**	**−1.61**	**0.75**	**3.35E-02**	**−2.42**	**0.39**	**6.84E-10**	**0.39**
5	**rs11743006**	**136550583**	**A**	**C**	**−1.71**	**0.75**	**2.33E-02**	**−2.43**	**0.39**	**5.67E-10**	**0.44**
5	rs11746206	136483914	A	G	−1.14	0.77	1.40E-01	−1.95	0.39	7.64E-07	0.44
5	rs17720840	136513869	C	T	−1.13	0.76	1.35E-01	−1.87	0.39	2.12E-06	0.51
5	**rs17777541**	**136522062**	**C**	**G**	**−1.35**	**0.76**	**7.39E-02**	**−2.33**	**0.39**	**3.13E-09**	**0.27**
5	**rs17778739**	**136551998**	**G**	**C**	**−1.63**	**0.76**	**3.24E-02**	**−2.41**	**0.39**	**8.18E-10**	**0.40**
5	rs6890599	136458109	A	G	−0.92	0.75	2.21E-01	−1.72	0.39	9.60E-06	0.42
6	rs117368522	109576943	A	G	−2.58	2.05	2.09E-01	−6.00	1.21	6.97E-07	0.08
6	rs140472982	65729359	A	G	4.12	2.58	1.10E-01	−4.23	1.21	5.07E-04	0.00
6	rs6927133	49543614	G	A	0.93	0.89	2.96E-01	−1.54	0.42	2.85E-04	0.00
7	rs61109185	149025920	G	T	−0.59	1.35	6.63E-01	−3.17	0.71	7.51E-06	0.08
9	rs10746628	82952311	C	T	−0.23	0.75	7.61E-01	−1.61	0.37	1.63E-05	0.02
11	rs117620298	17960297	C	T	0.48	1.53	7.53E-01	−2.72	0.66	4.39E-05	0.05
13	rs17076102	52492509	C	T	2.32	2.09	2.69E-01	−3.57	1.25	4.17E-03	0.00
15	rs3751566	22940670	T	C	−0.22	0.89	8.05E-01	−1.85	0.48	1.26E-04	0.09
15	rs956120	22937838	G	A	−0.17	0.88	8.42E-01	−1.85	0.46	5.84E-05	0.06
16	rs1345872	73500800	G	A	−0.31	0.79	6.94E-01	−1.55	0.41	1.40E-04	0.15
17	rs11657625	21381802	C	T	1.05	0.91	2.53E-01	−1.65	0.44	2.01E-04	0.00
18	rs12968137	33796927	T	C	2.85	1.09	9.14E-03	−1.39	0.57	1.45E-02	0.00
19	rs74260502	45101336	T	C	0.93	2.03	6.49E-01	−4.37	0.88	6.46E-07	0.01
20	rs11698429	13009926	T	C	0.87	0.68	1.99E-01	−1.06	0.35	2.30E-03	0.00
20	rs3761896	12989901	C	T	0.75	0.67	2.60E-01	−1.17	0.34	6.12E-04	0.00
20	rs3859619	12993893	C	A	0.79	0.67	2.40E-01	−1.14	0.34	8.23E-04	0.00

Table 4. Results of association of 28 SNPs with mathematics ability in replication and combined meta-analysis. *Meta-analysis of all three populations. **P value from fixed model. #P_Q is the p value for Cochran's Q statistic.

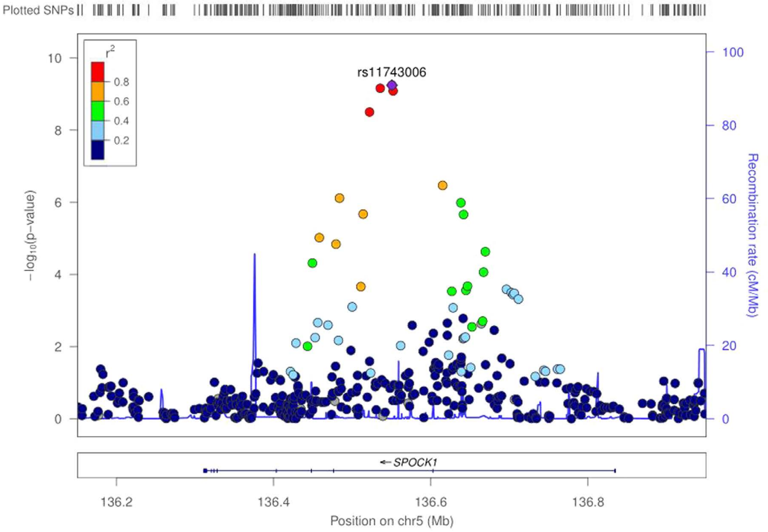

Figure 2. Signal plot of the discovery-stage GWAS meta-analysis for new math score associated locus. Signal plot of GWAS meta-analysis results and recombination rates in the GWAS discovery stage. The results ($-\log_{10}P$) are shown for SNPs around the region of *SPOCK1* on chromosome 5. The genes within the region of interest are annotated, and the direction of the transcripts is shown by arrows. The key SNP (rs11743006) are shown in purple and the linkage disequilibrium values (r^2) for the other SNPs are indicated by the heat scale.

SNP	Stage	β	SE	P	$P_Q^{\#}$
rs1012694 T/C* chr5:136535675**	Discovery 1	−2.90	0.56	3.13E-07	
	Discovery 2	−2.36	0.81	3.82E-03	
	Discovery combine	−2.66	0.49	8.41E-08	
	Discovery meta	−2.73	0.46	3.17E-09	0.58
	Replication	−1.61	0.75	3.35E-02	
	Combined meta	**−2.42**	**0.39**	**6.84E-10**	**0.39**
rs11743006 A/C* chr5:136550583**	Discovery 1	−2.90	0.56	3.13E-07	
	Discovery 2	−2.27	0.80	4.99E-03	
	Discovery combine	−2.61	0.49	1.28E-07	
	Discovery meta	−2.69	0.46	4.33E-09	0.52
	Replication	−1.71	0.75	2.33E-02	
	Combined meta	**−2.43**	**0.39**	**5.67E-10**	**0.44**
rs17778739 G/C* chr5:136551998**	Discovery 1	−2.90	0.56	3.13E-07	
	Discovery 2	−2.28	0.80	4.89E-03	
	Discovery combine	−2.62	0.49	1.18E-07	
	Discovery meta	−2.70	0.46	4.22E-09	0.52
	Replication	−1.63	0.76	3.24E-02	
	Combined meta	**−2.41**	**0.39**	**8.18E-10**	**0.40**
rs17777541 C/G* chr5:136522062**	Discovery 1	−2.89	0.56	3.89E-07	
	Discovery 2	−2.28	0.80	4.89E-03	
	Discovery combine	−2.61	0.49	1.25E-07	
	Discovery meta	−2.69	0.46	5.23E-09	0.53
	Replication	−1.35	0.76	7.39E-02	
	Combined meta	**−2.33**	**0.39**	**3.13E-09**	**0.27**

Table 5. Summary of GWA scan and replication studies for the significant SNPs. Discovery 1: Liangshan population. Discovery 2: Dongming population. Discovery combine: Liangshan and Dongming populations combined directly. Discovery meta: meta-analysis of discovery 1 and 2 association results. Replication: Cao population. Combined meta: meta-analysis of association results in three populations. *Minor allele/Major allele. **Chromosomalposition (Build 37). #P value for Cochran's Qstatistic.

sample size of our study were relatively small compared with genome-wide studies of chronic diseases such cancer or diabetes. However, the strengths of our study include its stringent quality control procedures and all participants genotyped by using the Affymetrix Axiom Genome-Wide CHB1 and CHB2 arrays which contain over one million SNPs specifically designed for Chinese population.

In conclusion, we reported four genetic variants of *SPOCK1* that showed genome-wide significant association with mathematic ability in Chinese children. Mathematic ability is a complex trait that involved polygenic and environmental factors. *SPOCK1* and its gene product tesican-1 showed potential functional relevance to neurodevelopment. Our study has identified a susceptibility gene, *SPOCK1,* which provides novel genetic insights into development of mathematics ability and the basis of human intelligence.

Methods and Materials

Participants. We recruited 2,425 grade two to grade six primary students aged 7 to 13 from three counties, Liangshan, Dongming and Cao, in Shandong Province in China. In the first step, Raven's Progressive Matrices test for nonverbal intelligence was administered to these eligible children individually, whose nonverbal intelligence scores lower than the 25th percentile were excluded from this study. In total, 1622 participants (Liangshan: 501, Dongming: 522, Cao: 599) were eligible for subsequent genotyping and association analysis. Mathematic ability was measured by children's academic performances of mathematics according to their mid-term and final exam of each semester. The examination papers were designed by education authorities of Shandong Province for each grade respectively according to the curriculum. Therefore, different tests will be applied to children in different grades but children in same grade will take exactly the same test. The tests aimed to evaluate children's academic performances of mathematics from three perspectives including "understanding numbers", "computing and knowledge" and "non-numerical processes". The teacher's rating process was double-blinded as student's answer sheet will be randomly and anonymously distributed to different teachers. Answers of all questions are clear and definite. There was no arbitrariness in scoring as teachers from different schools received unified training to ensure that their rating criteria for each student are standardized and objective. The mean score of mid-term and final exam within in same semester that were usually conducted within a 3-month interval was calculated for the analysis. This study was approved by the ethical committee of Tsinghua University School of Medicine. The methods were carried out in accordance with the relevant guidelines. Informed consent was obtained from all subjects.

Genotyping and quality control in the GWAS. DNA was extracted from blood samples and SNP genotyping was performed with the Affymetrix Axiom Genome-Wide CHB1 and CHB2 arrays (1,284,609 SNPs) by CapitalBio Technology (Beijing, China). Quality control was performed followed by standard quality control metrics[27]. Six samples in Dongming were excluded as they had sex discrepancies between the records and the genetically inferred data, three and four samples in Liangshan and Dongming respectively were excluded as they had overall successful genotyping call rates <95% orhad outlying autosomal heterozygosity rates (out of range of mean ± 3 SD). Next, we removed four and eight individuals in Liangshan and Dongming respectively who had unexpected duplicates or probable relatives (all PI_HAT > 0.20). Finally, we detected population outliers using a method based on the principal component analysis. Common autosomal SNPs in each cohort were employed to identify population outliers in the samples that had passed the quality control, with four original HapMap populations (CEU, CHB, JPT and YRI). In the next step, we performed basic quality control on genotyping data. In total, 40428 and 117542 SNPs in Liangshan and Doming respectively were excluded with call rate of <95%, 57985 and 56307 SNPs in Liangshan and Doming respectively were excluded with minor allele frequency (MAF) of <0.01, 3679 and 2323 SNPs in Liangshan and Doming respectively were excluded with genotype distribution that deviated from the Hardy–Weinberg equilibrium ($P < 1.0 \times 10^{-5}$). After quality control procedures had been performed, 494 children with 1182517 SNPs from Liangshan and 504 children with 1108437 SNPSs from Dongming were included in the final analysis.

Cao sample genotyping. Replication samples were typed at CapitalBio Technology (Beijing, China) with Sequenom MassARRAY platform (San Diego, U.S) according to the manufacturer's protocol. Briefly, genomic DNA was extracted from saliva of each individual through Oragene^{TM} DNA self-collection kit according to the manufacturer's instructions (Ottawa, Canada). DNA concentration was determined by Nano Drop 1000 (Waltham, U.S). Specific assays were designed using the MassARRAY Assay Design software package (v3.1). Mass determination was carried out with the MALDI-TOF mass spectrometer and Mass ARRAY Type 4.0 software was used for data acquisition.

Genome-wide association analysis. After quality control, association analyses and meta analyses were performed using PLINK1.9[28], fitting an additive model to the data by linear regression model with adjustment for sex, age and principle components in GWAS Liangshan and Dongming samples respectively. SNPs with a P-value < 0.01 were further analyzed by the meta-analysis based method to combine the results from Liangshan and Dongming samples as the discovery phase. SNPs with a P-value $< 1.0 \times 10^{-5}$ were selected for replication in Cao population. Finally, a meta-analysis was conducted to combine results from the three populations. A fixed-effect model with inverse variance weighting was used when there was no indication of heterogeneity (P for Cochran's Q statistic >0.05); otherwise, a random-effect model for the corresponding SNPs was adopted. A Manhattan plot of $-\log_{10}P$ was generated using the ggplot2 package[29] in R 2.15.1.

Power. Power calculations were performed using Quanto version 1.2.4 (http://biostats.usc.edu/Quanto.html). Under the additive model, it had 80% power at the $p < 0.05$ level to detect association with an allelic variant of 20% frequency accounting for 1.58% and 1.55% of the variance in math score in Liangshan and Dongming populations respectively.

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Author Contributions

Y.S. and L.H.T. directed the study, obtained financial support and were responsible for the study design, interpretation of results and manuscript writing. H.C., X.G. and Y.Z. performed overall project management and drafted the initial manuscript. Z.G., B.W., W.S. and G.W. were responsible for genotyping experiments and statistical analyses. M.H and Y.J. were responsible for subject recruitment and sample preparation. All authors approved the final manuscript.

Additional Information

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OPEN

Erratum: A Genome-Wide Association Study Identifies Genetic Variants Associated with Mathematics Ability

Huan Chen, Xiao-hong Gu, Yuxi Zhou, Zeng Ge, Bin Wang, Wai Ting Siok, Guoqing Wang, Michael Huen, Yuyang Jiang, Li-Hai Tan & Yimin Sun

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This Article contains errors in Table 2. The correct Table 2 appears below:

	Math score*			
	<80	80–90	≥90	P value
Grade				
2	10 (5.41)	36 (12.54)	271 (24.09)	<0.001
3	37 (20)	89 (31.01)	212 (18.84)	
4	44 (23.78)	43 (14.98)	273 (24.27)	
5	42 (22.7)	73 (25.44)	174 (15.47)	
6	52 (28.11)	46 (16.03)	195 (17.33)	
Sex				
Male	72 (38.92)	121 (42.16)	505 (44.89)	0.27
Female	113 (61.08)	166 (57.84)	620 (55.11)	
Region				
Liangshan	18 (9.73)	49 (17.07)	427 (37.96)	<0.001
Dongming	92 (49.73)	149 (51.92)	263 (23.38)	
Cao	75 (40.54)	89 (31.01)	435 (38.67)	
Raven score**	45.76 ± 5.63	45.13 ± 5.75	46.69 ± 5.67	<0.001

Table 2.

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ORIGINAL ARTICLE

Genetic polymorphism of nonsyndromic cleft lip with or without cleft palate is associated with developmental dyslexia in Chinese school-aged populations

Bin Wang[1,2,8], Yuxi Zhou[1,2,8], Song Leng[3,8], Liyuan Zheng[1,2], Hong Lv[1,2], Fei Wang[1,2], Li-Hai Tan[4,5] and Yimin Sun[1,2,6,7]

Developmental dyslexia (DD) is a neurodevelopment disorder characterized by reading disabilities without apparent etiologies. Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a structural craniofacial malformation featured by isolated orofacial abnormalities. Despite substantial phenotypic differences, potential linkage between these two disorders has been suggested as prevalence of DD among NSCL/P patients was much higher than that in general populations. Previous neuroimaging studies observed impaired short-term memory in patients with DD and NSCL/P, respectively. Genetic factors have a fundamental role during neurodevelopment and craniofacial morphogenesis but there lacks of evidence to support the linkage between DD and NSCL/P at genetic level. A recent genome-wide association study in Chinese populations identified a number of genetic polymorphisms associated with NSCL/P. Herein, we selected three risk variants of NSCL/P namely rs8049367, rs4791774 and rs2235371, and performed association analysis with DD in a Chinese population consisting 631 elementary school-aged children with 288 dyslexic cases without NSCL/P and 343 healthy controls. After Bonferroni correction for multiple comparisons, the T allele of rs8049367 showed significant association with DD (OR = 1.41, *P* = 0.0085). It is an intergenic variant between *CREBBP* and *ADCY9* located at 16p13.3. The *CREBBP* gene was reported to have an essential role during memory formation, although *ADCY9* was involved in dental development. In future studies, understanding functional effects of rs8049367 on *CERBBP* and *ADCY9* might contribute to explain underlying etiologies shared by DD and NSCL/P.

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INTRODUCTION

Developmental dyslexia (DD), also known as reading disability, is characterized by unexpected difficulties in reading and spelling without apparent intellectual and neurological impairment.[1] The prevalence of reading disability among children and adolescents ranges from 5 to 17.5%.[2] Notably, it was more prevalent among children with nonsyndromic cleft lip with or without cleft palate (NSCL/P), a common birth defect characterized by isolated orofacial cleft with unknown but complex etiology.[3] The rate of moderate and severe degree of reading disability were reported to be 35 and 17%, respectively, in a group of NSCL/P children.[4]

Although DD and NSCL/P are apparently different, they might have potential common etiologic pathways. Compared with age-matched non cleft controls, children with NSCL/P scored significantly lower in a series of reading measurements.[5,6] It has been suggested that DD and NSCL/P shared similar neurological endophenotypes. Short-term verbal memory is the ability to keep verbal information in conscious awareness for brief periods of time.[7] Deficiency in short-term verbal memory was known to be an important endophenotype of DD.[7] Among children with NSCL/P, reading disability were also featured by short-term verbal memory defects which fitted into a classic model of DD.[8,9] Therefore, there might be some common etiologies underlying DD and NSCL/P. Neuroimaging studies indicated a neurological basis of DD could be explained by aberrant structure and function of reading and language networks throughout the left hemisphere.[10] Consistent with this view, the most severely affected brain region among NSCL/P patients was reported to be the left temporal lobe in which observed structural abnormalities were directly related to cognitive dysfunctions.[11]

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In addition to neurological findings, DD and NSCL/P might have similar etiologies at genetic level as well. Both DD and NSCL/P are complex developmental disorders with genetic and non-genetic components to their perspective etiologies. Current genetic understanding of DD is mainly based on linkage analysis and candidate gene association study. Linkage studies of DD identified nine DD susceptibility loci termed DYX1 to DYX9. Further refinement of these loci reported a number of candidate genes associated with DD. The well recognized candidate genes of DD includes DYX1C1 in DYX1, DCDC2 and KIAA0319 in DYX2 and ROBO1 in DYX5.[12–15] In recent years, some of these associated genes have been replicated in different populations through association studies.[16] Meanwhile, genetic research of NSCL/P has recently identified multiple genes associated with disease susceptibility through genome-wide association study (GWAS). Previous GWAS in western populations identified a number of representative single nucleotide polymorphisms (SNPs) strongly identified as NSCL/P susceptibility loci.[17–20] Given substantial differences in genetic backgrounds, their exact contribution to risk in Chinese populations remains unknown. Recently, the first GWAS of NSCL/P in Chinese reported several risk SNPs of NSCL/P.[21] In particular, rs8049367 located in 16p13.3 was found to be a risk variant exclusive to Chinese.[21] We also confirmed variants within previously reported susceptibility loci including rs4791774 located in 17p13.1 and rs2235371 located in 1q32.2.[21]

Given the substantial genetic components underlying DD and NSCL/P, we aimed to explore observed linkage between these two disorders from genetic perspective. In the present study, we selected NSCL/P susceptibility SNPs from our recent GWAS report and evaluated their contribution to DD in a Chinese case–control study. Our study provided additional evidences to justify underlying genetic etiologies shared by DD and NSCL/P.

MATERIALS AND METHODS

Subjects

The criteria for participants with DD and healthy individuals were described elsewhere previously.[22] Briefly, DD screening underwent the two-stage procedures. First, primary school students aged between 7 and 13 from Shandong province of China were subjected to a Chinese reading test consisting of character-, word- and sentence-level questions. The participants whose reading scores were above 87th percentile or below the 13th percentile among all students in the same grade were chosen for further evaluation. These participants were subjected to a character reading test composed of 300 Chinese characters individually for the assessment of reading ability. Then the Raven's standard test was performed to exclude individuals with intelligence deficiency. Finally, a total of 631 children, 288 dyslexic participants and 343 controls, were selected for subsequent analysis. This study was approved by the ethical committee of Tsinghua University School of Medicine. All participants were informed with written consent.

Candidate SNPs genotyping

Three SNPs reported to be strongly associated with NSCL/P, rs2235371, rs8049367 and rs4791774, were genotyped on Sequenom MassARRAY platform (Sequenom, San Diego, CA, USA) at CapitalBio Corporation (Beijing, China). Genomic DNA samples were extracted from saliva samples using Oragene DNA self-collection kit (DNA Genotek, Ottawa, Ontario, Canada) and DNA quantity was determined by Nanodrop spectrophotometry (Nanodrop 1000 Spectrophotometer, Thermo Scientific, Wilmington, DE, USA). A PCR reaction based on a locus-specific primer extension reaction was designed using the MassARRAY Assay Design software package (v3.1, Sequenom, San Diego, CA, USA). MALDI-TOF mass spectrometer (Sequenom) and MassARRAY Type 4.0 software (Sequenom) were used for mass determination and data acquisition.

