NeuroImage 202 (2019) 116068

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Thoughts of death affect reward learning by modulating salience network activity

Siyang Luo [b,**], Bing Wu [c], Xiaoyue Fan [a], Yiyi Zhu [b], Xinhuai Wu [c,***], Shihui Han [a,*]

[a] School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University, Beijing, China

[b] Department of Psychology, Guangdong Key Laboratory of Social Cognitive Neuroscience and Mental Health, Guangdong Provincial Key Laboratory of Brain Function and Disease, Sun Yat-Sen University, Guangzhou, 510006, China

[c] Department of Radiology, The 7th Medical Center of PLA General Hospital, Beijing, China

ARTICLE INFO

Keywords:
fMRI
Mortality salience
Resting state
Reward learning
Salience network

ABSTRACT

Thoughts of death substantially influence human behavior and psychological well-being. A large number of behavioral studies have shown evidence that asking individuals to think about death or mortality salience leads to significant changes of their behaviors. These findings support the well-known terror management theory to account for the psychological mechanisms of existential anxiety. However, despite increasing findings of mortality salience effects on human behavior, how the brain responds to reminders of mortality and changes the activity underlying subsequent behavior remains poorly understood. By scanning healthy adults (N = 80) of both sexes using functional magnetic resonance imaging, we showed that, relative to reading emotionally neutral sentences, reading sentences that evoke death-related thoughts decreased the salience network activity, reduced the connectivity between the cingulate cortex and other brain regions during a subsequent resting state, and dampened the speed of learning reward-related objects and cingulate responses to loss feedback during a subsequent reward learning task. In addition, the decreased resting-state cingulate connectivity mediated the association between salience network deactivations in response to reminders of mortality and suppressed cingulate responses to loss feedback. Finally, the suppressed cingulate responses to loss feedback further predicted the dampened speed of reward learning. Our findings demonstrate sequential modulations of the salience network activity by mortality salience, which provide a neural basis for understanding human behavior under mortality threat.

1. Introduction

Thoughts of death occur early in human development (Kenyon, 2001) but counteract our overwhelming desire to survive. Behavioral studies have revealed remarkable influences of reminders of death or mortality salience (MS) on cognition, emotion, and behavior (Burke et al., 2010; Juhl and Routledge, 2016). These behavioral findings support the terror management theory (Greenberg et al., 1986; Rosenblatt et al., 1989; Pyszczynski et al., 1999), which posits that the conflict between awareness of mortality and desire to survive leads to existential anxiety and that cultural worldview and self-esteem provide two buffers of existential anxiety. Despite increasing findings related to MS effects on behavior and related psychological accounts, the neural underpinnings of MS influences on behavior remain poorly understood. In a real-life situation, an individual could be reminded of mortality by a specific event (e.g., being informed of fatal sickness) and, after a period of time, may change his/her decision making and behavior substantially. How does the brain respond to reminders of mortality. How do reminders of mortality affect subsequent resting-state activity? Does MS modulation of the resting state activity mediate the relationship between the initial reminder of mortality and later MS influences on neural activities engaged in other cognitive tasks? To address these questions is critical for understanding the neural mechanisms underlying MS influences on human behavior.

Recent behavioral research has revealed significant MS influences on behavioral responses to salient stimuli or events. For example, it has been reported that, relative to a control condition, MS derogated men's

* Corresponding author. School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Peking University, No. 52, Haidian Street, Beijing, 100080, China.

** Corresponding author. Department of Psychology, Sun Yat-Sen University, Guangzhou 510006, China.

*** Corresponding author. Department of Radiology, PLA Army General Hospital, 5 Nanmencang, Beijing, 100070, China.

E-mail addresses: ljc520ida@163.com (S. Luo), bei925@sina.com (X. Wu), shan@pku.edu.cn (S. Han).

https://doi.org/10.1016/j.neuroimage.2019.116068
Received 23 November 2018; Received in revised form 3 July 2019; Accepted 1 August 2019
Available online 7 August 2019
1053-8119/© 2019 Elsevier Inc. All rights reserved.

favorable views of advertisements featuring female sexuality (Lee et al., 2017). There is also evidence that MS decreased an impulsive approach tendency toward luxury brands (Audrin et al., 2018), although opposite effects have been reported in other studies in which high-status items (e.g., luxury cars) were evaluated more favorably by individuals who received MS priming than by control subjects (Mandel and Heine, 1999). These behavioral findings suggest possible modulations of brain activity in response to salient stimuli. Viewing both luxury brands and sexual stimuli has been associated with activations in the cingulate and anterior insular (AI) cortices (e.g., Arnow et al., 2002; Schaefer and Rotte, 2007), which are key nodes in the salience network (SN) and are densely connected with other brain regions (Van Den Heuvel and Sporns, 2011). The SN serves to integrate salient stimuli and interoceptive information to guide motivated behavior (Seeley et al., 2007; Uddin, 2015) and brain regions in the SN such as the insula have a major role in evoking associations between salient cues and approach behavior (Droutman et al., 2015). Given these brain imaging findings, one may suggest that some of the behavioral findings (e.g., Audrin et al., 2018; Lee et al., 2017) implicate that MS may weaken brain responses to salient stimuli to dampen relevant behavioral tendencies. In line with this proposal, early functional magnetic resonance imaging (fMRI) studies found that death-related words/sentences compared to words/sentences related to negative affect (NA) decreased activity in the anterior midcingulate (aMCC) and AI (Han et al., 2010; Shi and Han, 2013; Klackl et al., 2013), though the insular and cingulate responses to mortality threats varied based on individuals' self-esteem (Klackl et al., 2013) and cultural traits (Luo et al., 2017). Recent studies further showed that priming MS also decreased aMCC activity in response to others' pain (Luo et al., 2014) and the discrimination of ingroup/outgroup identity (Feng et al., 2017).

While these neuroimaging findings suggest that the SN is implicated in responses to MS, there has been no direct evidence for the potential link between modulations of SN activity and MS-induced behavioral changes. The current work tested the hypothesis that MS leads to sequential modulations of SN activity in response to reminders of mortality and during a subsequent resting state and a cognitive task. Moreover, we predicted that MS-induced sequential modulations of SN activity are associated with behavioral changes due to mortality threat. We tested these hypotheses by scanning healthy adults (N = 40), using fMRI, during MS priming (i.e., reading sentences that evoke death-related thoughts or neutral sentences), a subsequent resting state, and then, a probabilistic learning task in which monetary loss vs. win feedback upon choices of imperative stimuli activate the key nodes of the SN (Holroyd et al., 2004) (Fig. 1). We also scanned an independent group of participants (N = 40) during NA priming (i.e., reading sentences that evoke negative emotion unrelated to death or neutral sentences), a subsequent resting state, and then, the probabilistic learning task to control for the effects of negative emotion on brain activity and behavioral performance. We sought to determine whether MS priming changes behavioral performance during reward learning and whether such effects are related to MS-induced sequential modulations of SN activity. To address issues is of translational relevance because SN dysfunction is a prominent feature of a number of psychiatric and neurological disorders that are characterized by impaired detection and mapping of salient external stimuli and internal events (Menon, 2011).