Table 1 Basic characteristics of participants

	Case	*Control*
Characteristics	(n = *288)*	(n = *343)*
Age, year	10.005 ± 1.456	10.007 ± 1.453
Sex		
Male	234	142
Female	54	201
Grade		
2	44	68
3	48	70
4	56	85
5	69	57
6	71	63

Data are expressed as *n* or mean ± s.d.

Data analysis

Statistical analysis was undertaken using PLINK software version 1.9 (http://pngu.mgh.harvard.edu/~purcell/plink/), which is an open-source whole-genome association analysis toolset and is commonly used to perform a range of basic, large-scale analyses.[23] Hardy–Weinberg equilibrium tests were undertaken for each SNP, and association tests were performed using additive, dominant or recessive genetic models under a logistic regression model with covariates of age and sex. Bonferroni corrections for multiple testing were used to determine statistical significance.

RESULTS

Basic characteristics of research participants are presented in Table 1. There were no differences of age distribution between DD cases and controls. The proportion of male in DD case group was higher than control groups. The effects of gender on DD were indicated in Supplementary Table S1. Three SNPs were genotyped in current study. In our sample, the allele T of rs2235371 was less frequent in cases than that in controls. The allele T of rs8049367 and the allele G of rs4791774 were more frequent in cases than that in controls.

SNP rs2235371 was not significantly associated with DD under allelic ($P = 0.1905$, odd ratio (OR) = 0.8569), additive ($P = 0.1780$, OR = 0.8487) and recessive ($P = 0.5807$, OR = 1.1470) models, except dominant model ($P = 0.0293$, OR = 0.6998). However, with the adjustment of age and sex, the association under dominant model became nonsignificant ($P = 0.1659$). SNP rs8049367 was significantly associated with DD under additive ($P = 0.0085$, OR = 1.4100), dominant ($P = 0.0256$, OR = 1.4960) and recessive ($P = 0.0374$, OR = 1.7280) model, after adjustment for age and sex. SNP rs4791774 was not significantly associated with DD under all models (Table 2).

After the Bonferroni correction for multiple comparisons, only SNP rs8049367 (OR = 1.4100, 95% CI = 1.0920–1.8220) on chromosome 17 was significantly associated with DD under additive models, indicating rs8049367 is a potential SNP marker for DD.

DISCUSSION

In total, three susceptibility SNPs of NSCL/P were evaluated for their contribution to risk to DD. These SNPs were initially reported in a recent GWAS report as novel risk variants for NSCL/P specific to Chinese populations.[21] In the present study, the T allele of rs8049367 showed 41% increased risk of DD, therefore rs8049367 appears to be susceptibility SNP for DD as well. However, the observed association between rs8049367 and DD should be interpreted with caution as this

Table 2 Association analysis of NSCL/P SNPs with DD

Chromosome/SNP	*Model*	*Crude OR (95% CI)*	*Unadjusted* P*-value*	*Adjusted OR (95% CI)*	*Adjusted* P*-value*
1/rs2235371 C/T[a]	Additive	0.8487 (0.6685,1.0780)	0.1780	0.9086 (0.7002, 1.1790)	0.4710
	Dominant	0.6998 (0.5077, 0.9646)	0.0293	0.7792 (0.5475, 1.1090)	0.1659
	Recessive	1.1470 (0.7057, 1.8630)	0.5807	1.1820 (0.6942, 2.0110)	0.5386
16/rs8049367 C/T[a]	Additive	1.2360 (0.9824, 1.5540)	0.0706	1.4100 (1.0920, 1.8220)	**0.0085**
	Dominant	1.2470 (0.9072, 1.7140)	0.1738	1.4960 (1.0500, 2.1310)	**0.0256**
	Recessive	1.4860 (0.9350, 2.3630)	0.0938	1.7280 (1.0320, 2.8920)	**0.0374**
17/rs4791774 A/G[a]	Additive	1.0600 (0.7824, 1.4350)	0.7086	1.0740 (0.7702, 1.4970)	0.6747
	Dominant	1.0710 (0.7661, 1.4970)	0.6880	1.0950 (0.7569, 1.5830)	0.6310
	Recessive	1.0210 (0.3393, 3.0740)	0.9701	0.9733 (0.3001, 3.1560)	0.9640

Abbreviations: CI, confidence interval; DD, developmental dyslexia; NSCL/P, nonsyndromic cleft lip with or without cleft palate; OR, odds ratio; SNP, single nucleotide polymorphisms.
[a] Risk allele.
$P<0.05$ were indicated in bold.

SNP is an intergenic variant located 50 kb upstream of *CREBBP* and 32 kb downstream of *ADCY9* with unknown function. The *CREBBP* gene encodes a nuclear protein that binds to cAMP response element binding protein (CREB) and acts a transcriptional coactivator with histone acetyltransferase activity.[24,25] Mutations in CREBBP gene are known to cause Rubinstein–Taybi syndrome, which is a genetic disorder characterized by developmental delay and abnormalities as well as intellectual disabilities.[26] In addition, the *CREBBP* gene was implicated in memory consolidation.[27,28] Knock-out of *CREBBP* in mice resulted in permanent impairments on both long- and short-term memory.[29] Meanwhile, upregulation of *CREBBP*-mediated transcriptional activity improved both long- and short-term memory.[30] Short-term verbal memory has a critical role in phonological processing, comprehension, and other reading-related processes. Previous studies of DD frequently observed impaired short-term verbal memory among dyslexics. In a recent study, Chinese dyslexics showed reduced short-term verbal memory during recall of Chinese characters than age-matched normal readers.[31] Given reported implication of *CREBBP* in memory impairment as well as developmental and intellectual abnormalities, understanding functional effects of rs8049367 on CREBBP in future studies might explain its contribution to DD.

There were considerable amounts of NSCL/P patients showed reduced reading abilities.[4] Previous observed reading disabilities among NSCL/P were also featured by impaired short-term verbal memory which suggested underlying etiologies shared by DD and NSCL/P.[8] As rs8049367 has been associated with NSCL/P in the recent GWAS, our preliminary results indicated rs8049367 as a shared risk factor for both disorders. During palate development, expression of *CREBBP* was constant. The CREBBP-mediated transcription is likely regulated by changes in its phosphorylation rather than by changes in its expression.[32] Meanwhile, expression of *ADCY9* was upregulated in the dental pulp stem cultures of NSCL/P patients.[21] Therefore, rs8049367 might contribute to DD and NSCL/P through its pleiotropic effects on *CREBBP* and *ADCY9*, respectively. Indeed, rs8049367 was in strong LD with an exon variant of a long non-coding RNA (lncRNA), namely RP11–462G12.2, which was predicted to interact with both *CREBBP* and *ADCY9*.[21] Thus, future investigation on lncRNA RP11–462G12.2 might contribute to explain the assumed pleiotropic effects of rs8049367.

In conclusion, we performed association study of three NSCL/P susceptibility SNPs with DD in a Chinese population and observed significant association of rs8049367. This intergenic variant might have pleiotropic effects on its two nearby genes *CERBBP* and *ADCY9*. Further functional investigations are warranted to validate the assumed pleiotropic effects which might explain the shared etiology underlying DD and NSCL/P.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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On the Counterfactual Nature of Gambling Near-misses: An Experimental Study

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ABSTRACT

Research on gambling near-misses has shown that objectively equivalent outcomes can yield divergent emotional and motivational responses. The subjective processing of gambling outcomes is affected substantially by close but non-obtained outcomes (i.e. counterfactuals). In the current paper, we investigate how different types of near-misses influence self-perceived luck and subsequent betting behavior in a wheel-of-fortune task. We investigate the counterfactual mechanism of these effects by testing the relationship with a second task measuring regret/relief processing. Across two experiments (Experiment 1, $n=51$; Experiment 2, $n=104$), we demonstrate that near-wins (neutral outcomes that are close to a jackpot) decreased self-perceived luck, whereas near-losses (neutral outcomes that are close to a major penalty) increased luck ratings. The effects of near-misses varied by near-miss *position* (i.e. whether the spinner stopped just short of, or passed through, the counterfactual outcome), consistent with established distinctions between upward versus downward, and additive versus subtractive, counterfactual thinking. In Experiment 1, individuals who showed stronger counterfactual processing on the regret/relief task were more responsive to near-wins and near-losses on the wheel-of-fortune task. The effect of near-miss position was attenuated when the anticipatory phase (i.e. the spin and deceleration) was removed in Experiment 2. Further differences were observed within the objective gains and losses, between "clear" and "narrow" outcomes. Taken together, these results help substantiate the counterfactual mechanism of near-misses.

KEY WORDS near-misses; counterfactual thinking; luck; reflection and evaluation model; cognitive distortions

INTRODUCTION

The outcomes of decisions we make have a pronounced effect upon our emotional state: we feel happy after obtained successes, and sad or disappointed following losses and defeats. This focus on factual outcomes fits the assumption of traditional economic theory that we wish to maximize the outcomes we obtain (Kahneman, 2011). However, it is increasingly recognized that our feelings are also influenced by "counterfactual outcomes": outcomes we could have obtained if only reality had taken another turn. Research on counterfactual thinking shows that we are often strongly affected by what might have happened, or what nearly happened. An anecdote by Kahneman and Tversky (1982) described two travelers, one who missed his flight by 5 minutes, and the other who missed the same flight by 30 minutes. Objectively, these two outcomes are equivalent in that neither traveler caught the plane, but 96% of participants expected the first traveler to feel worse. Here, the closeness to the desired outcome creates an upward counterfactual ("He almost made the flight!"), such that a narrowly missed desirable outcome intensifies the emotional response (in this case, regret).

A more extreme example arises when people who perform objectively better in a contest can ultimately feel worse than those who perform less well, a phenomenon termed "satisfaction reversal" (Medvec & Savitsky, 1997). Olympic silver medalists describe less satisfaction at their achievements than bronze medalists (Medvec, Madey, & Gilovich, 1995), presumably due to the opposite influences of the counterfactual thoughts "I nearly won the gold" (silver) and "I nearly missed out on a medal" (bronze). Medvec and Savitsky (1997) developed these observations into a model of categorical cutoff points: values that impose qualitative boundaries on quantitative outcomes (which are frequently arbitrary, such as exam thresholds) can thereby induce counterfactual thoughts. As part of their model, they showed that the simple act of surpassing a grade cutoff elicits downward counterfactuals and increases positive affect, and conversely just missing a cutoff triggers upward counterfactuals and decreased satisfaction.

These effects of counterfactual outcomes are ubiquitous in gambling behavior, which itself offers a paradigm for studying decision-making more broadly (see Clark et al., 2013, for a review). The classic gambling "near-miss" refers to a non-win outcome that falls tantalizingly close to the jackpot (Clark, Lawrence, Astley-Jones, & Gray, 2009; Reid, 1986), such as a horse finishing in second place in a neck-to-neck finish. Previous research has shown that these events (henceforth labeled "near-wins" (NW) to avoid any semantic

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confusion between nearly winning and nearly losing) are experienced as aversive, but increased motivations to continue gambling (Clark, Crooks, Clarke, Aitken, & Dunn, 2012; Clark et al., 2009). Recent studies have extended the near-miss phenomenon into the loss domain, enabling a comparison between NW and near-losses (NL). Experiments measuring subjective responses to neutral outcomes that were close to jackpot (i.e. NW) as well as neutral outcomes that were close to a major penalty (i.e. NL) indicate that NL are also processed as a discrete class of event (Dillon & Tinsley, 2008; Wohl & Enzle, 2003; Wu, van Dijk, & Clark, 2015; Zhang & Covey, 2014).

Past work on these types of decision outcomes has tended to focus on anecdotal scenarios (Dillon & Tinsley, 2008) or single-shot decisions (Wohl & Enzle, 2003). In the present study, we developed a multi-shot task based on a wheel-of-fortune game to compare responses to these various outcome types within the same participant. We looked at how these outcomes influenced perceptions of luck, and betting decisions on the subsequent trial. Luck ratings capture the element of chance in decision outcomes and are known to be sensitive not only to the objective outcome valence, but also to close counterfactuals (Teigen, 1995). We also measured trial-by-trial bet amount change as a function of the preceding trial (see also Demaree, Burns, Dedonno, Agarwala, & Everhart, 2012).

Our first aim was to investigate how people respond to null outcomes that differed only in whether they were close to a win (i.e. NW) or close to a loss (i.e. NL) (see Figure 1). In a prior study using a wheel-of-fortune task where we highlighted each wheel segment successively, we found that null outcomes that were close to a significant penalty elicited downward counterfactuals and increased self-perceived luck, whereas null outcomes that were close to a jackpot elicited upward counterfactuals and decreased self-perceived luck (Wu, van Dijk, Clark, 2015; see also Wohl & Enzle, 2003). We sought to corroborate these effects of NW and NL using an improved version of the task with a spinner that allowed a continuously varying position, so that outcomes could fall close to the boundary to the next segment.

The second aim was to explore if these effects of near outcomes were further moderated by their position relative to the missed outcome; that is to say, when the spinner stopped just *before* the win/loss segment, compared to when the spinner stopped just *after* the win/loss segment. Using a slot machine task, we have previously described how NW that stop just short of the winning line primarily act to increase motivation to continue, whereas NW that pass through the winning line generate a more aversive effect (Clark, Liu, et al., 2013). These differential effects can be explained in terms of counterfactual thinking, drawing upon the distinction between additive and subtractive counterfactual thoughts. Additive counterfactuals refer to those that add hypothetical events to reality (e.g. “If only I had an umbrella, I would not have gotten wet”), whereas subtractive counterfactuals involve removing or “undoing” events from reality (i.e. “If only it hadn’t rained today, I would not have gotten wet”) (Roese & Olson, 1993). Additive and subtractive counterfactuals have differential effects upon mood and behavioral regulation (Roese, 1994). On a slot machine, NW where the reel stops before the payline position would likely generate a counterfactual thought that the reel’s trajectory might have continued to the jackpot position (an additive counterfactual), whereas for the NW after the payline, the gambler must mentally reverse the subsequent step, a subtractive counterfactual (Clark et al., 2013). This difference in the type of counterfactual thought may explain the contrasting emotional and motivational effects engendered by these two events (Clark et al., 2013; see also Markman & McMullen, 2003).

In the present study (Experiment 1 and 2), the spinner decelerated in a clockwise direction, and it could stop fractionally before entering a winning (or losing) segment, or just

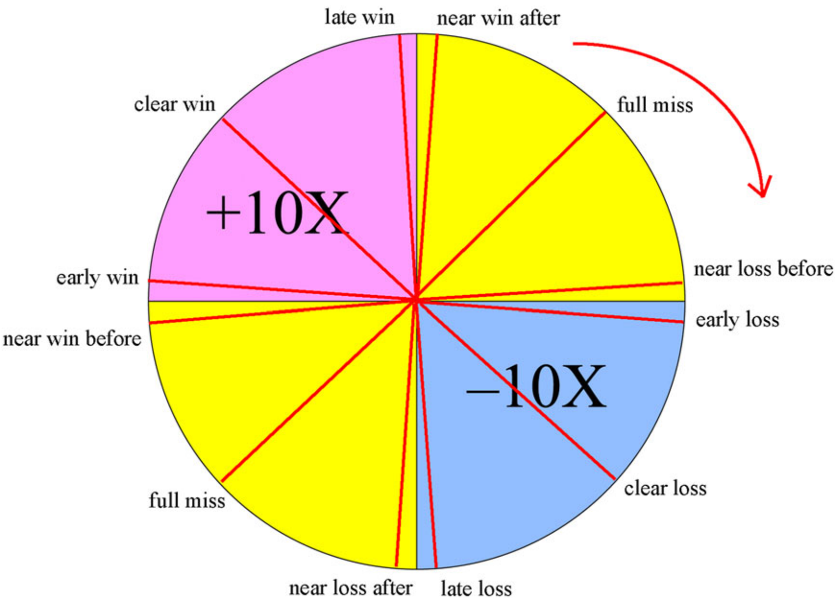

Figure 1. The wheel-of-fortune task. The arrow outside of the wheel indicates the movement direction of the spinner. [Colour figure can be viewed at wileyonlinelibrary.com]

after exiting a winning (or losing) segment. Based upon the slot machine data, we predicted that the spinner stopping just after a win location (NW after, henceforth a "NW-after") would be perceived as unluckier than when it stopped just before (henceforth a "NW-before"), and that a NW-before may increase the subsequent amount bet as a reflection of increased motivation.

The other major aim of this study was to examine the relationship between individual differences in reactions to near-miss events, and the behavior on a second task assessing regret and relief processing (Mellers, Schwartz, & Ritov, 1999). Prior work using anecdotes has asked participants to endorse counterfactual statements (Medvec et al., 1995; Medvec & Savitsky, 1997) or to reflect on "how things could be different" (Wohl & Enzle, 2003). These studies illustrate that narrowly missing more or less desirable outcomes elicited regret or relief (respectively), but these methods may be considered prone to demand characteristics. Other research on NW has primarily described these events as triggering frustrative non-reward (Wadhwa & Kim, 2015) or attributions of skill acquisition (Clark et al., 2013), mechanisms that need not inherently rely on counterfactual processing. In the present study, we sought to test the link between gambling near-misses and counterfactual thinking using a different approach, looking at individual differences in "counterfactual potency" on an independent task (Camille et al., 2004; Camille et al., 2010; Gillan et al., 2014; Wu & Clark, 2015). Previous research has characterized counterfactual potency as the multiplicative combination of "if likelihood" and "then likelihood", and showed this parameter correlated with intensity of emotional responses (Petrocelli, Percy, Sherman, & Tormala, 2011). In Experiment 1, we used a second decision-making task where participants choose between two gambles, and after viewing their obtained outcome, the non-obtained outcome from the unselected gamble was displayed. In this task, affect ratings taken after each trial are sensitive not only to size of the obtained outcome, but also to the non-obtained outcome. For example, negative affect is strongest when the obtained outcome is a loss *and* the non-obtained outcome is revealed to be a major win (Camille et al., 2004, 2010; Gillan et al., 2014; Mellers et al., 1999). We quantified counterfactual potency as the slope of function for affect ratings based upon the difference between obtained and non-obtained outcomes, such that steeper slopes indicate greater modulation by the difference between the outcomes. We analyzed the correlation between this index and the luck ratings following near events on the wheel-of-fortune task, predicting that participants with higher counterfactual potency would be more sensitive to near-misses.

Our task also enabled a more exploratory analysis decomposing the analogous subtypes of objective wins and losses. In the present study, the spinner could stop either centrally in the win (or loss) segment—a *clear* outcome—or near the boundary to the adjacent null segment, a *narrow* outcome. These events are commonplace within both gambling games (e.g. winning a horserace by a clear distance or a neck-to-neck finish) and other aspects of daily life (e.g. making it to the airport with 2 hours to spare, or 5 minutes). In a stock market simulation (Markman & Tetlock, 2000), participants gave more negative ratings when their chosen stock outperformed the un-chosen stock by a narrow margin (henceforth a "narrow win") compared to when the chosen stock substantially outperformed the other stock (henceforth a "clear win"). These effects were mirrored for losses, and outcome closeness further impacted subsequent willingness to invest. In an analysis of NBA basketball games, teams that were losing by narrow margin at half-time increased their effort and were ultimately more likely to win the match, compared to teams that were winning by narrow margin at the interval (Berger & Pope, 2011). Similar to the processing of NW and NL, these responses to clear versus narrow wins/losses likely also involve counterfactual thinking (Markman & Tetlock, 2000). We hypothesized that narrow wins compared to clear wins would prompt downward counterfactuals and make our participants feel luckier, and conversely narrow losses compared to clear losses would elicit upward counterfactuals and make people feel unluckier. These analyses further considered the narrow event position, distinguishing early events that have just entered the win/loss segment (henceforth a "early win" or "early loss") against late events that have almost left the win/loss segment (henceforth a "late win" or "late loss").

EXPERIMENT 1

Methods

Participants

We recruited 51 healthy volunteers (26 men, mean age = 24.69, *SD* = 4.16, age range = 19 – 35) from the student population at the University of Cambridge for a study of gambling behavior. Our advert stated that participants should be psychiatrically healthy, and it was directed toward students with some interest in gambling ("Do you enjoy gambling?"). We excluded psychology and economics students. The Problem Gambling Severity Index (Ferris & Wynne, 2001) was administered to screen for potential gambling problems. No participants were classified as problem gambler (a score of 8 or more), and the majority of participants (61%) scored 0. The study was conducted in accordance with Declaration of Helsinki and was approved by the University of Cambridge Psychology Research Ethics Committee. Written informed consent was obtained from all participants. Volunteers attended an individual testing session, which comprised the wheel-of-fortune task and the counterfactual thinking task. They were paid a fixed fee as reimbursement for their time, plus a financial bonus that was proportional to their actual earnings in the gambling tasks. Additional psychophysiological data collected in this sample have been reported elsewhere with different purposes (Wu & Clark, 2015; Wu, van Dijk, Aitken, and Clark, 2016).