2. Materials and methods

2.1. Participants

This study recruited 86 Chinese college students as paid volunteers. Half of the participants were randomly assigned to the MS group and half to the NA group. Six participants were excluded from data analyses due to head movements over 3 mm or 3° during fMRI scanning, leaving forty participants in the MS group (mean age = 21.45 ± 2.15 years, 18 males) and forty participants in the NA group (mean = 21.48 ± 2.20 years, 20 males) for data analyses. All participants were right-handed, had normal or corrected-to-normal vision, and reported no neurological diagnoses. A proper sample size for the present study was estimated using G*Power (Faul et al., 2007) prior to data collection. To detect a medium effect size for main effects of within-factors and between-within interactions with 95% power (f = 0.25, with alpha = 0.05, correlation among repeated measures = 0.30), a sample size of 38 per group was required. Informed consent was obtained prior to the experiment. This study was approved by the local ethics committee at the School of Psychological and Cognitive Sciences, Peking University.

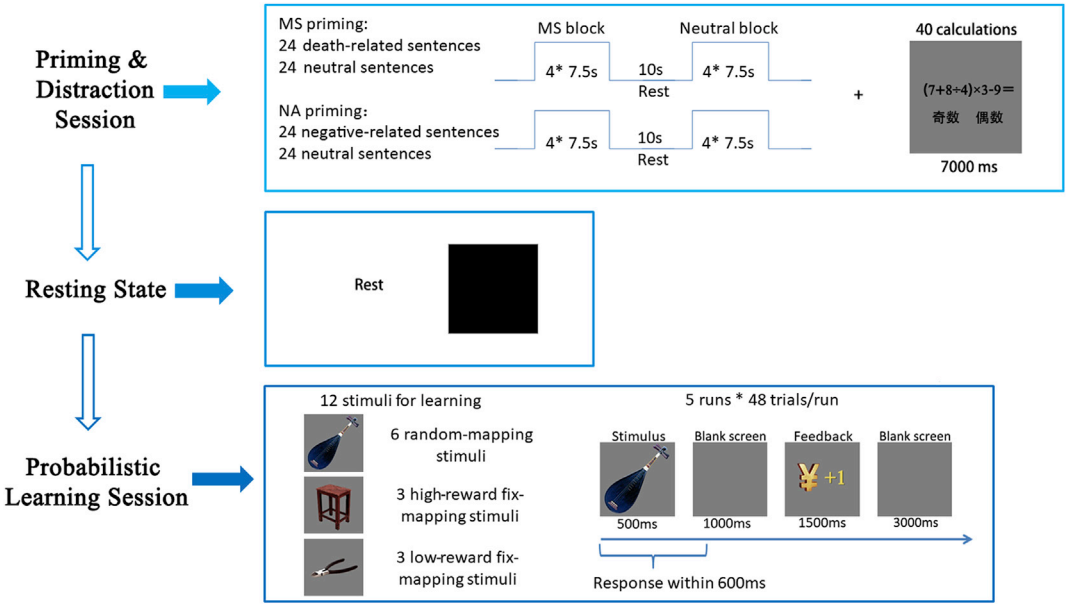

Fig. 1. Illustration of the experimental procedure of our study. The scanning procedure consisted of 3 sessions. During the first (priming) scanning session, participants were scanned while reading sentences evoking thoughts of death and neutral sentences (MS group) or reading sentences evoking negative affect and neutral sentences (NA group). These were followed by a distraction task in which participants had to finish 40 calculations. The second (resting state) scanning session followed and lasted for 5 min. During the third (learning) scanning session, participants had to learn reward-related objects according to monetary feedback.

2.2. Stimuli and procedure

Before fMRI scanning, participants completed questionnaires to assess their psychological traits related to death anxiety, including Templer's Death Anxiety Scale (Templer, 1970), Templer's Death Depression Scale (Templer et al., 2002), Life Orientation Test (a measure of optimism) (Scheier and Carver, 1985), Neuroticism subscale in Eysenck Personality Questionnaire (Eysenck and Eysenck, 1975), and Rosenberg Self-Esteem Scale (Rosenberg, 1965).

fMRI scanning consisted of three sessions (Fig. 1). The first (priming) session consisted of two 244 s functional scans and there were 6 blocks of 4 trials during each scan. Forty-eight statements similar to those used in the previous studies (Greenberg et al., 1990; Luo et al., 2014) were used for priming. MS group read 24 statements related to death (e.g. 'I won't feel terrible even if I would die lonely', 'My body would rot after death') for MS priming. NA group read 24 statements referred to negative but death-unrelated emotions (e.g. 'I feel anxious about my future life') and anxiety ('The coming exam makes me uneasy') for NA priming. Both MS and NA groups read 24 neutral statements (e.g., 'I would stay calm whatever happens', 'My body functions well') in the control condition. To estimate valence and arousal associated with MS, NA and neutral statements, we recruited an independent sample (n = 36, 8 male/28 female, mean age = 23.69 ± 5.67 years) to report subjective feelings evoked by each statement on a Likert 9-point scale (arousal: 1 = not at all, 9 = extremely strong; valence: 1 = extremely negative, 9 = extremely positive). The participants were asked to rate neutral statements first. The order of rating MS and NA statements was counterbalanced across participants. During fMRI scanning each statement was presented for 7 s, and participants responded to agree or disagree each statement by button press. There were 4 statements in each block (all death-related, all negative, or all neutral) and 2 statements were intervened by 0.5 s. There were 3 blocks of death-related statements and 3 blocks of neutral statements for the MS group. There were 3 blocks of negative statements and 3 blocks of neutral statements for the NA group. All blocks were presented in a random order in each scan.

After MS or NA priming, each participant conducted 40 calculations in 5 min. Participants had to judge whether each calculation would give an odd or even number by button press. Each calculation (e.g., 7 + 7 × 3–10 ÷ 2) was presented for 7 s, and two consecutive calculations were intervened with 0.5s. In a typical MS study, participants are asked to complete a distraction task between MS priming and a dependent measure that taps their distal death defenses. This delay/distraction between MS priming and a dependent measure was included to allow for thoughts of death to fade from consciousness, in keeping with terror management theory's contention that the distal death defenses occur only when thoughts of death take place subconsciously (Pyszczynski et al., 1999), which is supported by the findings that removal of this delay/distraction eliminates the effects of MS priming on following dependent measures (e.g., Greenberg et al., 2000). The calculation task employed in our work served as a delay between MS/NA induction and the measures of resting state activity and brain activity during reward learning.

The second (resting-state) scanning session was conducted after the calculation task. Participants were scanned for 5 min while they were instructed to hold still and keep their eyes closed, but not fall asleep or think of anything in particular.

The third (learning) scanning session consisted of five 300-s functional scans during which participants performed a probabilistic learning task (Holroyd et al., 2004). This task required responses to images of 12 imperative stimuli (e.g., a chair, a musical instrument) within 600 m s by pressing one of two buttons. Each response was followed by one of the three types of feedback stimuli (i.e., +¥1 (low reward) or +¥5 (high reward) for 'correct' feedback, -¥1 (low lost) or -¥5 (high lost) for 'incorrect' feedback, and a clock with -¥10 for 'too late' feedback if response times were longer than 600 m s, see Fig. 1). Participants were informed that each correct response would win 1 or 5 yuan and that each incorrect response would lose 1 or 5 yuan. To encourage fast responses during reward learning, participants were told that a late response would incur a penalty of 10 yuan. During the probabilistic learning task, 3 of the 12 imperative stimuli were mapped to the left button and 3 of the 12 imperative stimuli mapped to the right button in a similar fashion (fixed-mapping stimuli). Participants were rewarded if they pressed the correct button but were penalized if they pressed the wrong button when responding to these stimuli. For the remaining 6 stimuli, however, one of the three types of feedback was delivered randomly (random-mapping stimuli), regardless of the given response, to increase task difficulty. Consequently, participants were rewarded on 50% of the trials and penalized on 50% of the trials. Participants were not informed of the appropriate stimulus-response mappings and were instructed to respond using trial-and-error to maximize the total amount of money earned by the end of the experiment. They began the task with ¥10 as a bonus and were paid based on their performance at the end of the task (approximately ¥50). Each trial began with the presentation of a central fixation cross for 1 s, followed by the presentation of an imperative stimulus for 0.5 s, and then, a blank screen for 1 s. A feedback stimulus was then presented for 1.5 s, followed by a blank screen for 2 s. All stimuli were scaled to a uniform size so that they subtended approximately 5° x 5°, and were presented in color against a black visual display projected into the scanner. Participants were given instructions for the task and performed 24 trials for practice outside the scanner. There were 5 blocks of 48 trials during scanning and each of the 12 imperative stimuli was presented 20 times in a random order. A new set of six matching images were chosen for each participant, and only these six images were presented to them throughout the practice and experimental phases.

A manipulation check was conducted after the third scanning session. Participants were asked to rate their feelings about the priming task to assess their feelings of closeness to death, fear of death and unpleasantness during the priming session. A likert-type scale was used for ratings (0 indicated no effect and 10 indicated a maximal effect) on the following questions: How close do you feel to death after reading the statements and making judgments? How fearful do you feel about death after reading the statements and making judgments? How unpleasant do you feel after reading the statements and making judgments?

2.3. Imaging parameters

Brain images were acquired using a 3.0T GE Signa MR750 scanner (GE Healthcare; Waukesha, WI) with a standard head coil. Functional images were acquired using T2-weighted, gradient-echo, echo-planar imaging (EPI) sequences sensitive to BOLD contrast (64 × 64 × 32 matrix with 3.75 × 3.75 × 5 mm 3 spatial resolution, repetition time = 2000 m s, echo time = 30 m s, flip angle = 90°, field of view = 24 × 24 cm). A high-resolution T1-weighted structural image (512 × 512 × 180 matrix with a spatial resolution of 0.47 × 0.47 × 1.0 mm^3, repetition time = 8.204 m s, echo time = 3.22 m s, flip angle = 12°) was acquired before the priming procedures and functional scans.

2.4. Functional imaging analysis

2.4.1. Preprocessing

Functional images were preprocessed using SPM12 software (the Wellcome Trust Centre for Neuroimaging, London, UK). Functional scans were first corrected for within-scan acquisition time differences between slices and then realigned to the first volume to correct for inter-scan head motions. This realigning step provided a record of head motions within each fMRI run. Head movements were corrected within each run and six movement parameters (translation; x, y, z and rotation; pitch, roll, yaw) were extracted for further analysis in the statistical model. Participants with head movements over 3 mm or 3° were excluded from further data analyses. The anatomical image was coregistered with the mean realigned functional image and then was normalized to the standard Montreal Neurological Institute (MNI) template. The functional images were resampled to 2 × 2 × 2 mm^3 voxels, normalized to the MNI space using

the parameters of anatomical normalization and then spatially smoothed using an isotropic of 8 mm full-width half-maximum (FWHM) Gaussian kernel. After the preprocessing, we also checked the extent of each participant's head movements and whether images after normalization were matched with the template.

2.4.2. Whole-brain analysis

In the first level fixed effect analysis of priming-session fMRI data, the onsets and durations of each condition were modeled using a general linear model (GLM) for each participant. All conditions were included in the model (GLM1 in MS group: regressor 1: death-related statements; regressor 2: neutral statements; regressor 3–8: head motions; GLM2 in NA group: regressor 1: negative affect statements; regressor 2: neutral statements; regressor 3–8: head motions; each statement block lasted for 30 s). A box-car function was used to convolve with the canonical hemodynamic response in each condition. The contrasts between death-related (or negative) vs. neutral statements were calculated to define neural activities in response to MS (death-related statements vs. neutral statements) or NA (negative affect statements vs. neutral statements) priming. Random effect analyses were then conducted based on statistical parametric maps from each participant to allow population inference. We further conducted a whole-brain two-sample *t*-test (GLM3) to assess the group differences in priming effects on brain activity ((death-related vs. neutral statements) vs. (negative affect vs. neutral statements)).

We also conducted whole brain analyses to examine neural activities in response to positive and negative feedback during the learning session. We first conducted fixed effect analyses of fMRI data to estimate neural responses to feedback at each voxel and to compare regionally specific effects in each participant using linear contrasts. In this GLM, based on feedback, there were 4 regressors (regressor 1–4: large win: +¥5, small win: +¥1, large loss: ¥5, small loss: ¥1, the duration for each feedback was 1.5s; regressor 5–10: head motions). To define feedback specific neural activations, we calculated two contrasts (contrast 1: win vs. loss and contrast 2: loss vs. win). Random effect analyses were then conducted across all participants based on statistical parametric maps from each individual participant to allow population inference for these two contrasts (GLM4 and GLM5). Significant activations were defined in the whole brain analysis using a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$ (Family-wise error (FWE) corrected).

2.4.3. Functional connectivity analysis

The Data Processing Assistant for Resting-State fMRI (DPARSF) (Yan and Zang, 2010) was used for functional connectivity analyses. BOLD signals were extracted from 5-mm-radius spheres centered at the key nodes of the salience network (including the aMCC and bilateral insula) and of the frontal-parietal control network (including the bilateral lateral frontal cortex and left angular gyrus) which showed significant activations or deactivitions during MS (vs. neutral) priming (see MNI coordinates of these brain regions in Result section). The extracted time series were used to calculate Pearson's correlation coefficients to construct task-specific undirected and weighted functional connectivity matrices for each participant. We computed connectivity matrices as the Pearson correlation between the time series of each pair of nodes in the two networks.

To complement our connectivity analysis within a priori hypothesized networks, we conducted a secondary analysis that applied the same analysis pipeline using a whole-brain parcellation to examine the robustness of our results. This analysis included 264 brain regions that were previously assigned to 13 large-scale functional brain systems (Power et al., 2011). The functional connectivity within the salience network and between the salience network and other networks was then compared between the MS and NA groups.

To analyze the functional connectivity between the aMCC and regions in the reward network during the learning session, BOLD signals were extracted from 5-mm-radius spheres centered at the aMCC which showed decreased response to loss feedback in MS than NA group and the key nodes of the reward network (including the left ventral striatum, right ventral striatum, and ventral medial prefrontal cortex) which showed significant activations in response to win feedback across all participants (see MNI coordinates of these brain regions in Result section). The extracted time series were used to calculate Pearson's correlation coefficients to construct task-specific undirected and weighted functional connectivity.