Wheel-of-fortune task

Participants completed 76 experimental trials on a computerized wheel-of-fortune task modified from Wu, van Dijk, and Clark (2015), using a spinner rather than highlighted

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segments to indicate gambling outcome. The task was programmed in Matlab, using the Psychophysics Toolbox extensions (Brainard, 1997). On each trial, the wheel was divided into four segments of different colors. The "+" or "−" symbols in each segment indicated the amounts stood to win or lose. Those segments without any symbols represented zero outcomes (neither win nor lose). The number (e.g. 10) indicated the size of win/loss, as a multiplier of the amount participants bet on that round. For instance, +10× meant that the participant would win 10 times the bet, and −10× would lose 10 times of the bet.

The trial sequence and timings are displayed in Figure 2. At the beginning of each trial, the participant was asked to choose a bet between £0.10 and £0.90, in £0.10 increments. Following bet selection, the spinner on the wheel spun for an anticipation interval (5.3 – 6.9 seconds), during which time the spinner decelerated to a standstill. The outcome phase then lasted 1 second, where the spinner stopped, and there was accompanying auditory feedback (applause sound for winning outcomes, booing sound for losing outcome and thud sound for null outcome), and the numeric outcome was displayed for 1 second. Following the outcome phase, a luck rating was displayed using a 9-point visual analogue scale ("How lucky did you feel?"), with 1 indicating "extremely unlucky" and 9 indicating "extremely lucky". No time constraints were imposed on the bet selection or luck rating.

The outcomes were fair, with each event type repeated five times. The closeness was manipulated in such a way that on the near event trials, the distance of the spinner to the segment boundary was 1.8°. For the clear outcomes (i.e. clear-wins, clear-losses and two types of full-misses (FM)), the spinner stopped 45° from the boundary of the segment. We interspersed 16 filler trials where the spinner landed at various other positions on the wheel in order to make the task more realistic. On average, participants won £9.59 (*SD* = 16.43) in this task.

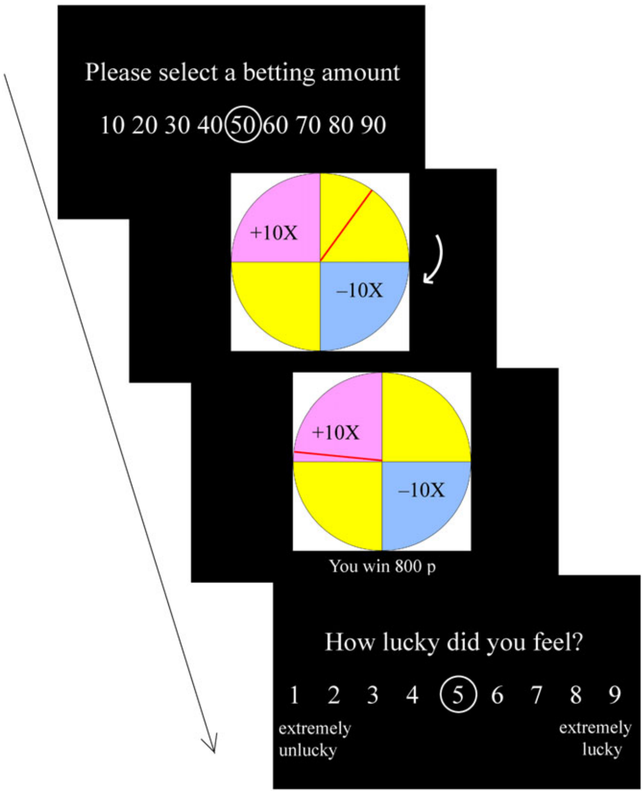

Figure 2. Trial timing for the wheel-of-fortune task. [Colour figure can be viewed at wileyonlinelibrary.com]

Counterfactual thinking task

Following the wheel-of-fortune task, participants completed a counterfactual thinking task adapted from Camille et al. (2004) (see Wu & Clark, 2015 for analysis of facial muscle responses on this task). On each of 112 trials, participants chose between two wheels that displayed different potential gains and losses, and their respective probabilities. Each wheel offered two of the following possible outcomes: +70, +210, −70, −210, representing monetary values in pence (i.e. British £). Outcome probability was illustrated by the segment size occupied by that outcome (0.25, 0.5 or 0.75, see Figure 3). As the participant selected a wheel, the wheel was highlighted with a red surround. The obtained outcome on that wheel was presented for 4 seconds, during which time the non-selected wheel was hidden. After a further 4 seconds of blank screen, the outcome on the non-selected wheel (i.e. the non-obtained outcome) was revealed for 4 seconds. Participants were then asked to rate "how pleased were you with the outcome", with 1 indicating extremely unpleasant and 9 indicating extremely pleasant. This was followed by a 4 second inter-trial interval (to optimize the task for psychophysiology, not reported here). No time constraints were imposed on wheel selection or affect ratings. Outcomes were prespecified in line with the displayed probabilities in order that the task was fair. On average, participants won £12.65 (*SD* = 5.51) on the task.

Statistical analysis

Wheel-of-fortune task: We used R and *nlme* (Pinheiro, Bates, DebRoy, & Sarkar, 2013) to perform two linear mixed effects analyses on the dependent variables: (i) luck ratings (centered; 0 means neither lucky nor unlucky); (ii) the change in the bet amount (from the current *n* trial to the next, *n* + 1, trial). We use linear mixed-effects (LME) modeling via restricted maximum likelihood for all repeated-measures analyses (Judd, Westfall, & Kenny, 2012). As a random effect, we had an intercept representing participant number. For the two dependent variables, we ran a series of LME models to test each set of hypotheses. In a preliminary model run as a manipulation check, we assessed the impact of the *objective* outcomes (e.g. wins, losses and null) as a fixed effect. In the second step, we compared three types of null outcomes, i.e. NW, NL and FM. In the third step, we considered whether near-miss position (before vs. after) was relevant, treating both near-miss type and position as fixed effects (with interaction terms). In the final step, we compared the three types of win outcomes (model 4a, i.e. early-wins, clear-wins and late-wins), and the three types of loss outcomes (model 4b, i.e. early-losses, clear-losses and

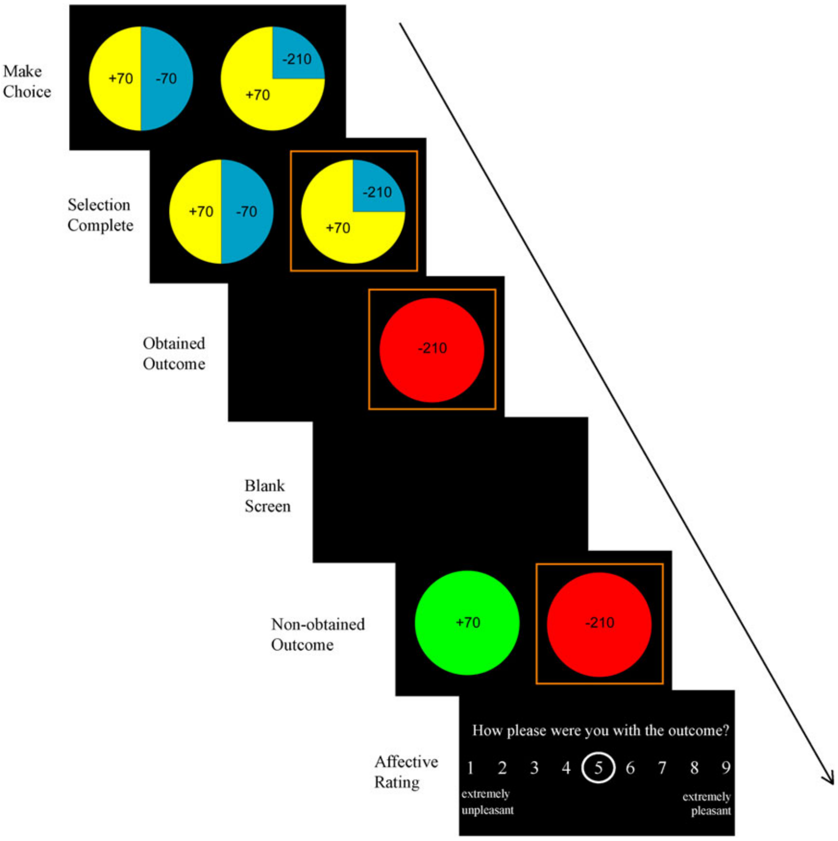

Figure 3. Trial timing for the counterfactual thinking task. [Colour figure can be viewed at wileyonlinelibrary.com]

late-losses). Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality. For the models on luck ratings, the bet amount at the start of current trial (i.e. before the outcome was delivered) was entered as a fixed factor of no interest. To assess the validity of the mixed effect analysis, we performed likelihood ratio tests comparing the models with fixed effects to the null models with only the random effects. We rejected results in which the model including fixed effects did not differ significantly from the null model.

Counterfactual thinking task: For the affect ratings following the non-obtained outcomes, the size of the obtained and non-obtained outcomes were entered as predictors, along with the interaction term. The counterfactual index was calculated by regressing the difference between what was obtained and what could have been obtained had the participant chosen the other wheel (obtained outcome minus non-obtained outcome on the non-selected wheel) against the subjective ratings. A steeper slope (i.e. more positive value) indicated greater relief for downward counterfactuals and stronger regret for upward counterfactuals.

Results and discussion

Wheel-of-fortune task

Objective outcomes. Luck ratings. The first model investigated the effect of the different objective outcomes (three levels: wins vs. losses vs. neutral) on luck ratings (see Figure 4A). There was a significant main effect of Outcome Type, $\chi^2(2) = 117.65$, $p < .001$, with participants feeling luckier following wins compared to neutral outcomes, $b = 1.05$, $t(100) = 6.64$, $p < .001$, and following neutral outcomes compared to losses, $b = 1.27$, $t(100) = 8.01$, $p < .001$.

Betting behavior. The objective outcomes also impacted differently upon betting behavior (see Figure 4B), $\chi^2(2) = 36.11$, $p < .001$, with participants reducing their bet following wins compared to both neutral outcomes, $b = -7.09$, $t(100) = -4.66$, $p < .001$, and losses, $b = -9.17$, $t(100) = -6.02$, $p < .001$. There was no statistical difference between losses and neutral outcomes on bet amount change, $b = 2.08$, $t(100) = 1.37$, $p = .18$.

Thus, as a manipulation check, our participants felt luckier following wins and unluckier following losses, confirming that the task effectively induced distinct luck perceptions for the basic objective outcomes. The finding that the amount bet reduced following wins is consistent with a broad definition of the "gambler's fallacy" that people do not expect runs to continue in a random sequence (Ayton & Fischer, 2004).

Decomposing neutral outcomes. Luck ratings. In the next set of tests, we compared the three types of neutral outcomes (see Figure 5A), i.e. NW versus NL versus FM. There was a significant main effect of Outcome Type, $\chi^2(2) = 81.89$, $p < .001$. NL significantly increased luck ratings compared to FM, $b = 0.68$, $t(100) = 5.70$, $p < .001$, while NW significantly reduced luck ratings relative to FM, $b = -0.66$, $t(100) = -5.50$, $p < .001$.

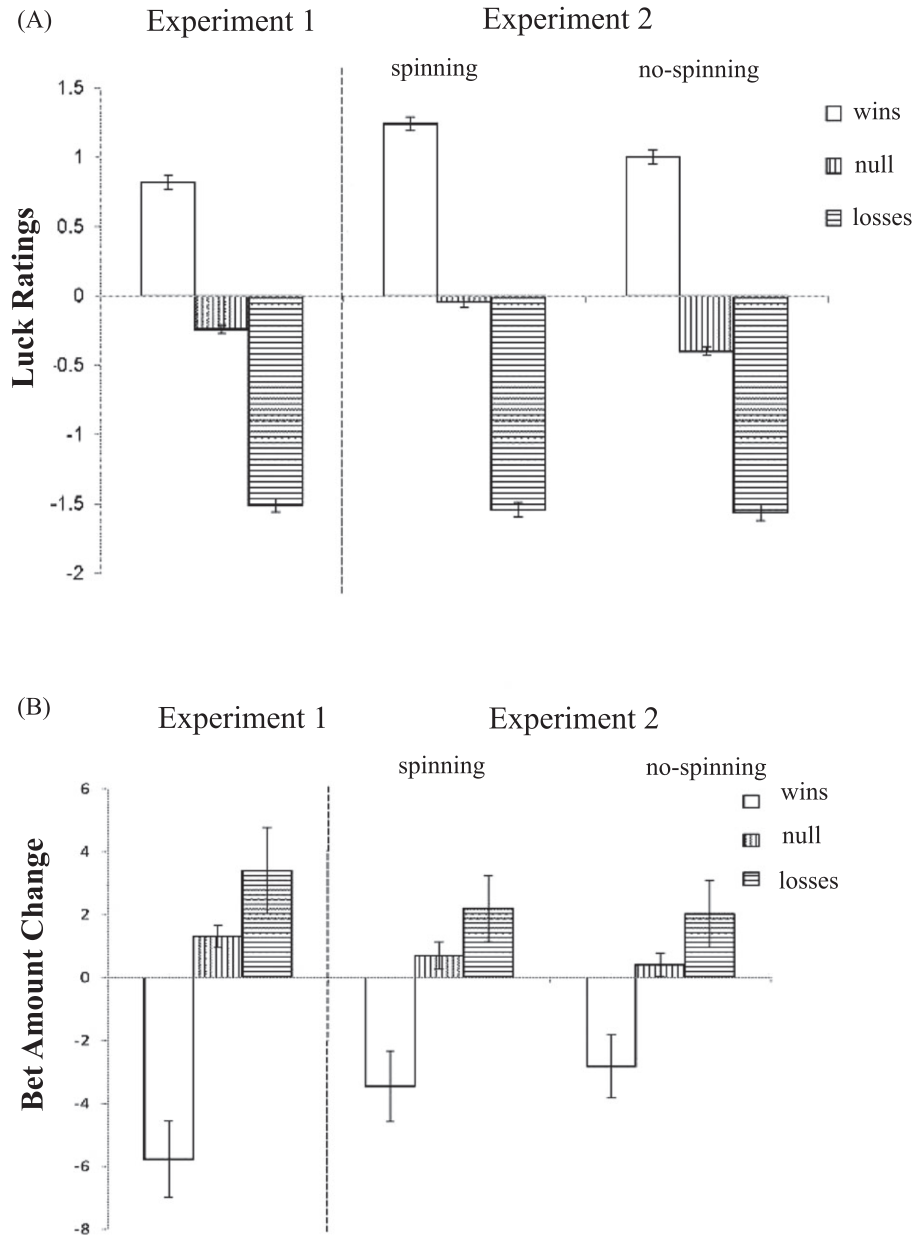

Figure 4. (A) Luck ratings following the three types of objective outcomes and (B) bet amount change following the three types of objective outcomes. Error bars represent standard errors of the mean

Betting behavior. There was no difference in betting behavior following the different types of neutral outcomes (see Figure 5B), $\chi^2(2) = .21$, $p > .1$.

Near outcomes by position. Luck ratings. The third model distinguished four types of near-misses based on both near-miss type (NW vs. NL) and near-miss position (before vs. after) (see Figure 5C). The interaction term was significant, $\chi^2(1) = 9.90$, $p = .001$. For NW, the NW-after were rated as unluckier than NW-before, $b = 0.19$, $t(50) = 2.27$, $p < .05$. For NL, NL-after were rated as luckier than NL-before, $b = -0.41$, $t(50) = -3.24$, $p < .01$.

Betting behavior. While we observed no overall effect of near-misses on betting in the previous model, a significant interaction was observed between near-miss type (NW vs. NL) and near-miss position (before vs. after) on betting behavior (see Figure 5D), $\chi^2(1) = 18.84$, $p < .001$. Following NW, participants reduced their bet for NW-after compared to NW-before, $b = 9.18$, $t(50) = 3.46$, $p = .001$. Following NL, participants increased their bet following NL-after compared to NL-before, $b = 6.16$, $t(50) = 2.72$, $p < .01$.

Thus, NW decreased self-perceived luck, whereas NL increased self-perceived luck. This effect was moderated by near-miss position, such that NW-after were perceived as unluckier than NW-before, consistent with the previous observation that an aversive response was stronger with NW-after (Clark et al., 2013). On betting behavior, NW-before increased subsequent bet amount compared to NW-after, replicating the motivational effect of NW in the slot machine task (Clark et al., 2009, 2013; Qi, Ding, Song, & Yang, 2011). Conversely, NL-after was rated as significantly luckier than NL-before, and NL-after increased bet amount more than NL-before.

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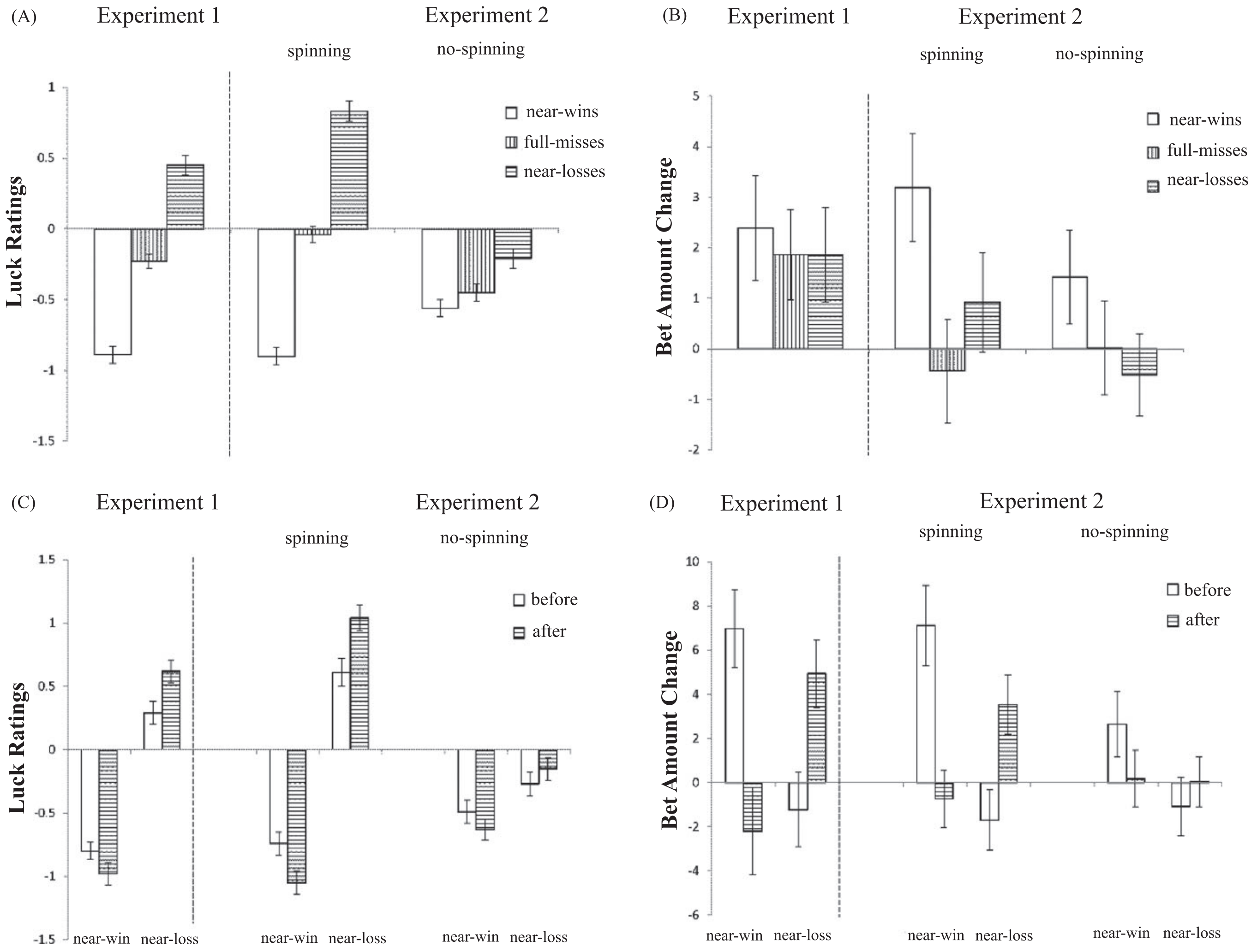

Figure 5. (A) Luck ratings following the three types of null outcomes, (B) bet amount change following the three types of null outcomes, (C) luck ratings as a function of Near-Miss Type (Near-Win, Near Loss) and Near-Miss Position (Before, After) and (D) bet amount change as a function of Near-Miss Type (Near-Win, Near Loss) and Near-Miss Position (Before, After). Error bars represent standard errors of the mean

Subtypes of objective wins. Luck ratings. In the next step, we distinguished the three types of win outcomes, i.e. early-wins versus clear-wins versus late-wins (see Figure 6A). There was a significant main effect of Outcome Type, $\chi^2(2) = 7.52$, $p < .05$, with late-wins increasing luck feelings compared to clear-wins, $b = 0.30$, $t(100) = 2.77$, $p < .01$. No difference between early-wins and clear-wins was found, $b = 0.13$, $t(100) = 1.15$, $p > .1$.

Betting behavior. The three subtypes of win outcomes also exerted a differential effect on the subsequent bet amount (see Figure 6B), $\chi^2(2) = 8.88$, $p = .01$, with the overall reduction in betting seen most strongly for early-wins and clear-wins, relative to late-wins, $b = -6.04$, $t(100) = -2.11$, $p < .05$, and $b = -8.35$, $t(100) = 2.92$, $p < .01$, respectively.