2.4.4. Resting-state activity analysis

Prior to the preprocessing of resting-state session fMRI data, the first 10 vol of each participant's data were discarded to allow for scanner stabilization and for participants to adapt to the resting state. The waveform of each voxel was finally passed through a band-pass filter (0.01–0.08 Hz) to reduce low-frequency drift and high-frequency physiological noise. Resting-fMRI data preprocessing was conducted using DPARSF.

We employed a measure of nodal degree centrality to detect functional hubs in the voxel-wise functional networks. Degree centrality is one of the most popular graphical measures used to identify global hubs in the brain (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). In graph theory, the degree k for voxel i in the network is defined as:

$$k_i = \frac{1}{N-1}\sum_{j \neq i} a_{ij}$$

where a_{ij} represents the edge between voxel i and j, N is the total number of voxels in the group gray matter mask (Dai et al., 2012; Opsahl et al., 2010; Rubinov and Sporns, 2010). The degree centrality of a voxel indicates its average functional connectivity strength with all of the other voxels, and characterizes its influence in the network. We computed the weighted degree centralities of the brain networks (Wang et al., 2010). Significant activations were defined in the whole brain analysis using a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$ (FWE corrected).

2.5. Mediation analysis

Standard multiple regression and mediation analytic techniques (Baron and Kenny, 1986) were used to explore the relationship between MS priming, subjective feelings during the priming session, brain activities in response to the priming, topological properties during the resting state and brain activities during the probabilistic learning session. For each mediation analysis, three regression models were constructed. First, we regressed a dependent variable on an independent variable and demonstrated that the independent variable was correlated with the dependent variable. Second, we regressed the mediator on the independent variable and demonstrated that the independent variable was correlated with the mediator. Finally, we regressed the dependent variable on both the mediator and the independent variable to test whether the mediator affected the dependent variable and whether the effect of the independent variable on the dependent variable was significantly reduced when the mediator was included in the regression model. The robustness of the estimation of mediation was further tested using a specific bootstrap procedure (10,000 iterations) for mediation models (Preacher and Hayes, 2008). An observation of zero outside the 95% confidence interval would indicate that the indirect (or mediated) effect was significantly different from zero at the threshold of $p < 0.05$.

2.5.1. Moderation analysis

Hierarchical regression analyses were also conducted to examine whether positive mental state (Self-esteem, Optimism, Neuroticism, and Life satisfaction) moderates the relationship between MS/NA priming (IV) and individuals' degree centrality during the resting state (DV). The IV (Priming group) and the moderator (Self-esteem, Optimism, Neuroticism, or Life satisfaction) were normalized before the hierarchical

regression analysis. The MS and NA group were coded as a dichotomous dummy variable with 0 representing NA priming and 1 representing MS priming. The interactions between the priming group and each positive mental state were calculated by multiplying the normalized variables (Aiken et al., 1991). The normalized priming group, positive mental state and their interactions were then sequentially entered into the hierarchical regression. The moderator effect was indicated by a significant interaction of priming group and positive mental state on individuals' degree centrality. As the result revealed a significant moderator effect of positive mental state on the degree centrality, we divided participants into MS and NA groups. We further conducted post hoc regression analyses to examine the association between positive mental state and degree centrality for MS and NA groups, respectively.

3. Results

3.1. Valence and arousal estimation

Rating scores of valence and arousal related to the statements in each condition were subject to an analysis of variance (ANOVA), which revealed a significant main effect of statements (MS, NA, neutral) on rating scores of valence ($F(2,68) = 21.84$, $p < 0.001$). Post hoc tests showed that rating scores of valence were significantly lower for MS statements (4.51 ± 0.88) and NA statements (4.30 ± 0.91) compared to neutral statements (5.64 ± 1.03, both $p < 0.001$; all post hoc tests were applied with false discovery rate (FDR) correction). However, rating scores of valence did not differ significantly between MS and NA statements ($p = 0.94$). Similarly, there was a significant main effect of statements (MS, NA, neutral) on rating scores of arousal ($F(2,68) = 8.94$, $p < 0.001$). Post hoc tests showed that rating scores of arousal were significantly higher for MS statements (5.85 ± 1.30) and NA statements (5.88 ± 1.32) compared to neutral statements (4.59 ± 1.39, both $p = 0.011$). However, the rating scores of arousal did not differ significantly between the MS and NA statements ($p > 0.99$). These results suggest similar subjective feelings of valence and arousal related to MS and NA statements.

3.2. Manipulation check of MS priming effects

The questionnaire measures collected before fMRI scanning were used to estimate trait personality of participants from each group. The results indicated comparable death anxiety, self-esteem, optimism, neuroticism and life satisfaction in the MS and NA groups (ps > 0.1, see Table S1 for statistical details). The rating scores were obtained after the third scanning session to assess individuals' feelings related to death and negative affect. Two sample t-tests confirmed higher rating scores of closeness to death in the MS than NA group (4.43 ± 3.01 vs. 1.54 ± 2.55, $t(78) = 4.65$, $p < 0.001$, Cohen's $d = 1.04$) but comparable rating scores of fear-of-death (2.43 ± 2.25 vs. 1.64 ± 1.83, $t(78) = 1.72$, $p = 0.09$, Cohen's $d = 0.39$) and unpleasantness (3.32 ± 2.44 vs. 3.12 ± 2.25, $t(78) = 0.38$, $p = 0.70$, Cohen's $d = 0.09$) in the MS and NA groups. These results suggest enhanced feelings of closeness to death in the MS than in the NA group but comparable negative affect in the two groups.

3.3. MS effects on performance during reward learning

We examined possible MS effects on behavioral performance during reward learning first by calculating reaction times (RTs) to the imperative stimuli during the probabilistic learning task. However, the results did not show a significant difference in RTs between the MS and NA groups (all stimuli: 431 vs. 442 m s, $t(78) = -0.69$, $p = 0.495$; fixed mapping stimuli: 434 vs. 442 m s, $t(78) = -0.50$, $p = 0.618$; random mapping stimuli: 429 vs. 443 m s, $t(78) = -0.85$, $p = 0.396$), suggesting similar task difficulty for the MS and NA groups.

Next, to evaluate participants' learning performance, we calculated response accuracies for fixed-mapping and random-mapping stimuli in the order of the presentations of each type of stimuli (each stimulus was presented 20 times during the learning procedure). We then conducted regression analyses to estimate the relationships between response accuracies and the order of presentation. The regression coefficient (β) reflects the speed of learning reward-related stimuli. β was significantly higher than zero for fixed-mapping stimuli (mean ± SD = 0.09 ± 0.26, $t(79) = 3.04$, $p = 0.003$, Cohen's $d = 0.35$, FDR corrected $p < 0.05$) but not for random-mapping stimuli (0.01 ± 0.17, $t(79) = 0.70$, $p = 0.489$, Cohen's $d = 0.06$, FDR corrected $p > 0.05$), indicating an increase in learning performance related to fixed-mapping stimuli. Moreover, β was significantly higher than zero for high reward stimuli (0.18 ± 0.27, $t(79) = 6.21$, $p < 0.001$, Cohen's $d = 0.69$, FDR corrected $p < 0.05$) but not for low reward stimuli (-0.06 ± 0.28, $t(79) = -1.78$, $p = 0.08$, Cohen's $d = 0.20$, FDR corrected $p > 0.05$), indicating that the increase in learning performance was mainly driven by high reward stimuli. Interestingly, two sample t-tests confirmed that, relative to the NA group, the MS group showed significantly smaller β for high-reward fixed-mapping stimuli (vs. random-mapping stimuli) (0.25 ± 0.26 vs. 0.08 ± 0.30, $t(78) = 2.65$, $p = 0.010$, Cohen's $d = 0.61$, FDR corrected $p < 0.05$) but not for low-reward fixed-mapping stimuli (vs. random-mapping stimuli) (-0.04 ± 0.32 vs. -0.11 ± 0.27, $t(78) = 1.05$, $p = 0.295$, Cohen's $d = 0.24$, FDR corrected $p > 0.05$, see Table S2 for statistical details). These results suggest that MS relative to NA priming decreased the speed of learning high-reward fixed-mapping stimuli.