Subtypes of objective losses. Luck ratings. In distinguishing the three subtypes of losses, i.e. early-losses versus clear-losses versus late-losses (see Figure 6C), the main effect of Outcome Type was at a trend level of significance, $\chi^2(2) = 5.52$, $p = .06$, and should thus be interpreted with caution. In the pairwise comparisons, clear-losses were rated significantly unluckier than late-losses, $b = -0.22$, $t(100) = 2.32$, $p = .02$.

Betting behavior. For the differential effect of loss type on bet amount change, there was a significant main effect of Outcome Type (see Figure 6D), $\chi^2(2) = 9.50$, $p < .01$, with late-losses increasing bet amount compared to both early-losses and clear-losses, $b = 4.56$, $t(100) = 2.30$, $p < .05$, and $b = 5.92$, $t(100) = 2.98$, $p < .01$, respectively.

Thus, betting behavior differed between clear-cut and close-call outcomes for objective gains and losses, with marginal evidence for an analogous effect on luck ratings. For objective wins, late-wins (one type of narrow-wins) were perceived as luckiest, and this appeared to attenuate the reduction in bet amount following wins. For objective losses, late-losses (one type of narrow-loss) were rated as luckiest, and elicited the largest increase in subsequent betting following losses.

Counterfactual thinking task. Affect ratings following the presentation of non-obtained outcomes were first analyzed

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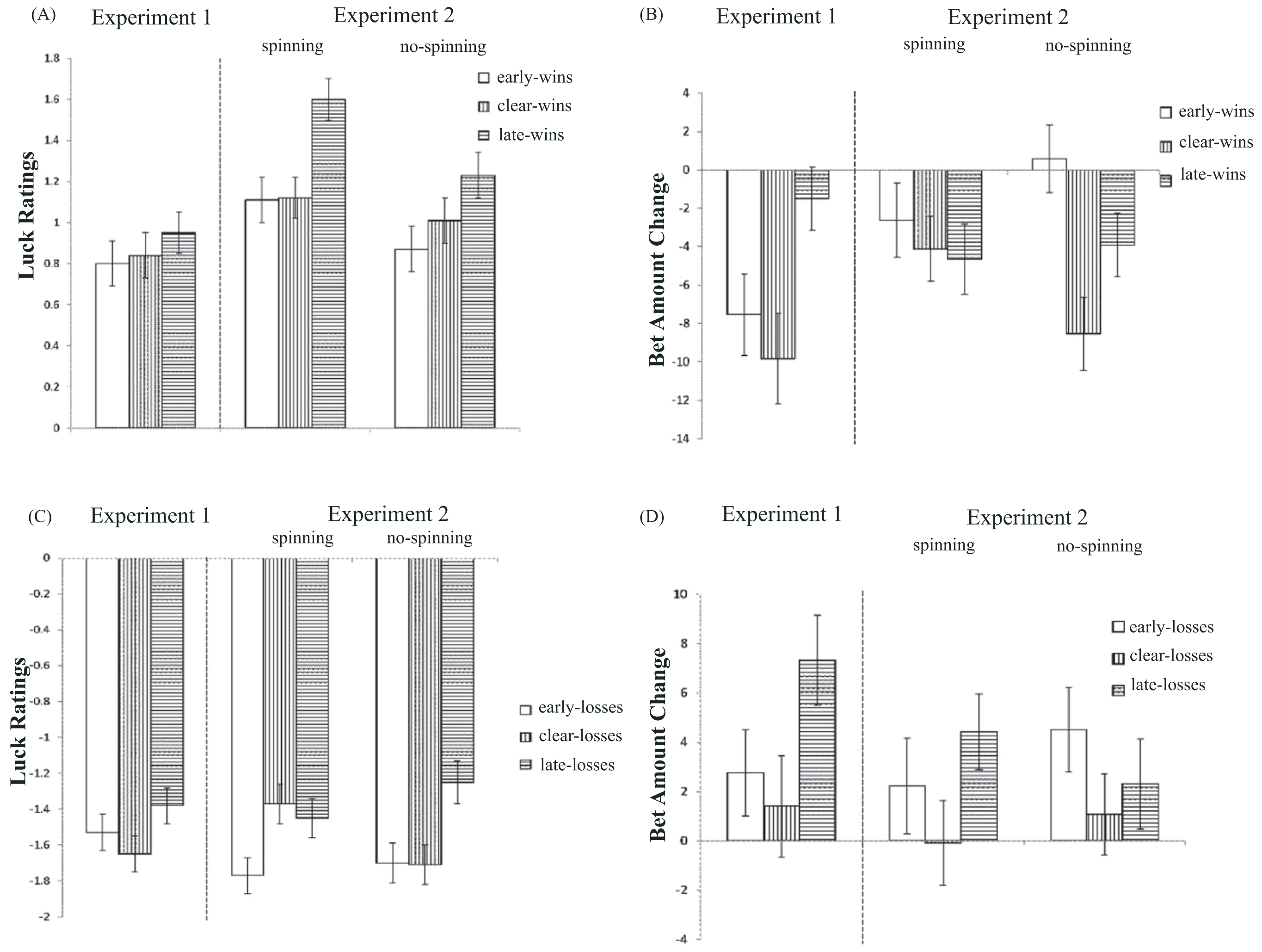

Figure 6. (A) Luck ratings following the three types of objective wins, (B) bet amount change following the three types of objective wins, (C) luck ratings following the three types of objective losses and (D) bet amount change following the three types of objective losses. Error bars represent standard errors of the mean

using the magnitude of the obtained and non-obtained outcomes as two predictors, as well as their interaction term. Importantly, the affect ratings were modulated by the non-obtained outcome, such that the participants felt worse when the non-obtained outcome was more positive (i.e. regret) and reported higher affect when the non-obtained outcome was more negative (i.e. relief), $b = -0.0057$, $t = -40.38$, $p < .001$. This confirms that the task effectively induced counterfactual comparisons. There was also an expected main effect of obtained outcome, as well as a significant interaction effect.[1]

The slope of the affect ratings as a function of the difference between the obtained and non-obtained outcomes was used to index counterfactual potency: a more positive value indicates greater sensitivity to regret and relief. The change of luck ratings from NW to NL (i.e. NL – NW) in the wheel-of-fortune task provided an index of sensitivity to near-misses (more positive values indicate greater responsivity to near-misses). Across individuals, the slope of the regression line in the counterfactual thinking task was *positively* correlated with the change score in the wheel-of-fortune task (see Figure 7), $r = 0.47$, $p < .001$. We also assessed the relationships between counterfactual potency and sensitivity to the subtypes of near-misses (see Table 1). Counterfactual potency was *negatively* correlated with NW-before (i.e. NW-before—FM, more negative values indicate greater responsivity, because NW-before decreased luck ratings compared to FM), $r = -0.36$, $p < .01$, and NW-after (i.e. NW-after—FM), $r = -0.47$, $p < .001$. Counterfactual potency was *positively* correlated with NL-before (i.e. NL-before—FM: more positive values indicate greater responsivity, because NL-before increased luck ratings compared to FM), $r = 0.34$, $p = .015$.

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[1] We decomposed the interaction effect by looking at the effect of non-obtained outcomes at each level of objective outcome. When participants objectively won the maximum amount (i.e. +210), they felt subjectively better if the non-obtained outcomes were more negative (i.e. relief), $b = -0.002$, $t = -4.39$, $p < .001$. When they objectively won a moderate amount (i.e. +70), they felt worse if the non-obtained outcome was more positive (+210, i.e. regret) and felt better if the non-obtained outcomes were more negative (−70 or −210, i.e. relief), $b = -0.005$, $t = -32.55$, $p < .001$. This slope was steepest when participants objectively lost a moderate amount (i.e. −70), $b = -0.007$, $t = -36.27$, $p < .001$. When participants lost the maximum amount (i.e. −210), they felt worse if the non-obtained outcomes were more positive (i.e. regret), $b = -0.005$, $t = -3.88$, $p < .001$, but this effect was somewhat blunted by a floor effect (see Wu & Clark, 2015).

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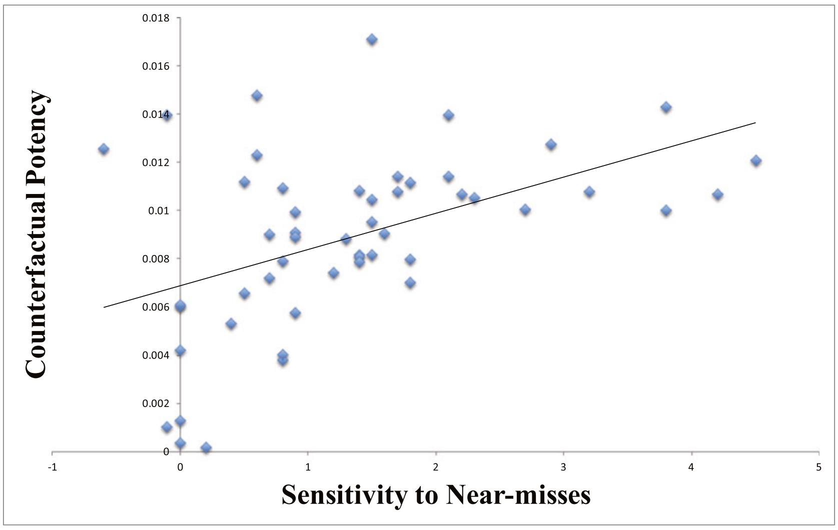

Figure 7. Correlation between individual's sensitivity to near-misses and counterfactual thinking. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Correlation between counterfactual potency and each subtype of outcome

	r	*p*
Near-win before	−0.36	.0086
Near-win after	−0.47	.0006
Near-loss before	0.34	.0151
Near-loss after	0.24	.0908
Early win	−0.24	.0865
Late win	−0.11	.4418
Early loss	−0.02	.8778
Late loss	0.09	.5499

EXPERIMENT 2

The correlation between near-miss and regret/relief tasks provides evidence for a counterfactual mechanism for near-misses. The position effect observed on the near-miss task is also consistent with a counterfactual account, based on additive versus subtractive thoughts. This counterfactual explanation for the position effect assumes a mental simulation that may be elicited by the motion trajectory of the spinner during the anticipation phase. However, other aspects of outcome processing may not require the visual input of the motion trajectory and depend only upon the final outcome. For example, participants' prior experience with spinning wheels may be sufficient to simulate the basic difference between NW, NL, and the objective gains and losses; the movement leading up to those outcomes may be psychologically irrelevant. For Experiment 2, we reasoned that a manipulation of the anticipatory phase would provide a further test of the counterfactual hypothesis. We compared two groups of participants: the first group played an identical wheel-of-fortune task as in Experiment 1. The second group performed the task with one modification: we removed the visual presentation of the wheel spin and deceleration, thus removing any visual influence of the motion trajectory in generating the near-miss position effect. We hypothesized that if the near-miss position effect depends on the motion trajectory, then the distinction between near-misses in the "before" and "after" positions should disappear in the modified ("no-spin") version. We also aimed to corroborate the main findings of Experiment 1 in a separate group of participants.

Methods

One hundred and four healthy Chinese students (51 men, mean age = 21.56, *SD* = 1.44, age range = 19 – 26) were randomized to two conditions of the wheel-of-fortune task. No participants were classified as problem gamblers based on a translated Chinese version of Problem Gambling Severity Index. For the spinning version ($n = 52$), the task was identical to Experiment 1. For the no-spin version ($n = 52$), following bet selection, the participants viewed a 4 second blank screen followed by a reveal of the final position of the spinner on the wheel, indicating the outcome for that trial. For data analysis, Condition (spinning vs. no-spinning) was added as a between-subjects factor.

Results and discussion

Objective outcomes

Luck ratings. In the first model investigating the objective outcomes (wins vs. losses vs. neutral) in the two Conditions (spin vs. no spin), the main effect of Outcome Type was significant (see Figure 4A), $\chi^2(2) = 287.54$, $p < .001$, with participants feeling luckier following wins compared to neutral outcomes, $b = 1.34$, $t(202) = 12.30$, $p < .001$, and following neutral outcomes compared to losses, $b = 1.34$, $t(202) = 12.14$, $p < .001$. There was no significant main effect of Condition, $\chi^2(1) = 0.28$, $p > .1$, or Outcome Type × Condition interaction, $\chi^2(2) = 2.06$, $p > .1$.

Betting behavior. There was a significant main effect of Outcome Type (see Figure 4B), $\chi^2(2) = 34.80$, $p < .001$. Wins reduced betting compared to both losses and neutral outcomes, $b = -5.25$, $t(202) = -5.88$, $p < .001$, and $b = -3.70$, $t(202) = -4.14$, $p < .001$, respectively. The difference between losses and neutral outcomes was only marginally significant, $b = 1.56$, $t(202) = 1.74$, $p = .08$. Neither the main effect of Condition nor the interaction term was significant, both $ps > .1$.

Thus, our participants felt luckier after wins and unluckier after losses, in both the spinning and no-spin conditions, with no discernible differences in the objective outcomes between the two versions of the task. Participants reduced their bets following wins, in line with a "gambler's fallacy" account and consistent with Experiment 1.

Decomposing neutral outcomes

Luck ratings. In the model distinguishing the three types of neutral outcomes (NW vs. NL vs. FM), there was a significant main effect of Condition (see Figure 5A), $\chi^2(1) = 5.18$, $p < .05$, with higher overall luck ratings on the spinning task than the no-spin task. A possible explanation is that the spinning and decelerating phase in the spin condition made it easier for the participants to generate counterfactuals, thus giving rise to heightened luck-ratings. The main effect of Outcome Type was significant, $\chi^2(2) = 86.94$, $p < .001$, and qualified by a significant Outcome Type × Condition interaction, $\chi^2(2) = 51.84$, $p < .001$. Simple effects analysis looked at the outcome types in the two conditions separately. In the spinning condition, there was a significant main effect of Outcome Type, $\chi^2(2) = 89.58$, $p < .001$: NL increased self-perceived luck relative to FM, $b = 0.87$, $t(102) = 6.01$, $p < .001$, while NW decreased luck ratings, $b = -0.85$, $t(102) = -5.89$, $p < .001$. In the no-spin condition, the main effect of Outcome Type was also significant, albeit with a smaller effect size ($R^2 = .02$ in the no-spin task vs. $R^2 = .18$ in the spinning task), $\chi^2(2) = 13.02$, $p = .0015$: NL increased luck ratings compared to FM, $b = 0.23$, $t(102) = 2.36$, $p < .05$, but there was no significant difference between NW and FM, $p = .19$.

Betting behavior. There was a significant main effect of Outcome Type (see Figure 5B), $\chi^2(2) = 8.47$, $p = .01$. NW increased bet amount compared to both NL and FM, $b = 2.16$, $t(202) = 2.27$, $p < .05$, and $b = 2.62$, $t(202) = 2.74$, $p < .01$, respectively, but there was no difference between FM and NL, $p > .1$. There was no significant main effect of Condition or interaction term, $ps > .1$.

Near outcomes by position

Luck ratings. There was a significant three-way interaction between Condition (spin vs. no-spinning), Near-Miss Type (NW vs. NL) and Near-Miss Position (before vs. after) (see Figure 5C), $\chi^2(1) = 4.97$, $p < .05$. For simple effects analysis, we tested the two-way interaction between near-miss type and near-miss position in the two conditions separately. In the (original) spin condition, there was a significant Near-Miss Type by Near-Miss Position interaction, $\chi^2(1) = 20.55$, $p < .001$. For NW, NW-after were rated as unluckier than NW-before, $b = -0.24$, $t(51) = -2.46$, $p = .017$. For NL, NL-after were rated as luckier than NL-before, $b = 0.44$, $t(51) = 3.55$, $p < .001$. In the no-spin condition, the interaction between Near-Miss Type and Near-Miss Position was only marginally significant, $p = .07$. There was a significant main effect of Near-Miss Type, $\chi^2(1) = 9.68$, $p = .0019$, such that NL increased luck ratings compared to NW, $b = 0.36$, $t(51) = 3.26$, $p = .002$. The main effect of Near-Miss Position was not significant, $p > .1$.

Betting behavior. There was a significant three-way Condition by Near-Miss Type by Near-Miss Position interaction on the bet amount change (see Figure 5D), $\chi^2(1) = 5.90$, $p = .015$. Considering the two-way interactions of near-miss type and near-miss position in the two conditions separately, there was a reliable interaction in the spinning condition, $\chi^2(1) = 20.26$, $p < .001$. NW-before increased bet amount change compared to NW-after, $b = 7.85$, $t(51) = 3.77$, $p < .001$, while NL-after increase bets relative to NL-before, $b = 5.23$, $t(51) = 2.81$, $p < .01$. In the no-spin condition, the near-miss Type by Position interaction was marginally significant, $p = .07$, and the main effect for Near-Miss Position was not significant, $p = .99$. The main effect of Near-Miss Type was significant, $b = 0.36$, $t(51) = 3.26$, $p < .05$, corroborating the finding in the last model that NL increased luck feelings compared to NW.

Thus, the effects of near-miss position from Experiment 1 were reproduced in the original condition with the spin and deceleration, but were attenuated (to the point of non-significance) in the no-spin condition.

Subtypes of objective wins

Luck ratings. In the model comparing the three types of objective wins (early-wins vs. clear-wins vs. late-wins), there was a significant main effect of Outcome Type (see Figure 6A), $\chi^2(2) = 36.34$, $p < .001$, with late-wins increasing luck feelings compared to both clear-wins and early-wins, $b = 0.48$, $t(202) = 5.91$, $p < .001$, and $b = 0.39$, $t(202) = 4.86$, $p < .001$, respectively. There was no significant main effect of Condition, $\chi^2(1) = 0.22$, $p > .1$, or Condition × Outcome Type interaction, $\chi^2(2) = 3.17$, $p > .1$.

Betting behavior. The main effect of Outcome Type was significant (see Figure 6B), $\chi^2(2) = 9.00$, $p = .01$. Both late-wins and clear-wins decreased bet amount compared to early-wins, $b = -3.27$, $t(202) = -1.84$, $p < .06$, and $b = -5.31$, $t(202) = -2.99$, $p < .01$, respectively. Neither the main effect of Condition nor the interaction term was significant, $p > .1$, and $p = .08$, respectively.

Subtypes of objective losses

Luck ratings. In the model comparing the three subtypes of losses (early-losses vs. clear-losses vs. late-losses), there was a significant main effect of Outcome Type (see Figure 6C), $\chi^2(2) = 15.69$, $p < .001$, and a significant Condition × Outcome Type interaction, $\chi^2(2) = 11.35$, $p < .01$. The main effect of Condition was not significant, $\chi^2(1) = 0.005$, $p > .1$. For simple effects analysis, we looked at differences between loss outcomes in the two conditions separately. In the spinning task, there was a significant main effect of Outcome Type, $\chi^2(2) = 11.08$, $p < .01$: early-losses were rated as unluckier than both clear-losses and late-losses, $b = -0.44$, $t(102) = -3.35$, $p < .001$, and $b = -0.29$, $t(102) = -2.23$, $p < .05$, respectively. In the no-spin condition, there was also a significant main effect of Outcome Type, $\chi^2(2) = 15.27$, $p < .001$, where both early-losses and clear-losses were rated as unluckier than late-losses, $b = -0.42$, $t(102) = -3.30$, $p = .001$, and $b = -0.45$, $t(102) = -3.49$, $p < .001$, respectively.

Betting behavior: There was no significant main effect of Outcome Type or Condition (see Figure 6D), both $p\text{s} > .1$. The Outcome Type × Condition interaction was not significant, $p > .1$.

Thus, significant differences emerged between clear-cut and close-call outcomes for both objective gains and losses, and these effects were further moderated by the visual display of the motion trajectory during the anticipatory phase.

GENERAL DISCUSSION

Using a wheel-of-fortune task, the present study investigated the subjective and behavioral responses to various near events within the framework of counterfactual thinking. Past work on near-misses has predominantly focused on NW, which are common events in the context of gambling behavior. The present study extended this work by also presenting NL, a logical counterpart of NW, and by further testing for a modulatory role of near-miss position (i.e. whether the spinner stopped just before or after the missed outcome). In Experiment 1, NL were perceived as much luckier, replicating a previous observation by Wu, van Dijk, and Clark (2015; see also Wohl & Enzle, 2003). Near-miss position had a differential impact on NW: NW-after were perceived as unluckier than NW-before, consistent with our past work using a laboratory slot machine task (Clark et al., 2013). Conversely, NL-after were rated as luckier compared to NL-before. In Experiment 2, this interaction between near-miss type and near-miss position was corroborated in an independent (and culturally distinct) sample, but the effect of near-miss position was attenuated in a modified task where the motion trajectory of the spinner during the anticipatory interval was removed. Experiment 1 also included a second decision-making task measuring affective responses to counterfactual comparisons. Individual differences in "counterfactual potency"—effectively, the degree of affect corresponding to regret and relief—were seen to predict the sensitivity to NW and NL on the wheel-of-fortune task.