3.4. Neural responses to MS and NA priming

To examine brain responses to MS and NA priming, we conducted whole-brain analyses of contrasts between MS vs. neutral sentences for the MS group and between NA vs. neutral sentences for the NA group. The results showed that MS (vs. neutral, GLM 1) sentences induced activations in the bilateral frontal cortices, bilateral superior temporal sulcus (STS), left angular gyrus, and supplementary motor area (SMA), and induced deactivations in the cingulate (including the anterior cingulate (ACC), aMCC, and posterior cingulate (PCC)), the retrosplenial cortex, bilateral anterior and middle insula, ventral medial prefrontal cortex (vmPFC), right middle temporal gyrus, and bilateral TPJ (combining a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected; Fig. 2A, Table S3 Fig. S1A). In contrast, NA (vs. neutral, GLM 2) sentences increased activities in the aMCC (and neighboring SMA and ACC) and bilateral AI, but decreased activities in the retrosplenial, left superior frontal, and left middle occipital cortices (combining a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected; Fig. 2A, Table S3, Fig. S1B). We also conducted whole-brain two-sample t-tests (GLM 3) to further confirm the difference in brain activations in response to MS and NA priming. The results revealed significantly decreased activities in the aMCC, bilateral anterior or middle insula, and retrosplenial cortex in MS vs. NA group (combining a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected; Fig. 2A, Table S3), indicating decreased SN activity specifically in response to MS priming.

3.5. MS effects on functional connectivity during MS/NA priming

Because MS vs. NA priming modulated neural activities in the SN and frontal-parietal control network including bilateral frontal cortices and left angular gyrus in opposite directions, MS vs. NA priming might weaken functional connectivity between the key nodes of the SN and between the SN and frontal-parietal control network. To test this hypothesis, we examined functional connectivity between the key nodes of the SN (i.e., aMCC and bilateral insula) and those of the frontal-parietal control network that were defined based on significant activations or deactivations during MS (vs. neutral) priming (see Table S3). BOLD signals were extracted from 5-mm-radius spheres centered at the aMCC (MNI coordinates x/y/z = −8/34/22), bilateral insula (34/6/6 and −38/0/8), bilateral lateral frontal cortex (−38/0/48 and 52/26/30), and left

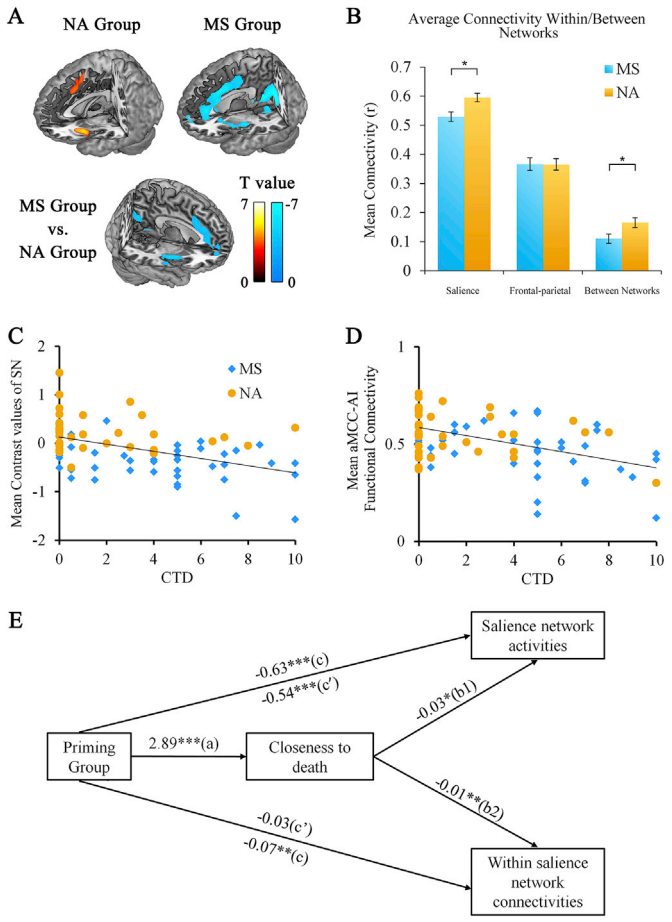

Fig. 2. MS-induced modulations of brain activity during priming. (A) The upper panel illustrates opposite effects MS and NA priming on SN activity. Relative to neutral sentences, reading sentences related to negative affect increased SN activity whereas reading sentences related to mortality decreased SN activity. The lower panel shows significantle decreased SN activity in the MS compared to the NA group. (B) MS vs. NA priming decreased functional connectivity within the SN and between the SN and frontal-parietal control network. (C) Larger subjective rating scores of closeness to death predicted decreased SN activity. (D) Larger subjective rating scores of closeness to death predicted decreased aMCC-AI connectivity. (E) Feelings of closeness to death mediate MS effects on SN activity and MS effects on functional connectivity within the SN. A voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected was used to identify and to visualize brain activations. CTD = closeness to death.

angular gyrus (−56/-60/28). We calculated connectivity matrices as the Pearson correlation between the time series of a pair of nodes from the same network and between the time series of a pair of nodes from two different networks. The mean strengths of functional connectivity within each network and between SN and frontal-parietal control network were then compared between the MS and NA groups. Two sample t-tests showed that the MS (vs. NA) group showed significantly decreased connectivity between the key nodes within the SN ($t(78) = -3.03$, $p = 0.003$, Cohen's $d = 0.69$, FDR corrected $p < 0.05$) and between the SN and frontal-parietal control network ($t(78) = -2.36$, $p = 0.02$, Cohen's $d = 0.53$, FDR corrected $p < 0.05$) but not between the key nodes within the frontal-parietal control network ($t(78) = 0.03$, $p = 0.98$, Cohen's $d = 0.01$, FDR corrected $p > 0.05$, Fig. 2B; see Table S4 for statistical details). These results were further confirmed in the edge-level analysis of functional connectivity (Fig. S2).

To further confirm the MS effects on functional connectivity during priming, we conducted additional analyses of functional connectivity that applied the same analysis pipeline but used the whole-brain parcellation of the SN and other networks. This analysis included 264 brain regions that were previously assigned to 13 large-scale functional brain systems (Power et al., 2011). The cingulo-opercular/salience network from the systems overlapped with the regions of the anterior and middle cingulate and bilateral insula in which activities were decreased by MS (vs. NA) priming. Similarly, we calculated both the within-network connectivity of the cingulo-opercular/salience network and the between-network connectivity between cingulo-opercular/salience network and other networks. The whole-brain analysis showed decreased connectivity within the cingulo-opercular/salience network during MS (vs. NA) priming ($t(78) = 2.58$, $p = 0.011$, FDR corrected $p < 0.05$; Fig. 3). We also observed weaker connectivity during MS (vs. NA) priming between the cingulo-opercular/salience network and the default mode network, frontal-parietal control network, visual network, subcortical network, dorsal attention network and sensory network ($t = -2.57 \sim -2.19$, $ps < 0.05$, FDR corrected; see Table S5 for statistical details).

3.6. Subjective feelings and MS effects on brain activity

To examine whether feelings of closeness to death mediate the group differences in brain responses to MS and NA priming, we first conducted linear regression analyses to explore associations between rating scores of closeness to death and brain activities across all participants. The results showed negative correlations between scores of closeness to death and the magnitudes of cingulate and insular activity in response to priming (aMCC: $r(80) = -0.34$, $p < 0.01$; left insula: $r(80) = -0.36$, $p < 0.01$; right insula: $r(80) = -0.38$, $p = 0.001$, all FDR corrected $p < 0.05$, Fig. 2C, see Table S6 for statistical details). Scores of closeness to death also reversely correlated with the strengths of functional connectivity between aMCC and bilateral insula (aMCC and left insula FC: $r(80) = -0.45$, $p < 0.001$; aMCC and right insula FC: $r(80) = -0.37$, $p = 0.001$, both FDR corrected $p < 0.05$, Fig. 2D see Table S7 for statistical details). These results suggest that individuals with greater feelings of closeness to death after priming showed a larger decrease of SN activity and of functional connectivity between the key nodes of the SN during priming.

To further assess whether the group difference in brain activities were mediated by feelings of closeness to death, we calculated the mean of ACC, aMCC and bilateral insular activities to index SN responses to priming and found a reverse correlation between scores of closeness to death and SN responses ($B = -0.07$, $p < 0.001$). A bootstrap resampling analysis further revealed a significant effect of Priming (IV) on scores of closeness to death (Mediator) ($B = 2.89$, $p < 0.001$) and a significant effect of the mediator on SN responses (DV) ($B = -0.03$, $p = 0.046$, Fig. 2E). The indirect effect of the IV on the DV through the mediator differed from zero with 95% confidence (95% CI: [-0.2330, −0.0096]). Similarly, we calculated the mean functional connectivity between the aMCC and bilateral insula to index SN connectivity during priming and found a negative correlation between scores of closeness to death and SN connectivity ($r(80) = -0.41$, $p < 0.001$). The bootstrap resampling analysis also revealed a significant effect of Priming (IV) on scores of closeness to death (Mediator) ($B = 2.89$, $p < 0.001$) and a significant effect of the mediator on SN connectivity (DV) ($B = -0.01$, $p = 0.005$, Fig. 2E). The indirect effect of the IV on the DV through the mediator differed from zero with 95% confidence (95% CI: [-0.0604, −0.0110]). These results provide evidence that MS (vs. NA) priming effects on SN responses were partially due to MS-induced feelings of closeness to death.

3.7. MS effects on resting-state brain activity

Next, we investigated whether and how MS (vs. NA) priming modulated the subsequent resting-state brain activity. We first conducted a region-of-interest (ROI) analysis to assess functional connectivity between the key nodes of the SN including the aMCC and bilateral AI in

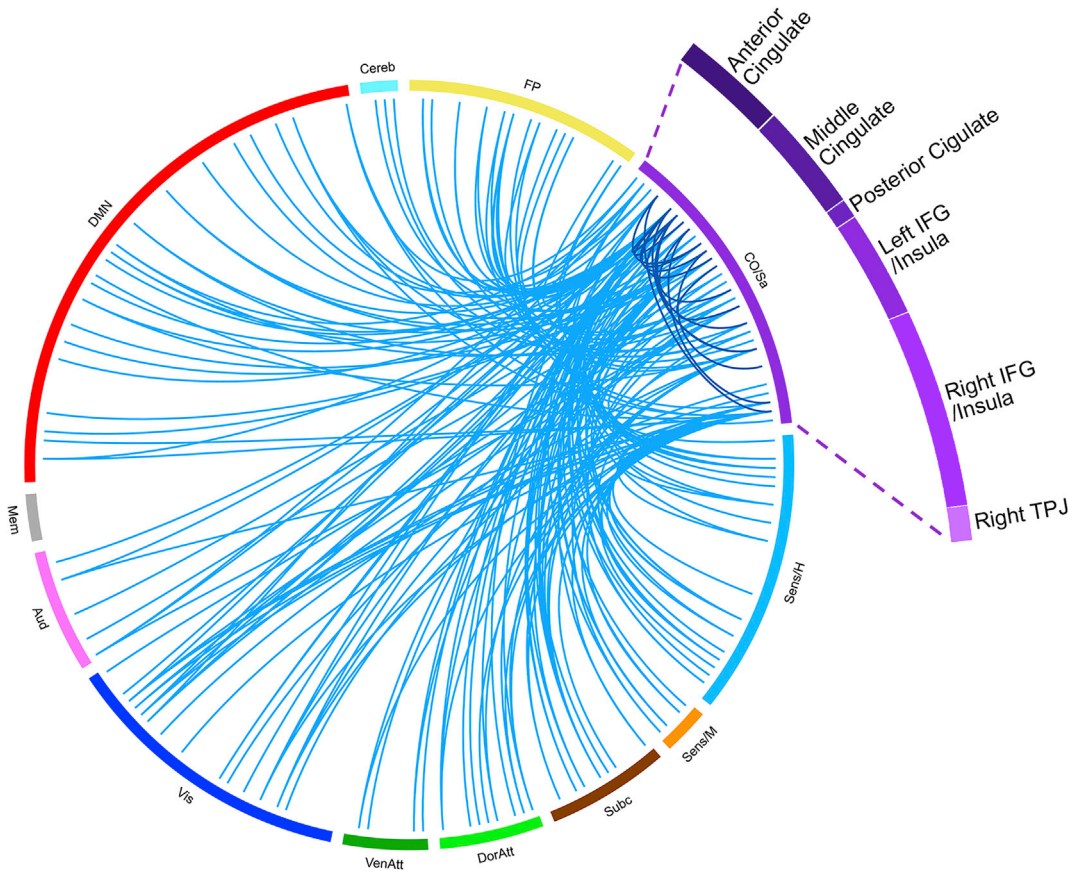

Fig. 3. Illustration of MS-induced changes in functional connectivity based on the parcellation of 13 large-scale functional brain systems. Decreased connectivity within the cingulo-opercular and salience (CO/Sa) network edges and between CO/Sa network edges and other network edges is illustrated in dark blue and light blue lines, respectively ($p < 0.01$, uncorrected, for exploratory purposes). Sens/M: mouth sensory-somatomotor network; Sens/H: hand sensory-somatomotor network; Subc: subcortical network; DorAtt: dorsal attention network; VenAtt: ventral attention network; Vis: visual network; Aud: auditory network; Mem: memory retrieval network; DMN: default mode network; Cere: cerebellar network; FP: frontoparietal task control network; * FDR corrected $p < 0.05$.

which activity was decreased during MS priming. Time courses of BOLD signals were extracted from 5-mm-radius spheres centered at the MNI coordinates (x/y/z) −8/34/22 (aMCC), −38/0/8 (left insula), and 34/6/6 (right insula) (see Table S3). Two sample t-tests showed significantly decreased functional connectivity between the aMCC and right insula in the MS group compared to that in the NA group ($t(78) = -2.60$, $p = 0.011$, Cohen's $d = 0.59$).