On the counterfactual nature of gambling near-misses

In Experiment 1, the counterfactual potency index on a standard choice between two lotteries task (Mellers et al., 1999) correlated with the sensitivity to both NW and NL on our gambling task. Elsewhere, the strength or magnitude with which a particular counterfactual is experienced (i.e. counterfactual intensity) has been found to correlate with subsequent affective and behavioral reactions (Sanna & Turley-Ames, 2000). A recent study characterized counterfactual potency as the multiplicative combination of "if likelihood" (the degree to which the antecedent condition of the counterfactual is perceived to be likely) and "then likelihood" (the perceived conditional likelihood of the outcome of the counterfactual). This measure predicted the extent to which counterfactual thinking influenced judgments of regret, causation and responsibility (Petrocelli et al., 2011). In our gambling task, the NW elicit upward counterfactuals (e.g. "I almost landed on the big one") whereas NL outcomes induce downward counterfactual (e.g. "I could have gone bankrupt") (Wohl & Enzle, 2003; Wu et al., 2015). The observed relationship between counterfactual potency and sensitivity to NW and NL provides correlative support for a counterfactual mechanism of processing near-miss outcomes. Note that in both Sanna and Turley-Ames (2000) and Petrocelli et al. (2011) who used scenario settings, the index to represent counterfactual strength was derived from the scenario itself to predict its effect upon emotional response. In the present study, the counterfactual potency was derived from an independent task.

The counterfactual account is further strengthened in our data by the interaction effect between near-miss type (NW vs. NL) and near-miss position (before vs. after), seen for both luck ratings and bet amount change. For both types of NW, participants compared a neutral outcome with the counterfactual possibility of a (non-obtained) win. NW-before implies a trajectory toward the win segment in which the spinner stops just before the win. In this case, the perception of having almost won requires that the participants mentally simulate the spinner moving forward; an additive upward counterfactual (Roese, 1994). For the NW-after, when participants first experienced the win state and then see the spinner move on to stop in the null segment, the counterfactual requires the mental undoing of the final move, a subtractive upward counterfactual. Our findings suggest that subtractive process makes participants feel unluckier, compared to the additive process. The finding of increased bet following NW-before compared to NW-after extended our observation that NW-before enhanced self-reported motivation (Clark et al., 2013) by showing a behavioral effect on actual bet adjustment.

The position effect for NL mirrored its effect on NW, such that NL-after were perceived as luckier compared to NL-before. Both types of NL made participants compare the neutral outcome with the undesirable loss alternative (i.e. a downward counterfactual). But participants experienced the escape from a major penalty most vividly on the NL-after outcomes, when the spinner exited the loss zone and entered the safe zone. To experience a downward counterfactual, the participant would need to mentally undo this escape (i.e. a subtractive counterfactual). For NL-before, the spinner stopped just short of the loss segment, and the participant would need to mentally simulate the extra motion to achieve the downward counterfactual (i.e. an additive counterfactual). As a result, the NL-after was reliably experienced as

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luckier compared to NL-before. The differential effect of additive versus subtractive counterfactual also affected subsequent bet behavior such that NL-after enhanced bet compared to NL-before. The interactive effect of near-miss type by near-miss position extends Wohl and Enzle (2003, Experiment 2) in which they manipulated near-miss position but did not find any modulatory effect on either subjective ratings or betting behavior. Taken together, the interactive effect of near-miss type and near-miss position is consistent with the established influence of types (upward vs. downward) and structure (additive vs. subtractive) of counterfactual thinking (Roese, 1994).

In Experiment 2, the near-miss position effect—the dissociable effects of near-misses occurring in the before versus after positions—was attenuated in the version of the task where the wheel spin and deceleration were no longer visible. This indicates that the visual stimulus of the anticipatory build-up is necessary to generate the additive versus subtractive thoughts. In contrast, the near-miss type effect (i.e. NW vs. NL) observed in Experiment 1 was replicated in both conditions in Experiment 2, although was somewhat attenuated in the no-spin task. Previous research has shown that individuals who are prone to vivid mental imagery tend to have stronger emotional reactivity to counterfactuals (Barlett & Brannon, 2007). These data suggest that the distinction between NW and NL seems to be outcome-related effects that are not dependent upon the visual input of the motion trajectory.

Our position effect can also be considered within the framework of the reflection and evaluation model (REM) of comparative thinking (see Markman & McMullen, 2003 for a review). The REM proposes that two psychologically distinct models of mental simulation operate during comparative thinking. In *reflection*, an experiential (“as if”) mode of thinking is characterized by vividly simulating that information about the comparison standard is true of, or part of, the self. In evaluation, information about the standard is used as a reference point against which to evaluate one’s present standing. Thus, in the case of the NW before outcome, the spinner initially decelerates within the neutral segment, but as the spinner begins to approach the win segment, the participant may mentally simulate the possibility of a win. According to the REM, when attention is focused *only* on the counterfactual outcome (McMullen, 1997), reflective processing triggers an assimilation effect (e.g. “Wow, that was exciting – I nearly won”). In the case of NW after, the participants momentarily expect to win as the spinner is slowing down in a win position. This focuses their attention on winning. At the very end, however, the win is withheld, and this unexpected result focuses their attention on both the NW and the obtained neutral outcome, triggering an *evaluative* contrast effect (e.g. “my loss feels really bad because I almost won”). Thus, the contrast effect for NW after reduced luck ratings compared to the assimilation effect for NW before. In a similar way, the NL before outcome engages reflective processing (i.e. assimilation effect) and thus induces negative affect, whereas the NL after outcome triggers evaluative processing (i.e. contrast effect) to induce positive affect.

Other accounts of near-misses

At this point, it may be appropriate to compare our insights to alternative accounts of near miss effects. One account drawing upon behavioral learning theory is based on frustrative non-reward. This posits that as goal pursuit is thwarted (e.g. on a NW), it elicits increased reward-seeking effort (Amsel, 1958). In the case of NW, the salience of the non-win outcome being proximal to the goal (e.g. the jackpot) induces a negative emotional state (Clark et al., 2012; Dixon, Harrigan, Jarick, Fugelsang, & Sheepy, 2011), which serves to increase the motivation to gamble further, putatively in order to alleviate the aversive state. Frustration theory is compatible with the motivational effects of NW, but is difficult to reconcile with the dissociations between motivational ratings and physiological arousal following NW-after and NW-before effects (Clark et al. 2013). Frustration theory also makes no strong predictions concerning NL events. A second contemporary explanation, grounded in the cognitive approach to gambling, is that NW are falsely interpreted as skill acquisition, and thereby foster the “illusion of control” (Billieux, Van der Linden, Khazaal, Zullino, & Clark, 2012; Clark et al., 2013, 2009). In games involving skill, NW outcomes may provide indication of skill acquisition and constitute useful signal of imminent success. Similar to the frustration theory, this learning account is compatible with the motivational effect of NW, but does not readily explain the differential effect of near-miss position.

By proposing a counterfactual account for near-misses, we do not seek to argue against these alternative accounts. Near-misses are psychologically complex events involving conflicting emotional responses and cognitive appraisals, both of which influence subsequent gambling decisions (Clark et al., 2013). Both the frustration and skill acquisition account are compatible with some aspects of the near-miss findings in the winning context (i.e. NW), but they do not readily explain the position effect, and they do not generate clear predictions for the reaction to a NL, a type of outcome that has received much less empirical attention to date. Recent research using anecdotes of natural disasters suggests that NL influence decision-making by reducing the future perceived risk of that event happening (Dillon & Tinsley, 2008). The counterfactual account complements these alternative accounts by accommodating both NW and NL findings within one framework.

Narrow versus clear objective outcomes

In the present study, we also obtained preliminary evidence showing that people process objective wins and losses differently depending on whether they constitute clear outcomes (i.e. winning or losing by a clear margin) or narrow outcomes (i.e. winning or losing by a narrow margin). Across the two experiments, one type of narrow-win (the late win) was consistently perceived as luckier, compared to clear-wins. For the narrow-wins, people compare the winning outcome with the anticipated, less desirable null outcome (i.e. a downward counterfactual), and this may enhance positive feelings. Some other effects were not consistent across both experiments, and we reserve interpretation of those events until further replications have been attempted. Future research is needed to elucidate the mechanism by which narrow outcomes impact on decision-making and the effect of the narrow-miss positions.

Relationships between luck ratings and betting behavior adjustment

The present study also demonstrated multiple influences on betting behavior. One line of research has shown that induced feelings of luck increase subsequent risky choice (Darke & Freedman, 1997; Jiang, Cho, & Adaval, 2009; Wohl & Enzle, 2003). Experiencing a lucky event increased self-reported confidence and betting on a subsequent gamble, and this effect was moderated by personal beliefs in luck conceptualized as a personality trait (Darke & Freedman, 1997). Priming participants with luck-related concepts also increased their self-perceived luck and risky financial choices (Jiang et al., 2009). Of more direct relevance to the present study, Wohl and Enzle (2003) found that NL increased luck feelings and increased the amount bet on a subsequent gamble on a separate event. Thus, under some circumstances, temporarily induced lucky states appear to enhance risk taking. Our data speak against this reasoning. As a manipulation check in the present study, objective wins increased self-perceived luck compared to null outcomes, whereas objective losses decreased self-perceived luck. However, the bet amount change responded in the opposite direction: objective wins were rated as the luckiest events, but reduced subsequent betting to the greatest extent, and vice versa for objective losses. In our view, these effects are best conceptualized in relation to a gambler's fallacy-type mechanism; people do not expect successive wins (losses) in a random task, and therefore strategically reduce (increase) their bet to maximize their earnings on the task.

Note that in the present study, after the participant made a bet, the wheel spin was controlled by the computer rather than by the player himself. Perceived control can have important effects in gambling decisions. For instance, individuals have inflated self-confidence when they can exert irrelevant control over chance event (i.e. illusory control; Langer, Marcus, Roth, & Hall, 1975). Future studies would benefit from testing the modulatory role of perceived control in processing near-miss outcomes (see Clark et al., 2009).

Conclusions

Characterization of the psychological processes that underlie near-miss experiences not only informs gambling research but also has implications for economic decision-making in general. The present study demonstrates that individuals who were more susceptible to counterfactual comparisons were also more sensitive to near-miss outcomes. NW and NL had differential effect upon subjective luck-feelings and betting behavior, and this was further moderated by near-miss position, consistent with established influence of upward versus downward and additive versus subtractive thoughts in counterfactual thinking. Critically, the near-miss position effect depends on the spinning and decelerating effect in the anticipatory phase. Taken together, these date help substantiate the counterfactual account of near-misses.

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Automatic Processing of Emotional Words in the Absence of Awareness: The Critical Role of P2

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It has been long debated to what extent emotional words can be processed in the absence of awareness. Behavioral studies have shown that the meaning of emotional words can be accessed even without any awareness. However, functional magnetic resonance imaging studies have revealed that emotional words that are unconsciously presented do not activate the brain regions involved in semantic or emotional processing. To clarify this point, we used continuous flash suppression (CFS) and event-related potential (ERP) techniques to distinguish between semantic and emotional processing. In CFS, we successively flashed some Mondrian-style images into one participant's eye steadily, which suppressed the images projected to the other eye. Negative, neutral, and scrambled words were presented to 16 healthy participants for 500 ms. Whenever the participants saw the stimuli—in both visible and invisible conditions—they pressed specific keyboard buttons. Behavioral data revealed that there was no difference in reaction time to negative words and to neutral words in the invisible condition, although negative words were processed faster than neutral words in the visible condition. The ERP results showed that negative words elicited a larger P2 amplitude in the invisible condition than in the visible condition. The P2 component was enhanced for the neutral words compared with the scrambled words in the visible condition; however, the scrambled words elicited larger P2 amplitudes than the neutral words in the invisible condition. These results suggest that the emotional processing of words is more sensitive than semantic processing in the conscious condition. Semantic processing was found to be attenuated in the absence of awareness. Our findings indicate that P2 plays an important role in the unconscious processing of emotional words, which highlights the fact that emotional processing may be automatic and prioritized compared with semantic processing in the absence of awareness.

Keywords: emotional word, continuous flash suppression, P2, unconscious, semantic processing

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INTRODUCTION

Emotional words hold an important place in social communication in the modern world. When we see an emotional word, it transmits two main kinds of information. One is semantic information, which contains the meaning of the word, which activates the left lateral occipitotemporal sulci (Dehaene and Cohen, 2011), inferior frontal gyrus (Mestres-Missé et al., 2008; Chou et al., 2012), and angular gyrus (Horwitz et al., 1998; Seghier, 2013); the other is emotional information, which includes the biological value or social significance (Fox et al., 2001), which activates the amygdala (Isenberg et al., 1999; Garavan et al., 2001; Tabert et al., 2001; Hamann and Mao, 2002; Compton et al., 2003; Kensinger and Schacter, 2006; Herbert et al., 2009; García-García et al., 2016), orbitofrontal gyrus and bilateral inferior frontal gyrus (Nakic et al., 2006), anterior cingulate gyrus (Posner et al., 2009), and lingual gyrus (Kuchinke et al., 2005). Although semantic and emotional information processing in the brain may largely overlap (Duncan and Barrett, 2007; Shackman et al., 2011; Raz et al., 2012, 2014), these results suggest that semantic and emotional processing of words activated different brain regions during a visible condition. However, it remains unclear to what extent the emotional and semantic processing of words can take place in the absence of conscious awareness.

In the invisible condition, to render the word stimuli invisible, we adopted an effective paradigm called continuous flash suppression (CFS). This interocular suppression technique has been known as a pivotal tool for exploring the visual awareness (Lin and He, 2009; Eo et al., 2016). In the CFS paradigm, some Mondrian-style images flash successively into the dominant eye steadily, which suppresses the experimental materials projected to the non-dominant eye (Kim and Blake, 2005; Tsuchiya and Koch, 2005).

At present, there is no consensus about unconscious processing of words, regardless of unconscious semantic processing or subliminal emotional processing. Firstly, there are conflicting findings for unconscious semantic processing. Some studies have reported the semantic processing of word stimuli in the unconscious conditions (Dehaene et al., 2006; Jiang et al., 2007; Wang and Yuan, 2008; Sklar et al., 2012; Ortells et al., 2016). However, other studies have provided the opposite evidence that the semantic processing of verbal stimuli cannot occur when they are rendered invisibly (Zimba and Blake, 1983; Kang et al., 2011; Heyman and Moors, 2014; Hesselmann et al., 2015). Secondly, the subliminal emotional processing of words remains questionable. For example, in a behavioral study, Sklar et al. (2012) reported that the negative word expression break suppression faster than the neutral word expression, while Yang and Yeh (2011) showed that the negative words took more time to break suppression than the neutral words. Using fMRI, Ortigue et al. (2007) showed that the masked lovers' names activated the fusiform and angular gyri, indicating that emotion-loaded word can activate the emotion-related and word-related areas. In contrast, Hoffmann et al. (2015) found that there was no significant difference of the relevant brain regions between masked emotional words and neutral words in the invisible condition. These fMRI results are inconsistent partly due to the fact that this technique has a low temporal resolution, which does not provide the temporal course for emotional word processing.

Due to high temporal resolution, ERPs are able to reflect the temporal processing of emotional stimuli. We focused on the P2 component for the following reasons. First, P2 was related to attention and categorization around 150–300 ms post-stimulus onset (Antal et al., 2001; Crowley and Colrain, 2004). Second, a plethora of research has found that emotional effect takes place in the P2 time-window in visible conditions (Begleiter and Platz, 1969; Begleiter et al., 1979; Williamson et al., 1991; Schapkin et al., 2000; Ortigue et al., 2004; Kanske and Kotz, 2007; Wang and Bastiaansen, 2014). For instance, Herbert et al. (2006) observed that negative words elicited larger P2 component compared with neutral words. Finally, at the time window around 250 ms after stimulus onset, there exists a positive-going wave (P250) at the whole brain, which is related to both automatic semantic processing and early word recognition (García-Larrea et al., 1992; Kim et al., 2001; Hill et al., 2002; Liu et al., 2009). For example, Chung et al. (2010) have found the P250 reflects that semantic memory network is activated in the semantic processing of Chinese words.

Until now, few studies have distinguished the two types of processing simultaneously in one experiment in absence of conscious awareness (Yang and Yeh, 2011). In the current study, we used an electroencephalography (EEG) method to clarify the extent to which the semantic and emotional processing of words can occur in the absence of awareness within the time-window of P2. The participants observed negative, neutral, and scrambled word stimuli during the visible condition and the invisible condition. In order to distinguish the semantic and emotional processing, we adopted a kind of scrambled stimuli that removed the semantic information and preserved the spatial location features (Yang and Yeh, 2011). We compared scrambled words with neutral words as the semantic process and also compared negative words with neutral words as the emotional process. Based on previous results (Bernat et al., 2001; Herbert et al., 2006; Kang et al., 2011), we predict that emotional processing of words would occur even when the stimuli were presented unconsciously, but semantic processing would be suppressed in absence of awareness.

MATERIALS AND METHODS

Participants

Sixteen right-handed students (10 female; mean age 22.9 years) from Liaoning Normal University participated in the experiment and were compensated \$4.50 after the experiment. They were all native Chinese speakers and had normal or corrected-to-normal visual acuity with no psychiatric or neurological history. All participants provided written informed consent. The protocol was approved by the Ethics Committee of Liaoning Normal University.

Stimuli

We used 30 negative Chinese two-character words (e.g., "悲伤," sadness) and an equal number of neutral words (e.g., "规则," rule), which were selected from the Chinese Affective Words System (CAWS; Luo and Wang, 2004; Yi et al., 2015). All of the words were nouns (see Supplementary Material). Negative words and neutral words differed significantly in valence [mean: negative = 2.72, neutral = 5.11; $t_{(58)} = -34.79$, $p < 0.001$] and arousal [mean: negative = 5.80, neutral = 4.54; $t_{(58)} = 5.75$, $p < 0.001$]. However, they were matched in stroke numbers [mean: negative = 16.77, neutral = 16.37; $t_{(58)} = 0.362$, $p = 0.719$], word frequency [mean frequency in 15 million words, negative = 0.002887%, neutral = 0.002357%; $t_{(58)} = -0.562$, $p = 0.578$; http://www.cncorpus.org/] and concreteness (this dimension rating was obtained through the use of a seven-point scale by 27 new participants [−3 to 3: very abstract to very concrete; mean: negative 0.72, neutral 1.07; $t_{(58)} = -1.154$; $p = 0.253$]). The scrambled word stimuli were constructed by dividing the negative and the neutral words into 5 × 10 blocks and then arranging them randomly. Separating the words removed the semantic information and preserved the effects of spatial location.

To create the interocular suppression, Mondrian images, and stimuli were projected onto each eye of the participant through a four-mirror stereoscope that included two intermediate mirrors (angled ± 45° orthogonally) between two adjustable mirrors. Mondrian patches (extended 7.16° × 10.03°, visual angle) were generated using Matlab 7.0 software and filled an outer frame (visual angle 8.60° × 11.36°) with the colors black and white. The stimuli were drawn using black characters at 28.6% contrast (the contrast of the stimuli was defined as the luminance difference between the background and the luminance of the words divided by the luminance of the background; Tsuchiya and Koch, 2005; Kang et al., 2011). The screen luminance of the word stimuli was set at 20% of the maximal screen luminance (dark gray), and the screen luminance of the background was set at 28% (light gray). Stimuli were presented on a 48 cm CRT (cathode ray tube) monitor (1024 × 768 resolution at 100 Hz frame rate) and controlled by E-Prime 2.0 software. Each participant was seated 60 cm in front of the computer screen with their head on a chin-rest, and responded by using a keyboard.

Design and Procedure

We used a within-subject experimental design in which two factors—word type (negative, neutral, and scrambled words) and awareness states (visible and invisible light conditions)—were manipulated. To control participants' fatigue, neutral, negative, and scrambled word stimuli were presented randomly within one block consisting of 108 trials in the visible condition. With the invisible condition, each block included 60 trials. Each observer took part in five visible blocks and nine invisible blocks of words. The participants could take a rest between the blocks. The total experimental session lasted ∼50 min. To assess their eye-dominance, the participants were asked to view an object through a hole made by their own fingers (the "Miles test"; Mendola and Conner, 2007; Axelrod et al., 2014). In the invisible condition, to make the word stimuli invisible, the Mondrian images were changed at a rate of 20 Hz (50 ms per image) and projected onto the dominant eye of each participant. At the same time, the negative, neutral, and scrambled words were presented to the non-dominant eye. At the beginning of each trial, the word stimuli and Mondrian images (extended 3.15° × 1.43°) were presented for 500 ms, and then followed by an 800–1200 ms randomized fixation (a dot, $d = 0.38°$) in an outer frame, representing an inter-trial interval (ITI; see **Figure 1A**). In the visible condition, in order to make the participants aware of the word stimuli, we replaced the Mondrian images that had been presented to the dominant eye with word stimuli. The other parts of the visible condition remained the same with the invisible condition (see **Figure 1B**).

In both the invisible and visible condition, the participants were asked to pay attention to the stimuli and complete a feature detection task. If the participants saw the word stimuli (negative, neutral, and scrambled words), they were instructed to press the "f" key; if not, they pressed the "j" key. The buttons "f" and "j," which were pressed by the right and left hands—associated with seen and unseen—were counterbalanced across participants. The sequences of the two conditions (invisible and visible) were also counterbalanced across participants. After completing the experiment, the participants were asked to recall the experience of each word when confronted with these words in the formal experiment, and rate the valences and arousals relating to each word using a 9-point Likert scale (valence 1–9; arousal 1–9). The results showed that an emotional word could effectively evoke an affective experience. The valence of the negative words (*M* ± *SE*, 3.28 ± 2821) were significantly lower than those of the neutral words (4.90 ± 0.11; $t = -7.01$, $df = 30$, $p < 0.001$). Moreover, the arousal induced by the negative words (5.26 ± 0.35) was much higher than that induced by the neutral words (3.60 ± 0.33, $t = 3.451$, $df = 30$, $p = 0.002$). Next, the participants completed a subjective report on whether the words presented in the experiment could be seen or not. All the participants reported that they did not see the word stimuli in the invisible condition but did distinguish the negative, neutral, and scrambled words in the visible condition. Moreover, the results of the feature detection task revealed that the participants reported not seeing any words in 99.89% of the trials.

Electrophysiological Recording and Analysis

Electroencephalographic (EEG) data were recorded from 64 electrodes using the Active Two system (BioSemi, the Netherlands). These electrodes were referenced online to averaged right and left mastoids. A horizontal electrooculogram (EOG) was recorded from an electrode placed 1.5 cm from the external canthus of the right eye. A vertical EOG was recorded from an electrode placed 1.5 cm below the left eye. The signal was recorded at a sampling rate of 512 Hz, filtered at 0–104 Hz and stored for offline analysis. EEG data were edited with NeuroScan equipment (Compumedics) after data conversion using PolyRex software (as described by Graux et al., 2014). Semiautomatic correction of eye movements was applied using an ICA filter transform developed by Brain Products (BP, Germany). A 0.1–30 Hz IIR bandpass filter was applied (24-dB/oct slope). The ERP waveforms were time-locked to the onset of the stimulus and corrected with a −100 to 0 ms baseline before the stimuli were applied. The remaining artifacts with amplitudes higher or lower than +80 μV were automatically rejected.

P2 was investigated in a time-window of 220–280 ms and was calculated as mean amplitude (Chung et al., 2010; Liu et al., 2013). We defined a mid-line ROI to test the P2 component (from five mid-line electrode sites: Fz, FCz, Cz, CPz, and Pz; Yun et al., 2011). Two-way repeated measures ANOVA was used to analyze the ERPs with awareness states and word types as within-subject

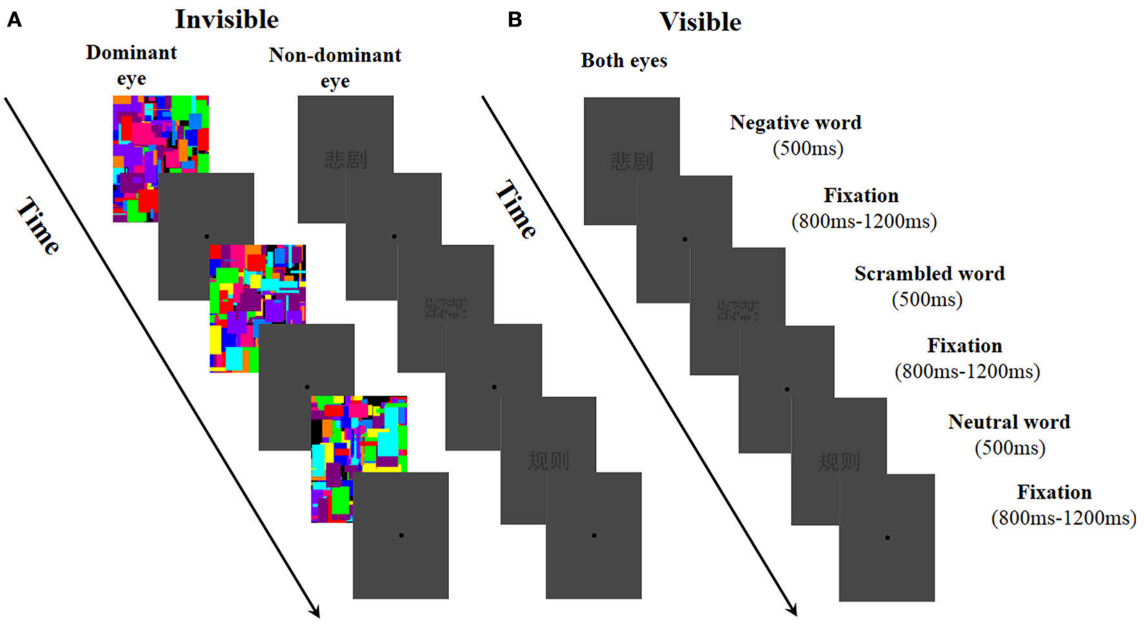

FIGURE 1 | Experimental procedure used in this study. (A) In the invisible condition, Mondrian-style images were presented to the dominant eye at a rate of 20 Hz while negative, neutral, and scrambled words were randomly presented to the non-dominant eye. **(B)** In the visible condition, negative, neutral, and scrambled words were randomly presented to both of the participant's eyes.

factors. Emotional effect was calculated by the differences in the P2 waves between the negative words and the neutral words. Semantic effect was calculated by the differences in the P2 waves between the neutral words and the scrambled words. We also used two-way repeated measures ANOVA to analyze the differences between waves, with awareness states and effects (emotional effect or semantic effect) as within-subject factors. When Mauchly's test indicated that the assumption of sphericity had been violated, Greenhouse–Geisser correction would be used to correct the degree of freedom. Bonferroni correction was used to adjust the *p*-value for all pairwise comparisons.

RESULTS

Behavioral Data

We rejected the outliers of reaction time (RT) that were outside the range of ±2.5 standard deviations from the mean (<1%) and error trials (mean error rate = 1.38%). The results showed that the interaction of word type and awareness state was not significant [$F_{(2, 30)} = 2.679, p = 0.119, \eta_p{}^2 = 0.152$]. However, the main effect of awareness state was found to be significant [$F_{(1, 15)} = 20.480, p < 0.001, \eta_p{}^2 = 0.563$]. The main effect of word type was also significant [$F_{(2, 30)} = 7.354, p = 0.013, \eta_p{}^2 = 0.329$]. The simple effect of word type in the visible condition [$F_{(2, 30)} = 4.656, p = 0.017, \eta_p{}^2 = 0.237$] revealed that negative words (mean RT, 394.169 ms) were faster than the scrambled words (435.437 ms) and neutral words (401.817 ms). The simple effect of word type in the invisible condition [$F_{(2, 30)} = 1.494, p = 0.241, \eta_p{}^2 = 0.091$, mean RT: negative word = 689.858 ms, neutral word = 691.949 ms, scrambled word = 695.799 ms] was not significant.

P2

A two-way ANOVA with awareness states (visible, invisible) and word types (negative, neutral, and scrambled) showed that the interaction of awareness states and word types was significant [$F_{(2, 30)} = 10.969, p < 0.01, \eta_p{}^2 = 0.422$]. The main effect of awareness states was also significant [$F_{(1, 15)} = 39.191, p < 0.0001, \eta_p{}^2 = 0.723$], and so was the main effect of word type [$F_{(2, 30)} = 5.646, p < 0.01, \eta_p{}^2 = 0.273$]. Subsequent analyses revealed that P2 showed no difference between the negative words and the neutral words in the visible condition ($p = 0.586$; see **Figures 2A,3**). However, the negative words (1.202 ± 0.748 μV) elicited more positive P2 than the neutral words in the invisible condition (0.640 ± 0.715 μV, $p < 0.01$). The neutral words (5.164 ± 0.473 μV) elicited a larger P2 than the scrambled words (3.336 ± 0.583 μV) in the visible condition ($p < 0.01$; see **Figures 2B,3**). In the invisible condition, the P2 amplitudes of the scrambled words (1.189 ± 0.665 μV) were larger than those of the neutral words (0.640 ± 0.715 μV, $p < 0.05$).

A 2 × 2 ANOVA with awareness states (visible and invisible) and effects (semantic and emotional) indicated that the interaction between semantic and emotional effects was also significant [$F_{(1, 15)} = 22.377, p < 0.001, \eta_p{}^2 = 0.599$]. The main effect of awareness states was significant [$F_{(1, 15)} = 7.037, p = 0.018, \eta_p{}^2 = 0.319$]. However, the main effect of all these effects was not significant [$F_{(1, 15)} = 1.288, p = 0.274, \eta_p{}^2 = 0.079$]. In the visible condition, the semantic effect (1.827 ± 0.561 μV) was larger than the emotional effect (−0.125 ± 0.225 μV, $p =$

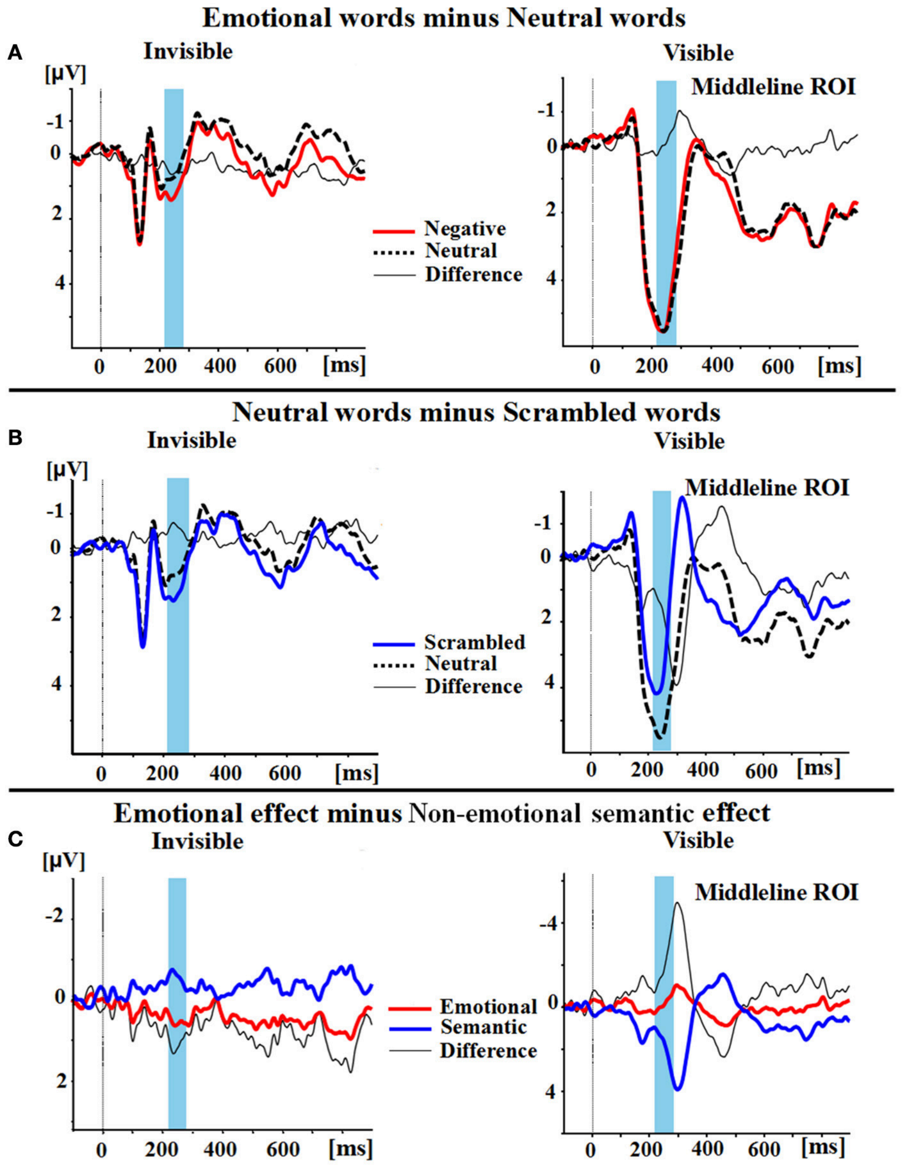

FIGURE 2 | Group Average differences in wave amplitudes from 220 to 280 ms. (A) Emotional effect: the P2 amplitudes of negative words (red line) minus the amplitudes of neutral words (black dotted line); the difference wave is represented as a thin black line. **(B)** Semantic effect: the P2 amplitudes of neutral words (black dotted line) minus the amplitudes of scrambled words (blue line); the difference wave also is again represented as a thin black line. **(C)** The difference wave (thin black line) between the emotional effect (red line) and the semantic effect (blue line).

0.006). In the invisible condition, the emotional effect (0.562 ± 0.186 μV) was larger than the semantic effect (−0.549 ± 0.197 μV, $p = 0.006$; see **Figures 2C,3**).

DISCUSSION

In this research, we investigated the P2 responses to the emotional, neutral, and scrambled words in the visible and invisible conditions. Negative words elicited larger P2 amplitudes than neutral words only in the invisible condition. Neutral words elicited larger P2 amplitudes than scrambled words in the visible condition, but smaller P2 amplitudes than scrambled words in the invisible condition. The results showed that emotional processing of words can occur unconsciously, while semantic processing of words can take place both consciously and unconsciously.

The emotional effect was significant in the invisible condition, whereas this effect was not significant in the visible condition. In the visible condition, consistent with the present study, Carretié et al. (2008) found that there was no significant difference between the neutral words and negative words. In our study, participants observed the negative, neutral, and scrambled words.

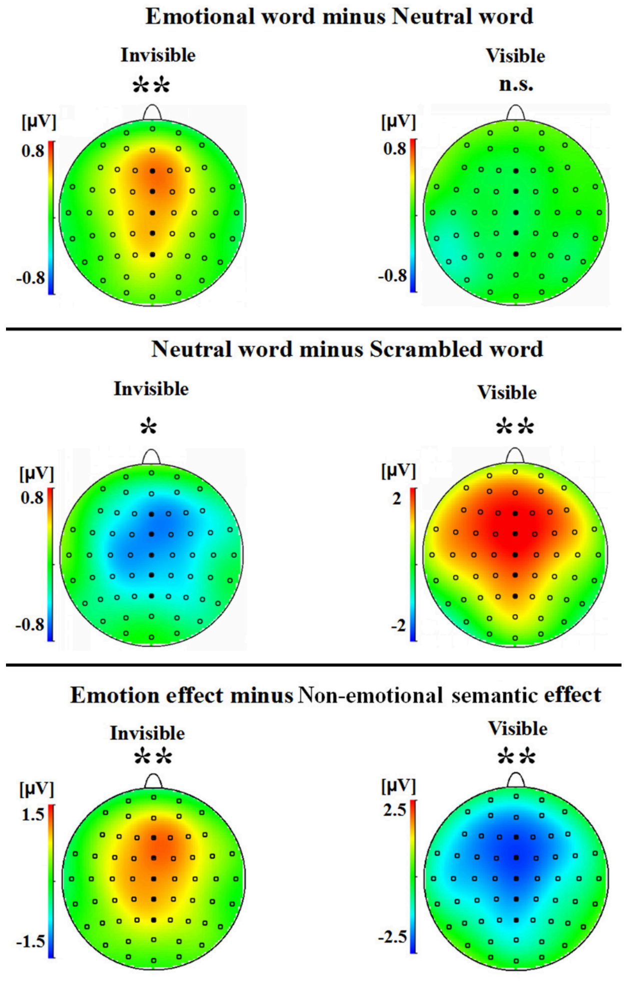

FIGURE 3 | A topographical representation of the difference waves shown in Figure 2. The asterisks indicate statistical significance (n.s., not significant; *$p < 0.05$, **$p < 0.01$).

They found the scrambled words much easier to distinguish than the intact words. This strong categorization effect may have suppressed the emotional effect. In contrast, the emotional effect of the P2 component occurred in the invisible condition. This result was similar to previous findings. For example, Bernat et al. (2001) found that in the supraliminal condition, no significant differences in the P2 component were exhibited between unpleasant and pleasant words, while in the subliminal condition, unpleasant words evoked larger P2 amplitudes than pleasant words. Moreover, Yun et al. (2011) also found that the masked threat words elicited a larger P250 amplitude than the neutral words in PTSD (posttraumatic stress disorder) patients. Thus, the P2 component was sensitive to emotional processing in the unconscious (invisible) condition, but not in the visible condition. This result suggested that P2 might play an important role in unconscious emotional word processing.

We found a difference in the P2 component (neutral words > scrambled words) in the visible condition. This result was similar to that of previous studies (Liu et al., 2009; Chung et al., 2010). Interestingly, when the participants were unaware of the stimuli, a P2 reversal (scrambled words > neutral words) was exhibited. Moreover, the semantic effect in the invisible condition was weaker than that in the visible condition. One possibility is that a reverse mode was operating between the different types of attentional processes adopted between the visible and the invisible condition. The participants adopted a "bottom-up" attentional style in the invisible condition, yet a "top-down" style in the visible condition. Another explanation is that the observed early semantic processing effect of the P250 component was related to the inferior frontal gyrus (Liu et al., 2013), which was activated by syntactic violations in the absence of conscious awareness (Batterink and Neville, 2013; Axelrod et al., 2014). Thus, the P2 reversal effect might imply that some early semantic processing was still operating in the invisible condition, but that this effect was much weaker than that in the visible condition. Further, studies are needed to verify our interpretation of the P2 reversal effect. In short, semantic processing appears to be suppressed in the invisible condition, but not in the visible condition.

Why was the emotional effect greater than the semantic effect in the invisible condition, yet the semantic effect was stronger than the emotional effect in the visible condition? Owing to the biological value of emotional stimuli, emotional processing holds a prioritized place (Yokoyama et al., 2013). Previous studies have provided evidence of two distinct networks of emotional processing: the cortical pathway and the subcortical pathway (Whalen et al., 1998; Morris et al., 1999; Damasio et al., 2000; Adolphs, 2004; Williams et al., 2006). For example, Naccache et al. (2005) recorded the amygdala-mediated reactions of three epilepsy patients when they observed threat words and neutral words in masked and unmasked paradigms by intracranial electroencephalography (iEEG). The brain regions relating to emotional memory were also activated, such as the dorsomedial prefrontal cortex (DMPFC, Cato et al., 2004) and ventromedial prefrontal cortex (VMPFC, Kuchinke et al., 2006). The cortical pathway of emotional processing was found to be reduced or absent in CFS, while the subcortical pathway—which included the amygdala and pulvinar—was still in operation. This is because the prefrontal cortex is related to "top-down" parieto-frontal networks, which bind conscious sensory processing (Dehaene et al., 2006). Although some researches hold the notion that cortical semantic processing still occurs in the absence of awareness (Dehaene et al., 2006; Axelrod et al., 2014), in our study the degree of semantic processing was definitely impaired. Therefore, the emotional effect was greater than the semantic effect in the invisible condition, which suggested that emotional processing is less dependent on conscious awareness than on semantic processing.

We also found the main effect of the awareness state to be significant: the amplitudes of P2 in the visible condition were larger than those in the invisible condition. This result reflected the fact that the brain reactions to the word stimuli in the visible condition were stronger than those in the invisible condition. The "unconscious binding" hypothesis suggests that, in the unconscious condition, registered and attentively grouped information can be integrated (Lin and He, 2009). However, compared with conscious binding (Crick and Koch, 1990), “unconscious binding” is fragile and weak. Thus, we found a larger P2 component in the visible condition than in the invisible condition. Moreover, the main effect of the word type was significant, which reflected that the emotional and semantic processing might exist in the visible and invisible conditions.

The behavioral data showed no differences between the RTs of negative words and those of neutral words in the invisible condition; negative words were faster than neutral words and scrambled words in the visible condition. To date, ample studies have been conducted looking at the differences in the processing of emotional vs. neutral words on the behavioral level (see Jończyk, 2016). Our findings support previous studies reporting facilitative processing of negative compared to neutral words (Kousta et al., 2009; Vinson et al., 2014; Yap and Seow, 2014). However, we did not find the behavioral differences between negative words and neutral words in the invisible condition. It was probably because the behavioral data was not as sensitive as the electrophysiological data. Moreover, in our experiment the time needed (500 ms) to break the CFS was not sufficient to elicit a behavioral reaction; Yang and Yeh (2011) found that the mean RT needed to break suppression was more than 1,600 ms. Therefore, the behavioral data in our study might be insufficient to elucidate the unconscious emotional word processing.

A limitation of our research might be the participants’ anticipation or some response strategies in our task. It appears to be better if the visible and invisible conditions are mixed within one block (see Yang et al., 2014). Therefore, we suggest that future studies should further investigate this line of thought.

In conclusion, our findings provide evidence that emotional words can be processed even when the stimuli are rendered invisible, which may contribute to the ongoing debate (Zimba and Blake, 1983; Dehaene et al., 2006; Jiang et al., 2007; Hesselmann et al., 2015; Ortells et al., 2016). Furthermore, emotional information is more sensitive to unconscious processing than semantic information; a semantic effect weakly occurs only in the absence of awareness. Besides, P2 plays an important role in unconscious emotional word processing. These findings appear to suggest that emotional processing remains automatic and prioritized compared with semantic processing in the unconscious condition. Since the issue is rather complex (context- and task-dependent), the debate on emotional vs. cognitive primacy remains ongoing (see Lai et al., 2012), and further studies are needed to clarify this issue.