We further conducted whole-brain analyses of the degree centrality of each voxel — an index of average functional connectivity strength between a voxel and all other voxels (Sporns et al., 2004) — during the resting state in the MS and NA groups, respectively. The whole-brain two-sample *t*-test revealed significantly decreased degree centrality of aMCC (x/y/z = −12/33/27) in the MS compared to the NA group (combining a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected; Fig. 4A).

As a regression analysis revealed that scores of closeness to death significantly reversely predicted the degree centrality of the aMCC during the resting state ($B = -0.06$, $p = 0.012$), we further tested whether SN activity in response to MS priming mediated the association between feelings of closeness to death and degree centrality of aMCC during the resting state. A bootstrap resampling analysis showed a significant effect of feelings of closeness to death (IV) on SN activity in response to priming (Mediator) ($B = -0.08$, $p < 0.001$) and a significant effect of the mediator on the degree centrality of the aMCC (DV) ($B = 0.41$, $p < 0.01$). The

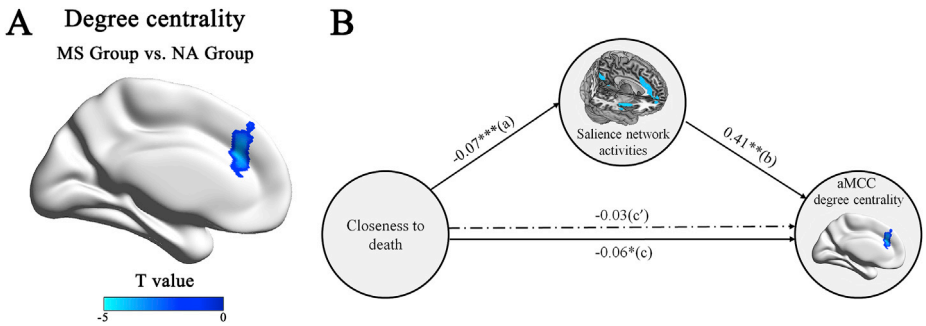

Fig. 4. MS-induced changes of the resting state activity. (A) aMCC-centered connectivity was decreased in the MS compared to the NA groups during the resting state. (B) Decreased SN activity in response to MS priming mediated the association between CTD feelings and decreased aMCC-centered functional connectivity in the resting state. A voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected was used to identify and to visualize brain activations.

indirect effect of the IV on the DV through the mediator differed from zero with 95% confidence (95% CI: [-0.0575, −0.0107], Fig. 4B). These results suggest that the association between MS-priming induced feelings of closeness to death and variations of the degree centrality of the aMCC during the resting state was at least partially due to the modulations of SN activity by MS priming.

Given the function of self-esteem in buffering MS effects on behavior (Greenberg et al., 1992; Harmon-Jones et al., 1997), we also assessed whether MS modulations of SN activities varied across individuals along self-esteem. Correlation analyses showed that self-esteem scores positively predicted the degree centrality of the aMCC during the resting state in the MS group ($r(80) = 0.41$, $p = 0.009$, FDR corrected $p < 0.05$) but not in the NA group ($r(80) = 0.10$, $p = 0.533$, FDR corrected $p > 0.05$; Fig. S3; see Table S8 for statistical details). The degree centrality of the aMCC during the resting state also showed trends of a positive correlation with self-reports of optimism ($r(40) = 0.34$, $p = 0.03$, FDR corrected $p = 0.06$) but a negative correlation with self-reports of neuroticism ($r(40) = -0.32$, $p = 0.04$, FDR corrected $p = 0.08$) in the MS group but not in the NA group ($p = 0.28$ and 0.96, see Tables S9 and S10 for statistical details). These results suggest that MS effects on the resting-state brain activity vary across individuals' psychological traits related to well-being.

3.8. MS effects on brain activity during reward learning

To investigate whether MS (vs. NA) priming influences SN activity during the probabilistic learning task, we first conducted whole-brain analyses to identify brain activations in responses to loss and win feedback on participants' choices. Both the MS and NA groups showed increased activities in the key nodes of the SN (i.e., aMCC, bilateral AI) in response to loss (vs. win, GLM 5) feedback in the reward system including the bilateral ventral striatum and ventral medial prefrontal cortex in response to win (vs. loss, GLM 4) feedback (combining a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected; Fig. 5, Table S11), replicating the previous findings (Holroyd et al., 2004; Varnum et al., 2014). However, whole-brain two-sample t-tests revealed significantly reduced aMCC activity in response to loss feedback in the MS compared with the NA group (combining a voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected; Fig. 6A, Table S11), providing evidence for modulations of SN activity during the subsequent learning task by MS priming. By contrast, the ventral striatum and ventral medial prefrontal activities in response to win feedback did not differ significantly between the MS and NA groups, suggesting a negligible influence of MS on the reward system activity.

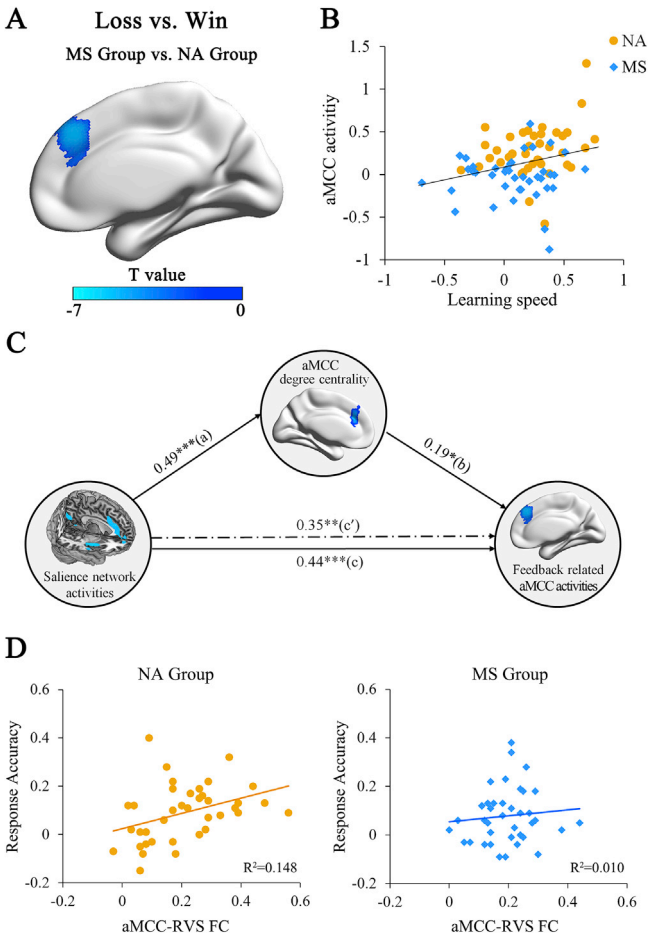

Fig. 6. MS-induced modulations of SN activity during reward learning. (A) The MS vs. NA group showed decreased aMCC activities in response to loss feedback during reward learning. (B) Greater aMCC activities in response to loss feedback predicted faster speed during reward learning across all participants. (C) The mediation role of aMCC-centered functional connectivity in linking MS-induced modulations of SN activity in response to priming and changes in monetary feedback related aMCC activities. (D) Stronger functional connectivity between aMCC and the right ventral striatum during reward learning predicted higher response accuracy for high reward fixed-mapping stimuli in the NA group but not the MS group. A voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected was used to identify and to visualize brain activations.