AUTHOR CONTRIBUTIONS

HD, QL, YL, and HL designed performed this study. HD performed the study. HD and ZZ analyzed the data. YL, HD, and WZ wrote the paper. YL and HD contributed equally to this work.

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SUPPLEMENTARY MATERIAL

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Self-reflection Orients Visual Attention Downward

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Previous research has demonstrated abstract concepts associated with spatial location (e.g., God in the Heavens) could direct visual attention upward or downward, because thinking about the abstract concepts activates the corresponding vertical perceptual symbols. For self-concept, there are similar metaphors (e.g., "I am above others"). However, whether thinking about the self can induce visual attention orientation is still unknown. Therefore, the current study tested whether self-reflection can direct visual attention. Individuals often display the tendency of self-enhancement in social comparison, which reminds the individual of the higher position one possesses relative to others within the social environment. As the individual is the agent of the attention orientation, and high status tends to make an individual look down upon others to obtain a sense of pride, it was hypothesized that thinking about the self would lead to a downward attention orientation. Using reflection of personality traits and a target discrimination task, Study 1 found that, after self-reflection, visual attention was directed downward. Similar effects were also found after friend-reflection, with the level of downward attention being correlated with the likability rating scores of the friend. Thus, in Study 2, a disliked other was used as a control and the positive self-view was measured with above-average judgment task. We found downward attention orientation after self-reflection, but not after reflection upon the disliked other. Moreover, the attentional bias after self-reflection was correlated with above-average self-view. The current findings provide the first evidence that thinking about the self could direct visual-spatial attention downward, and suggest that this effect is probably derived from a positive self-view within the social context.

Keywords: self, attention orientation, above-average, self-enhancement, social context

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INTRODUCTION

> "Look down and see the beggars at your feet, look down and show some mercy if you can."
> - - - - - - -From *Les Miserables*.

Nobles in the upper class look down with pride to see others at the bottom of the heap, while those of the underclass, look up to dignitaries, and show their respect. "Up" and "down" are not only used for concrete spatial location, but also as metaphors for abstract concepts, such as attitudes toward others (e.g., "look up to the leader" and "look down upon the beggar"). These kinds of metaphors have also been used to describe the higher position one possesses relative to others within the social hierarchy. For example, "I am at the top of the class" or "I am above average people." The belief that we are above average is a robust cognitive bias that helps to maintain self-esteem (Alicke, 1985; Taylor and Brown, 1988; Chambers and Windschitl, 2004; Beer and Hughes, 2010). In the current study, we focused on the spatial metaphor of the superiority of the self, and demonstrated its influence on visual attention.

Psychological researchers have been interested in the influence of abstract concepts on visual attention. These abstract concepts include ones associated with vertical spatial information, such as God and devil. Individuals responded faster to target stimuli presented at compatible locations, such as God is up and devil is down (Meier et al., 2007; Chasteen et al., 2010). Meier and Robinson (2004) also demonstrated that positive/negative words (e.g., hero/liar) were evaluated faster when they appeared at the up/down position of the screen. Using pictorial stimuli, Schubert (2005) showed that power was aligned to a vertical schema, in which a powerful agent (e.g., master) is on top of a powerless one (e.g., servant). These findings demonstrate that abstract concepts with implicit spatial information could trigger automatic visuospatial attention orientation toward locations compatible with their meanings. In the social context, to maintain self-esteem, people also tend to show cognitive bias that place themselves as better or "above" average people (Alicke, 1985; Taylor and Brown, 1988; Chambers and Windschitl, 2004; Beer and Hughes, 2010). This positive bias of the self suggests an association between the self-concept and an above-average position within the social context. However, whether thinking about self could show similar attention orientation effects is still unknown. Previous research has demonstrated that self-reflection on personality traits resulted in self-bias in memory, which is called "self-reference effect." That is, compared with other-related trait adjectives, self-related traits were better remembered, suggesting that self functions as a superordinate schema deeply involved in memory (Rogers et al., 1977; Klein and Kihlstrom, 1986). Investigating whether self-reflection could result in self-bias on visuospatial attention is crucial for understanding self-reflection. Attention orientation effect provides a good way to investigate this issue.

A theoretical account for the attention orientation effects of abstract concepts is the Perceptual Symbol Systems (PSS) theory, introduced by Barsalou (1999). The PSS theory proposes that our perceptual systems capture sensorimotor information during processing of a stimulus (e.g., the clouds are up in the sky). Then, during the conceptualization process (e.g., thinking about clouds in mind), the same perceptual systems are activated and the sensorimotor state is reenacted (e.g., looking up). For abstract concepts without a concrete physical basis (e.g., God), we often use sensory-based metaphors to describe them. Therefore, cognitive processes rely on these perceptual metaphors (Lakoff and Johnson, 1999) and the representational processes that are embodied in nature (Boroditsky and Ramscar, 2002). Using the study by Meier et al. (2007) as an example, God is closely tied to a vertical representation processes because we cannot directly perceive God, thus the conceptualization strongly relies on a perceptual metaphor, such as God is the "most high" and resides in the "high Heavens." The activation of spatial cues when thinking about God in this study increased the speed of responses to targets at the upper position. While for the self-concept, the "above average" cognitive bias for the self and look "down" upon others during social comparison reminds us of the higher position in which we exist relative to others within the social group. Thus, this raises the questions of whether thinking about the "self" directs visuospatial attention, and if so, in which direction (up or down) attention is oriented.

In previous studies, visual attention was guided by the position of external objects or concepts, using the self as the frame of reference. For example, God is located above the earth on which the self resides, so one would look up for God. Since the "above average" bias of the self uses others as the frame of reference, and the existence of others below us builds our sense of pride in the social environment, the higher position of the self would lead us to look down for others to maintain the superiority of the self. From the view of clinical psychology, when patients look up to the analyst (e.g., when lying down on the coach), the posture increases their feeling of smallness. By contrast, when the patients' posture allowed them to look down on others, the superiority of the self will be established (Steiner, 2006). Thus, we hypothesized that thinking about the self would direct visual attention downward.

The aim of the current study was to test whether thinking about the self could direct visual attention, and if so, in which direction (up or down) would attention orient. We adopted the target discrimination task (Estes et al., 2008) after self- or other-reflection on personality traits to investigate this issue. In Study 1, we used the self and a friend as target persons to show the effects of self-reflection and other-reflection on visual attention. The self-specific attention orientation effect was expected. Because our participants were all from an Eastern culture, in which individuals tend to regard their friend as part of the self (Markus and Kitayama, 1991), in Study 2, we used a disliked other as a control to replicate the attention orientation effect after self-reflection. Moreover, in Study 2, we also measured the subjective status of participants using the above-average paradigm (Beer and Hughes, 2010) to confirm that the attention orientation after self-reflection was derived from a positive self-view.

STUDY 1

Methods

Participants

Forty-two adults (19 males, mean age = 21.12 years, standard deviation $SD = 1.89$ years) participated in this study for monetary compensation. All participants were right-handed and had normal or corrected-to-normal vision. Written informed consent was obtained prior to participation. This study was approved by the ethics committee of Shenzhen University. In previous studies on attention orientation, the η^2 were approximately 0.2 in 2 × 2 designs (Meier et al., 2007; Estes et al., 2008; Gozli et al., 2013).

An *a priori* power analysis using G*Power (Faul et al., 2009) revealed that assuming an $\eta^2 = 0.2$, 34 participants were adequate to detect a medium- to large-sized interaction, with an α of 0.05, and power of 0.80.

Stimuli and Procedure

Attention Orientation Task

We selected 64 personality trait adjectives (32 positive and 32 negative) from an established personality trait adjective pool (Liu, 1990). These 64 words were classified into two blocks, one positive block with 32 positive adjectives, and one negative block with 32 negative adjectives. Each block was presented twice, once for self and once for a gender-matched friend. The "friend" condition existed as a control to clarify whether the attention orientation effect of trait reflection was self-specific. The four blocks (self-positive, self-negative, friend-positive, friend-negative) were presented in a random order, and with a 10 s interval between each two blocks. On each trial, a 550 ms fixation was followed by the word of "I" or "Friend" presented at the center of the screen as a person cue for 150 ms. Then, a trait adjective describing the person was presented for 350 ms in the center of the screen subtending 1° of the visual angle. After a 50 ms delay, a target letter "X/O" appeared at the upper or lower position of the screen relative to the fixation, 2.3° vertically from the center of the display. The target letter was presented for 2000 ms as the longest duration waiting for responses (**Figure 1**). Participants were asked to think about the trait descriptions for the cued person and judge the target was X or O by pressing one of the two buttons using their right hand when the letter appeared. Both accuracy and speed were instructed. The target (X or O) and its position were balanced within subjects and the button press was balanced across subjects.

In this paradigm, attention orientation speeds up the responses to targets at positions congruent with the spatial association of the concepts and slows down the responses to targets at incongruent positions (Meier and Robinson, 2004; Meier et al., 2007; Estes et al., 2008). Thus, in current study, the expected downward attention orientation should be defined as the differential RTs to higher targets and lower targets after self-reflection.

Questionnaires

According to our hypothesis, the attention orientation effect was rooted in the psychological status one holds of the self within the social context. We therefore measured the socioeconomic status (SES) of participants, as an index of subjective social status, and measured self-construal as an index of the sensitivity to social information. SES were measured using the subjective SES scale (Adler et al., 2000) with the drawing of a ladder of 10 rungs. People at the higher position of the ladder have more money, higher level of education, and better jobs than people at the lower position of the ladder. Participants were asked to indicate the position they feel they stand on the ladder. Self-construals were measured using the Self-Construal Scale (SCS, Singelis, 1994), which consists of 24-items that assess independent and interdependent self-construals, on a 7-point Likert scale (1 = *strongly disagree*, 7 = *strongly agree*). In addition, we also asked participants to rate to what extent they were familiar with, and similar to the friend, as well as how much they liked this friend, on 8-point scales (1 = *not at all*, 8 = *very much*).

Results

Inaccurate trials in the X/O discrimination task were removed from data analysis, and the reaction times (RTs) were log-transformed to normalize distribution (Ratcliff, 1993). Trials that were 2.5 SDs below or above the mean RTs for each subject were also removed from data analysis (similar to Meier and Robinson, 2004). The mean accuracy was high (95.94%) and the average removal rate was 6.19%.

The mean RTs and SDs are reported in **Table 1** and the log-transformed RTs were subjected to a 2 (Person: self vs. friend) × 2 (Valence: positive vs. negative) × 2 (Position: up vs. down) repeated measures analysis of variance (ANOVA). The main effect of Position was significant [$F(1,41) = 19.265$, $p < 0.001$, $\eta^2 = 0.320$], with faster responses to the targets at the lower position than those at the upper position ($p < 0.001$). This effect of Position was significant for both self ($t(41) = 2.440$, $p = 0.019$, $d = 0.379$, 95% confidence interval of the difference (CI) = [−0.0243, −0.0090]), and friend ($t(41) = 4.393$, $p < 0.001$, $d = 0.678$, 95% CI = [−0.0170, −0.0016]; **Figure 2A**). The differential RTs to targets at up vs. down positions for individual subjects are demonstrated in **Supplementary Figure S1**. However, other main effects or interactions were not significant [Person: $F(1,41) = 0.201$, $p = 0.656$, $\eta^2 = 0.005$; Valence: $F(1,41) = 0.167$, $p = 0.685$, $\eta^2 = 0.004$]; Person × Valence: $F(1,41) = 0.092$, $p = 0.763$, $\eta^2 = 0.002$; Person × Position: $F(1,41) = 1.972$, $p = 0.168$, $\eta^2 = 0.046$, **Figure 1**; Position × Valence: $F(1,41) = 1.492$, $p = 0.229$, $\eta^2 = 0.035$; Person × Valence × Position: $F(1,41) = 2.929$, $p = 0.095$, $\eta^2 = 0.067$). These results suggest downward attention orientation after both self- and friend-reflection. The attention bias of self was consistent with our hypothesis, however, the similar effect of the friend was unexpected. One possibility of the similar effect of friend-reflection was the "friend" was regarded as part of the self, thus similar attention orientation to that seen in the "self" condition was observed. We noticed that friend-reflection induced downward attention orientation ($lgRT_{up}$-$lgRT_{down}$) was positively correlated with the likeability scores of the friend [$r(41) = 3.63$, $p = 0.020$; **Figure 2B**], that is, the more an individual liked the friend, the stronger attention orientation effect was induced by friend-reflection. Additionally, we checked the self-construals of the participants. The self-construal scores were higher in interdependent than in independent subscales (interdependent: 61.45 ± 7.34, independent: 54.93 ± 7.18, $t(41) = 5.471$, $p < 0.001$, $d = 0.844$, 95% CI = [4.116, 8.932]). It is well known that interdependent individuals tend to regard a friend or close other as a part of the self (Markus and Kitayama, 1991). Thus, these results suggest that the attention orientation effect observed following friend-reflection relies on the attitude of the individual (i.e., likeability) toward the friend, and

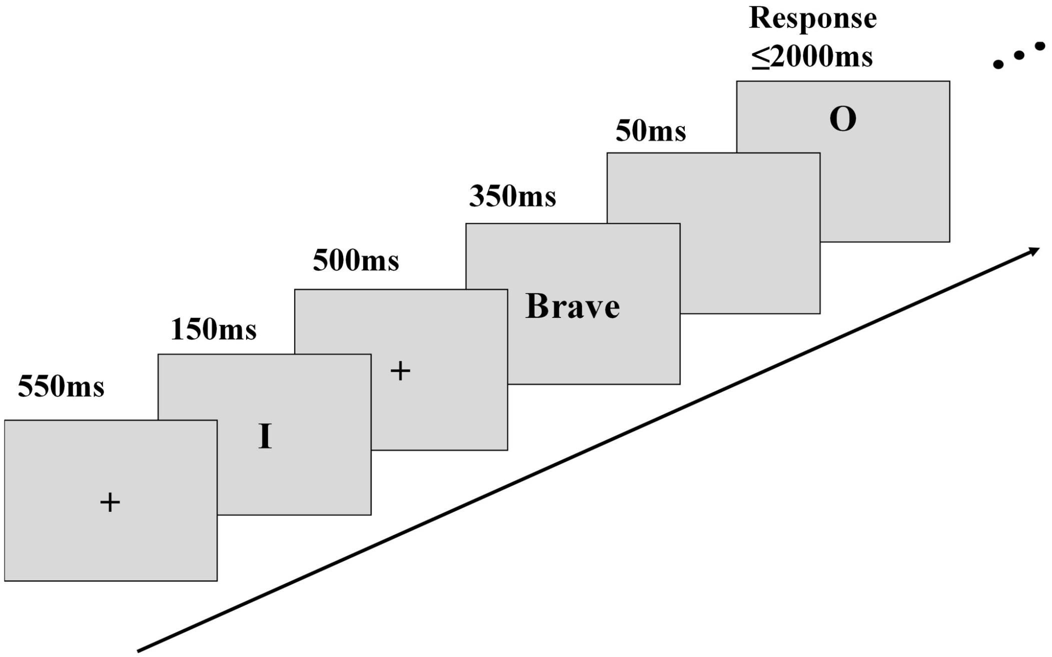

FIGURE 1 | Illustration of experimental procedure of the attention orientation task.

possibly, this attitude is rooted in the individual's self-construal. The possible confounding of a general attention facilitation to lower positions is more thoroughly addressed in Study 2.

Other ratings or questionnaires were not correlated with self- or friend-reflection induced attentional orientation ($ps > 0.1$).

Identical analyses were conducted on accuracy data. The 2 × 2 × 2 ANOVA did not show any significant main effects or interactions ($ps > 0.1$). The accuracy results suggest that there was no speed-accuracy trade-off for the faster responses to targets appearing at the lower position.

Discussion

As expected, we found self-reflection resulted in faster responses to targets that appeared at a lower position compared with responses to targets at higher position, suggesting a downward attention bias after self-reflection. However, a similar effect was also found after friend-reflection. There were two possibilities to explain these results. First, it is possible that the attention orientation was induced by general trait evaluation or just a facilitation effect on the targets appearing at the lower position. A second possibility is that the attention orientation was specifically related to self-concept. In Eastern cultures, friends are often considered as part of the self (Markus and Kitayama, 1991), thus resulting in the similar effect observed between the reflection on self and the friend. Here, we prefer the latter possibility, as there was evidence showing that self-enhancement could extend to others who are construed as part of self. For example, Gardner et al. (2002) manipulated self-construals and found that, when participants were primed to hold an expanded sense of self that included close others, they expected better performance for their friend as well as for themselves. This means that, if our participants regarded the friend as part of the self, they would also hold a positive view of their friend. Thus, it is reasonable to show similar attention orientation after friend-reflection. Our participants were more interdependent than independent in self-construals, and evidence that individuals who liked their friend more showed stronger attentional bias, supports this possibility. To explore this further, in Study 2 we replaced the friend with a disliked other to test whether the downward attention bias is specific to the self, or instead, general to all persons.

Additionally, we did not find correlations between attention orientation effects and the social status measured with the SES scale. The SES scale contains only one question (indicating a position they stand on the ladder) integrating information regarding money, education, and jobs, but with no evaluation of personality traits. Since the positive bias of self typically refers to an above-average view of personality traits (Beer and Hughes, 2010), we inferred that the SES scale may not be a sensitive index of positive self-view within the social context. Therefore, in Study 2, we adopted the above-average paradigm to measure the subjective social status considering personality traits, and correlated the above-average self-view with the attention orientation effect following self-reflection.

STUDY 2

In Study 1, although we observed the expected downward attention orientation after self-reflection, the similar results of the friend condition confounded the effects with the possibility

TABLE 1 | Mean RTs and SDs in the attention orientation task.

Mean (*SD*) (ms)		**Self-positive**	**Self-negative**	**Friend/disliked positive**	**Friend/disliked negative**
Study 1	Up	505.42 (88.32)	509.70 (85.95)	513.63 (97.88)	524.40 (110.81)
	Down	492.41 (76.59)	500.25 (92.03)	505.96 (116.97)	496.64 (93.74)
Study 2	Up	517.45 (81.83)	552.93 (92.88)	521.62 (92.20)	518.73 (68.97)
	Down	502.96 (76.38)	539.28 (87.73)	522.93 (116.79)	521.27 (73.07)

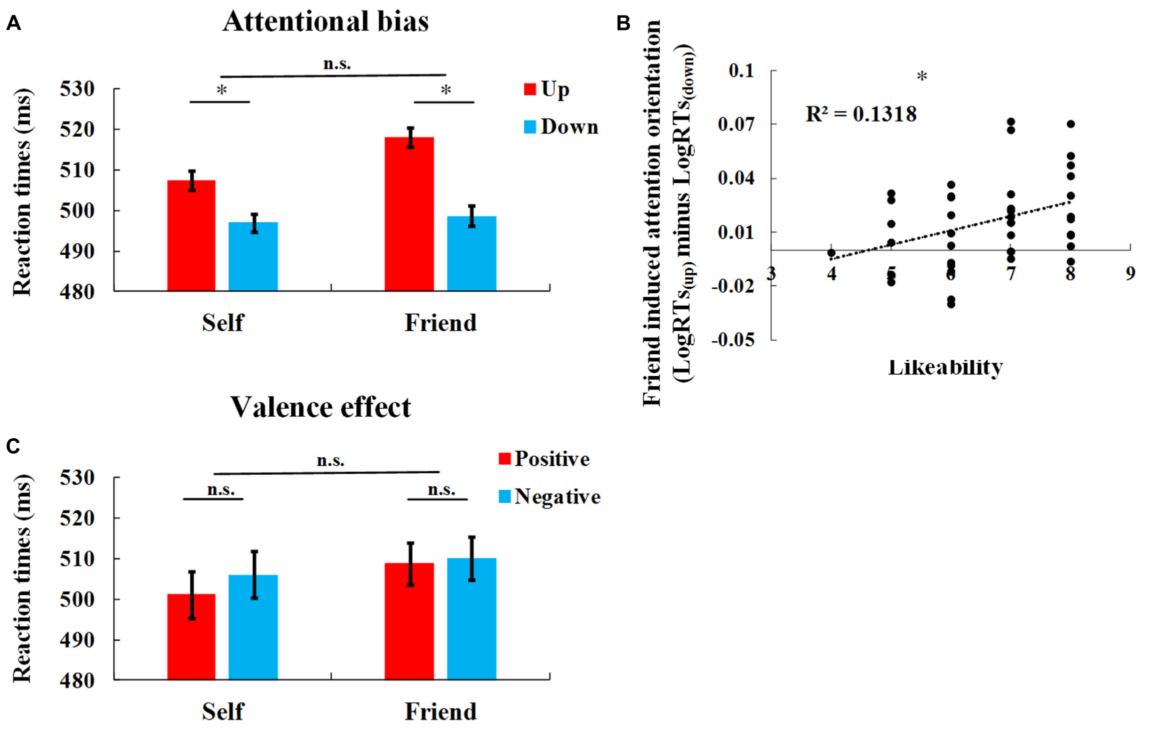

FIGURE 2 | Results of study 1. **(A)** RTs to targets in different conditions. The RTs are reported in raw millisecond values to facilitate comprehension. Error bars represent within-subject standard error of the mean. **(B)** Correlation between downward attention orientation effect ($lgRT_{up} - lgRT_{down}$) after friend-reflection and the likeability of the friend. **(C)** RTs to targets after positive and negation reflection. The RTs are reported in raw millisecond values to facilitate comprehension. Error bars represent within-subject standard error of the mean. $^{*}p < 0.05$.

that the attention orientation was a general facilitation effect on targets at lower positions. To rule out this confound, we conducted Study 2. In this study, a disliked other was compared with the self to test whether the downward attention orientation is general to all persons or self-specific. Moreover, theoretically, we inferred that the downward attention orientation was due to the automatic tendency of self-enhancement during social comparison. Therefore, in Study 2, we also measured self-enhancement effects, using the above-average paradigm, to test whether downward attention orientation after self-reflection were correlated with above-average self-view.

Methods

Participants

Forty-two adults participated in this study for monetary compensation. Gender and age were matched to the participants in Study 1. One participant was deleted because of low accuracy (6.25%), resulting in 41 total participants (19 males, mean age = 21.24 years, *SD* = 2.23 years) included in data analysis. One participant was left-handed, and all others were right-handed, and all had normal or corrected-to-normal vision. Written informed consent was obtained prior to participation.

Stimuli and Procedure

Attention Orientation Task

The stimuli and procedure were identical to Study 1, except that a "disliked" other was used as a control. The disliked other was defined as a gender-matched person, known to the participant in daily life, but whom the participant did not like. For example, the participant may disagree with his or her ideas or behaviors. The cue word presented to indicate the disliked person was "he/she."

Above-Average Measurement

To test whether downward attention bias is related to the psychological status or position one possesses within their specific social environment, we also measured the subjective status of participants, using the above-average paradigm (Beer and Hughes, 2010). Forty-eight positive trait adjectives were selected from the personality trait adjective pool (Liu, 1990). The participants were asked to judge, in relation to the average students in their university, to what extent they were above or below the average on each personality trait. An 8-point scale was used for the judgments, from *much lower* to *much higher*. Each adjective was presented on the screen until participants made their judgments. The inter-trial fixation was presented for 500 ms.

Questionnaires

The questionnaires (i.e., SES, SCS) used in Study 1 were also used in Study 2.

Results

Attention Orientation Task

Similar to Study 1, inaccurate trials in the target discrimination task were removed from data analysis, and the RTs were log-transformed to normalize distribution (Ratcliff, 1993). Trials that were 2.5 SDs below or above the mean RTs for each subject were also removed from analysis. The mean accuracy was high (95.46%) and the average removal rate was 6.63%.

The mean RTs and SDs are reported in **Table 1**, and the log-transformed RTs were subjected to a 2 (Person: self vs. disliked) × 2 (Valence: positive vs. negative) × 2 (Position: higher vs. lower) repeated measures ANOVA. The main effect of Valence was significant [$F(1,40) = 5.208$, $p = 0.028$, $\eta^2 = 0.115$], with faster responses to the target after positive adjectives. The main effect of Person was not significant [$F(1,40) = 0.608$, $p = 0.440$, $\eta^2 = 0.015$]. The main effect of Position was marginally significant with the tendency of faster responses to lower targets than higher targets [$F(1,40) = 3.854$, $p = 0.057$, $\eta^2 = 0.088$]. The Person × Position interaction was significant [$F(1,40) = 5.155$, $p = 0.029$, $\eta^2 = 0.114$]. A simple effect analysis showed that, after self-reflection, the RTs to lower targets were faster than to higher targets ($t(40) = -3.318$, $p = 0.002$, $d = 0.520$, 95% CI = [−.0194, −0.0047]), while for the disliked other, there was no difference ($t(40) = 0.028$, $p = 0.978$, $d = .004$, 95% CI = [−0.0078, 0.0080]; **Figure 3A**). The differential RTs to targets at up vs. down positions for individual subjects are demonstrated in **Supplementary Figure S1**. These results suggest a downward attention orientation after self-reflection, but not after disliked other-reflection. In addition, we also found a significant interaction between Person and Valence [$F(1,40) = 5.253$, $p = 0.027$, $\eta^2 = 0.116$]. A simple effect analysis showed that, in the self condition, RTs to targets after positive adjectives were faster than after negative adjectives ($t(40) = -3.178$, $p = 0.003$, $d = 0.496$, 95% CI = [0.0107, 0.0480]), while this difference in the disliked other condition was not significant ($t(40) = -233$, $p = 0.817$, $d = 0.037$, 95% CI = [−0.0158, 0.0199]; **Figure 3C**). This interaction suggests a positive bias of self-reflection on the discrimination task. However, neither the interaction between Valence and Position [$F(1,40) = 0.129$, $p = 0.721$, $\eta^2 = 0.003$], nor the three-way interaction of Person, Valence, and Position [$F(1,40) = 0.016$, $p = 0.901$, $\eta^2 < 0.001$] was significant.

Identical analyses were conducted on accuracy data. The 2 × 2 × 2 ANOVA showed significant main effect of Position [$F(1,40) = 7.669$, $p = 0.008$, $\eta^2 = 0.161$] with higher accuracy for higher position than lower position. However, other main effects or interactions were not significant (ps > 0.1).

Above-Average Measurement

Participants showed a significant above-average effect, which was consistent with previous findings (Lee et al., 2010). Subjects thought they were better than the average level of the students at their university on the personality traits presented ($t(40) = 9.495$, $p < 0.001$, $d = 1.483$, 95% CI = [0.7036, 1.084]).

Correlations

Moreover, the downward attention orientation effect ($\mathrm{lgRT}_{\mathrm{up}}$-$\mathrm{lgRT}_{\mathrm{down}}$) after self-reflection were positively correlated with the above-average scores [$r(41) = 0.315$, $p = 0.045$; **Figure 3B**]. In addition, we also calculated the positive bias of self-reflection, using RTs to targets after presentation of negative adjectives minus those recorded after positive adjectives ($\mathrm{lgRT}_{\mathrm{negative}}$-$\mathrm{lgRT}_{\mathrm{positive}}$) in the self condition, and found that the positive bias of self-reflection was positively correlated with SES of the individual [$r(41) = 0.330$, $p = 0.035$; **Figure 3B**]. These correlation results suggest that the downward attention orientation after self-reflection linked with above-average self-view on personality traits. While the SES of the self was sensitive to the valence of the reflection.

Although the interaction between Valence and Position was not significant, the valence of the reflection modulated the association between the interdependence of individuals and the attentional bias. Interdependence was defined by the difference between the sum score of the 12 interdependent items and the sum score of the 12 independent items. Higher difference scores indicated greater interdependent cultural orientation (Ma et al., 2014). After positive self-reflection, individuals with higher interdependence showed stronger downward attention orientation effects [$r(41) = 0.423$, $p = 0.006$], while after negative self-reflection, the correlation was not significant and showed a negative tendency [$r(41) = -0.199$, $p = 0.213$]. Fisher-z transformation confirmed the correlations were significantly different ($z = 2.85$, $p = 0.004$).

Discussion

The results of self-reflection in Study 2 replicated the results observed in Study 1, specifically that thinking about the self showed downward attention bias to targets appeared at a lower position. Through using a disliked other as a control, we clarified that the downward attention orientation effect specifically occurred after self-reflection, but not after disliked other-reflection. These results exclude the possibility that the downward attention orientation is general attention bias to targets appeared at lower position, or attention orientation after trait evaluation on all persons. Moreover, we found that individuals who regarded themselves as above average also showed stronger attentional bias after self-reflection, suggesting that the mechanism of the downward attention orientation after self-reflection is the psychological status within the social

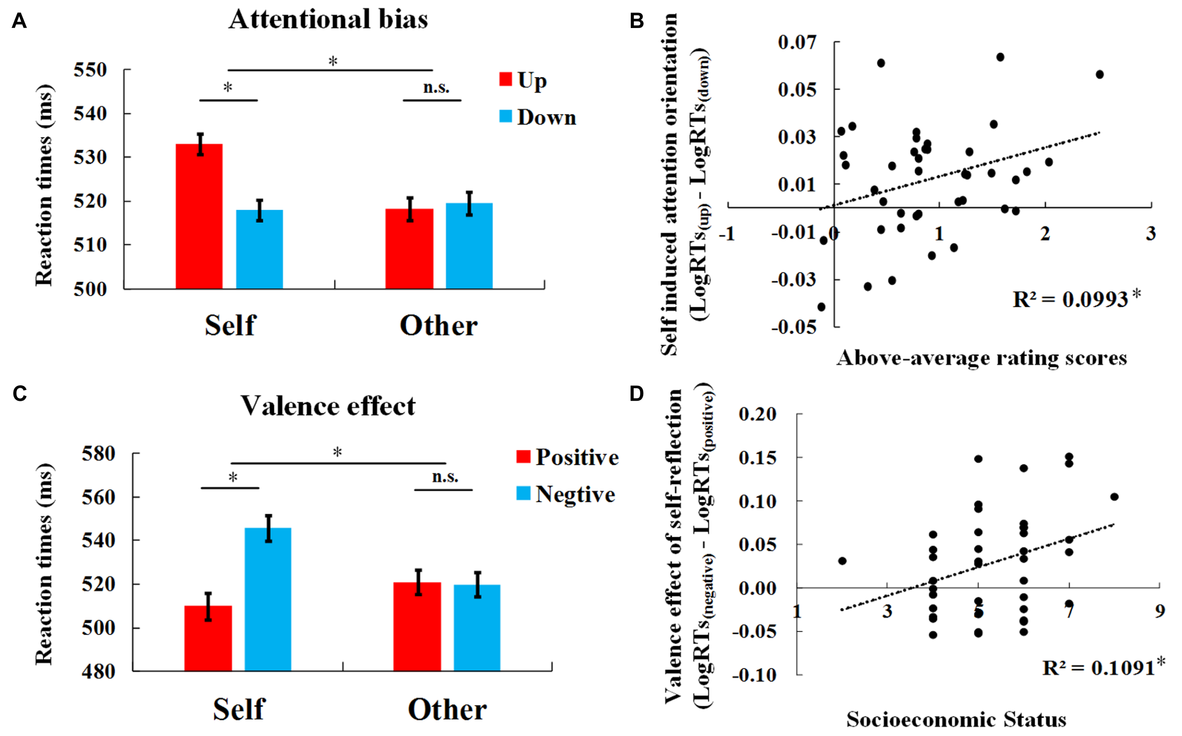

FIGURE 3 | Results of Study 2. **(A)** RTs to targets at up and down positions after reflection. The RTs are reported in raw millisecond values to facilitate comprehension. Error bars represent within-subject standard error of the mean. **(B)** Correlation between downward attention orientation effects ($\lg RT_{up} - \lg RT_{down}$) after self-reflection and above-average scores. **(C)** RTs to targets after positive and negation reflection. The RTs are reported in raw millisecond values to facilitate comprehension. Error bars represent within-subject standard error of the mean. **(D)** Correlation between valence effect of self-reflection ($\lg RT_{negative} - \lg RT_{positive}$) and subjective SES. *$p < 0.05$.

environment. These results were consistent with our hypothesis that thinking about the self would direct visual attention downward, and this effect was associated with a positive self-view.

Apart from the attention bias, we also found a positive bias of self-reflection. That is, positive self-evaluation resulted in faster responses than negative self-evaluation in the following target discrimination task, regardless of the targets' position. Meier et al. (2004) showed an association between brightness and evaluation of objects (i.e., bright objects are good, whereas dark objects are bad). Consistent evaluations (e.g., good–white) speeded responses, while inconsistent evaluations (e.g., bad–white) delayed responses, suggesting that evaluations could activate perceptual cues (i.e., brightness variations). People have an automatic association between the self and positivity, known as the self-enhancement or self-serving bias (Alicke, 1985; Taylor and Brown, 1988; Chambers and Windschitl, 2004). Thus, it was reasonable that an inconsistent description (e.g., self-negative) induced conflict, which interfered the discrimination task. While the consistent pairs (e.g., self-positive) required less mental resources, making it easier to respond to targets. For others, there are no specific associations of other-positive or other-negative. Therefore, no valence effect was found after other-reflection. Moreover, it was found that the positive bias of self-reflection was stronger for individuals with higher SES. This correlation suggests that individuals with higher SES possess a stronger positive association of the self than individuals with lower SES. This finding is consistent with the results that individuals with higher SES, especially young adults, reported higher self-esteem (Twenge and Campbell, 2002). However, the valence effect in Study 1 was not significant (*ps* > 0.1), but displayed similar patterns for both self and friend (i.e., faster responses to targets after positive traits than negative traits, **Figure 2C**). One possible reason for the increased valence effect in Study 2 compared to Study 1 was the presence of the disliked other. A disliked other may have served as a potential threat that increased the motivation to defend from negative self-evaluation. When participants were only evaluating themselves and/or their friend, the salience of valence information may have decreased, resulting in the null valence effect observed in Study 1.

In addition, the downward attention orientation effects after self-reflection of positive traits were stronger for individuals with higher interdependence. However, this association tended to be reversed after self-reflection of negative traits. For the role of the interdependent self-construal, there is evidence that the effect of social influences on the self-concept are stronger in individuals with interdependent self-construals rather than independent self-construals (Liew et al., 2011). Thus, when thinking about self, especially when considering positive traits, individuals with higher interdependence were more likely to activate a high social status of the self relative to others, which resulted in stronger attentional bias. However, for negative self-reflection, participants were more likely to perceive the negative self-description as a kind of self-threat for interdependent individuals (Park and Kitayama, 2014), under which the positive self-view was reduced, resulting in a weaker attentional bias.

GENERAL DISCUSSION

Our results in Studies 1 and 2 demonstrated that self-reflection directed visuospatial attention downward, which were consistent with our hypothesis. In Study 1, similar attention orientation effects were observed in both friend- and self-reflection. While in Study 2, reflection on a disliked other showed a null effect of attentional orientation, which ruled out the possibility that the downward attention orientation after self-reflection was a general facilitation to lower targets. Of note, the attention orientation effects observed after self-reflection were stronger for individuals that thought of themselves, to a large extent, above average.

The consistent effects of self-reflection in Studies 1 and 2 suggest that ones' self-concept contains vertical spatial information. It might be that the "above-average" self-schema associated self-concept with a higher status relative to others, as a type of self-enhancement to maintain self-esteem (Heine et al., 1999). In a previous work, a new learned association of the self and a perceptual symbol (a geometric shape) enhanced sensitivity to the self-relevant stimuli in a perceptual matching task (Sui et al., 2012). In our study, the association of self-concept and high status is deep-seated and learned over long periods of time. Thus, according to the PSS theory (Barsalou, 1999), it was reasonable that reminding of the self activated the higher social status, which is a spatial symbol of the self. With others below the self as the frame of reference, the high position one possesses resulted in downward attention orientation to see others below the self, which would likely strengthen the sense of pride. In addition, the null results of the disliked other clarified that the downward attention orientation effect was not a general facilitation effect to targets appearing at the lower position. Self-reference effect demonstrated self-reflection could affect memory (Rogers et al., 1977; Klein and Kihlstrom, 1986). Our results provide first evidence that the effect of self-reflection on personality could extend to attention, which enriches our understanding of the superiority of self.

However, the attention effects of other-reflection in Studies 1 and 2 were different. The downward attention orientation occurred after friend-reflection (Study 1), but not after disliked other-reflection (Study 2). One conceptual difference between a friend and a disliked other is that a friend may be considered part of the self for individuals with an interdependent self-construal, while the disliked other is decidedly considered a separate entity (Markus and Kitayama, 1991). Since the results of self-reflection suggested an association between downward orientation of attention and self-enhancement, and since this self-enhancement could extend to close others construed as part of the self (Gardner et al., 2002), it was not surprising that reflecting upon a close other (i.e., friend) showed similar effects as self-reflection. We noticed that our participants were more interdependent than independent, and the more that participants liked their friend, the stronger the attention orientation effect that they exhibited. These findings support the inference that the friend-reflection directed visual attention downward because the friend was regarded as part of the self. Our participants were all from an Eastern culture (i.e., China). It is possible that participants from a Western culture, who do not necessarily consider friends as part of self, may not exhibit the same downward attention orientation effect after friend-reflection.

Taking the cultural orientation or self-construal into account, we speculate the self-related attention orientation would show some culture differences. As is stated above, the attention effect of the self depends on two factors: ones' positive self-view and the sensitivity to social context. The downward attention orientation will occur only when the positive self-view (e.g., I am clever) was held in social comparison (e.g., I'm cleverer than others). It is well known that the positive self-view and/or self-enhancement is more typical in Western cultures than Eastern cultures (Gardner et al., 2002). However, there has also been some evidence that individuals in Western culture are less sensitive to social influences, i.e., they are less likely to compare themselves with others to define the self (Markus and Kitayama, 1991; Heine et al., 1999; Liew et al., 2011). Thus, the culture difference of the self-related attention orientation depends on which factor, i.e., positive self-view or sensitivity to social context, dominants the effects. Future research is needed to investigate this issue in more depth.

Although the downward attention orientation effects after self-reflection were found consistently in Studies 1 and 2, we noticed that the differential RTs to up and down targets were quite small (9–15 ms). The small differences of RTs were also shown in previous work using the target discrimination paradigm. For example, in Chasteen et al. (2010), God/Devil facilitated target discrimination in up/down (vs. down/up) position. The differential RT (up minus down) was 14 ms in God condition and −5 ms in Devil condition. There were also some studies showed relatively larger differential RTs. For example, Estes et al. (2008) showed head/foot related words facilitated target discrimination in up/down position (vs. down/up position). The size of the difference was about 40 ms. According to the PSS theory (Barsalou, 1999), the attention orientation effect depends on the association of the concept and its spatial information. Head/foot are concrete concepts explicitly associated with up/down positions, while god/devil and self/other are abstract concepts only implicitly associated with up/down metaphors. Since the association of self-concept and higher position one possesses is not quite tight, i.e., depends one cultural orientation and social context, it was not surprising that the attention orientation effect after self-reflection was small, and was not found for all subjects.

According to previous findings, one thing that should be addressed is whether the attention orientation effect observed was manifestations of facilitation (Meier and Robinson, 2004; Chasteen et al., 2010; Zanolie et al., 2012) or interference (Bergen et al., 2007; Estes et al., 2008). The two types of RTs to targets at the compatible locations were shorter or longer, than at the incompatible location. If the effects can be conceptualized as interference, and not facilitation, the faster responses to targets at the lower position after self-reflection might indicate an upward attention orientation. Gozli et al. (2013) clarified that interference would not occur unless the procedure met two conditions. The first is the use of multiple concept categories (e.g., clothing, house, animals). Multiple categories might lead to a between-trial category switching cost, and might prevent stimulus-response mappings between a subset of the category and a target location. The second is the use of short cue–target stimulus onset asynchrony (SOA; 150–350 ms). In the current study, we used only trait adjectives as a single concept category, and the self and other conditions were in different blocks, with a 10 s interval to prevent unwanted category switching. We also used a long SOA, with the time from the cue word reminding self or others to the target appearing being 1050 ms. Therefore, we believe that the results observed were facilitation effects, not interference. That is, the faster responses to lower targets after self-reflection suggest a downward attention orientation, which was consistent with our hypothesis.

One may argue that the valence of the person might drive the attention effects, i.e., reflection on a positive person (self and friend) induced downward attention orientation effect, while reflection on a negative person (disliked other) showed null effect. The affective valence of a person is individuals' subjective attitudes to the self and others. Actually, the positive attitude to the self is just what we called "positive self-view" or self-enhancement, which is the theoretical bases for the attention effect we found. One limitation of current study is we did not include a neural person as control. The friend could be regarded as part of self and share the positive association with the self, while the disliked other is obviously negative to the self. Therefore, whether a neutral person could induce downward attention orientation needs to be tested in future research. Another direction of future research is investigating how to induce the attention orientation by priming the positive or negative view of self or others. Resent work of "good true self" proposes that people tend to believe that every person (both self and others) is motivated to behave in morally good ways (Newman et al., 2015; De Freitas et al., 2017a). This positive belief ties to moral essence of a person and is consistent across cultures (De Freitas et al., 2017b). In future research, we could use morally good events to induce the positive belief about others, and test whether the belief of "good true self" could result in attention orientation.

CONCLUSION

Our study demonstrated the visuospatial influence of self-reflection, provided the first evidence that thinking about the self directs attention in a downward fashion, and that this attentional bias was associated with an above-average self-evaluation.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Shenzhen University with written informed consent from all subjects. The protocol was approved by the ethics committee of Shenzhen University.

AUTHOR CONTRIBUTIONS

YL collected and analyzed data, wrote the paper. YT provided theoritical bases of the study and revised the manuscript. HL paid for the study and revised the manuscript.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2017.01506/full#supplementary-material

FIGURE S1 | Attention orientation effect of self- and other-reflection for each participant in Study 1 and Study 2.

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