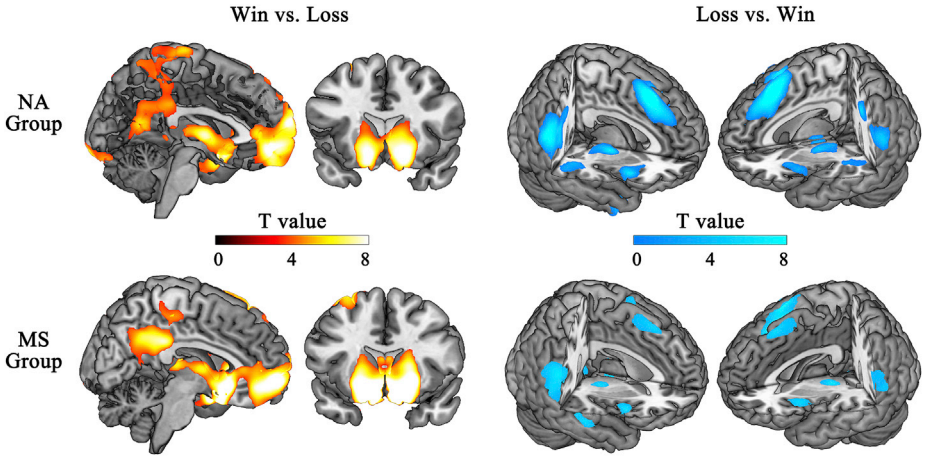

Fig. 5. Whole-brain analysis results of neural responses to win vs. loss feedback and loss vs. win feedback during reward learning. Both the MS and NA groups showed increased activities in the key nodes of the SN (i.e., aMCC, bilateral AI) in response to loss (vs. win) feedback and in the reward system including the bilateral ventral striatum and ventral medial prefrontal cortex in response to win (vs. loss) feedback. A voxel-level threshold of $p < 0.001$ and a cluster-level threshold of $p < 0.05$, FWE corrected was used to identify and to visualize brain activations.

3.9. MS-induced modulations of SN activity and reward learning speed

To examine the relationship between MS-induced modulations of SN activity and the speed of learning reward-related objects, we first tested correlations between learning performance and neural responses to monetary feedback. The results showed that stronger aMCC activities to high loss feedback predicted faster learning speeds for high reward fixed-mapping stimuli ($r(80) = 0.26$, $p = 0.020$; Fig. 6B), whereas there was no evidence for correlation between learning speed and neural responses to win feedback, suggesting a key role of aMCC activity in driving reward learning.

To further assess the link between MS priming and reward learning, we tested whether MS (vs. NA) priming influenced aMCC activity to loss feedback during reward learning by modulating the resting-state activity. We first confirmed that SN responses (the mean of aMCC and bilateral AI activities) during MS/NA priming predicted the aMCC activity in response to loss feedback across all participants ($r(80) = 0.45$, $p < 0.01$). We then conducted a mediation analysis to assess whether the association between SN activity to priming and aMCC activity to loss feedback was mediated by aMCC-centered functional connectivity during the resting state. A bootstrap resampling analysis revealed a significant effect of the SN activity during priming session (IV) on the degree centrality of the aMCC during the resting state (Mediator) ($B = 0.49$, $p < 0.001$) and a significant effect of the mediator on aMCC activity in response to loss feedback (DV) ($B = 0.19$, $p = 0.018$). The indirect effect of the IV on the DV through the mediator differed from zero with 95% confidence (95% CI: [0.0185, 0.2184], Fig. 6C). Given the direct link between the speed of learning reward-related objects and aMCC activity in response to loss feedback, these results suggest that MS-induced sequential modulations of SN activities during MS priming and later aMCC-centered functional connectivity during the resting state also contributed to the changes in learning performance by modulating aMCC activity in response to loss feedback during the reward learning task.

Finally, because there has been evidence for the universal role of the ventral striatum in reward-based learning (Daniel and Stefan, 2014), we explored whether neural responses to win feedback would predict learning performance, but we failed to find reliable results. Then, since the aMCC activity in response to loss feedback was decreased by MS (vs. NA), as shown above, we further assessed whether functional connectivity between aMCC and the reward network would be associated with performance during reward learning and whether such associations might be different between the MS and NA groups. To do this, we extracted BOLD signals from 5-mm-radius spheres centered at aMCC (MNI coordinates, x/y/z = 12/38/40) which showed decreased response to loss feedback in MS than NA group and the key nodes of the reward network (including the left ventral striatum(-12/14/-20), right ventral striatum(12/14/-22) and ventral medial prefrontal cortex (0/60/-4)) which showed significant activations in response to win feedback across all participants (see Table S12). Interestingly, we found that stronger functional connectivity between the aMCC and right ventral striatum predicted higher accuracy of choosing high reward fixed-mapping stimuli, but this association was evident in the NA group ($r(40) = 0.39$, $p = 0.014$, FDR corrected $p < 0.05$, Fig. 6D) but not in the MS group ($r(40) = 0.10$, $p = 0.532$, FDR corrected $p > 0.05$; Table S13 for statistical details), suggesting a potential contribution of aMCC-striatum connectivity to the performance of reward learning, which, however, might be disrupted by MS.

4. Discussion

The current work investigated how the brain responds to reminders of mortality and then changes its activity underlying subsequent behavior. Our findings provide evidence that thoughts of death induced sequential modulations of the SN activity that predicted the speed of learning reward-related objects. We first showed that MS and NA priming modulated SN activity in opposite directions. Relative to a neutral condition, NA priming activated whereas MS priming deactivated the aMCC and AI. Moreover, we showed that MS compared to NA priming decreased functional connectivity within the key nodes of SN and undermined the SN connectivity with other neural networks (e.g., the frontal-parietal control network). These results complement previous brain imaging results (Han et al., 2010; Shi and Han, 2013; Klackl et al., 2013) by showing that verbal reminders of mortality not only decrease the magnitude of SN activity but dampen its functional connectivity with other neural networks as well. MS-induced modulations of subsequent brain activities were also characterized by looser aMCC-centered functional connectivity during the resting state and reduced aMCC responses to loss feedback during reward learning. In addition, we showed evidence that MS-induced modulations of SN responses were mediated by individuals' feelings of closeness to death. These results uncover a unique pattern of sequential modulations of SN activity by verbal reminders of mortality which are essentially different from the modulations of the SN activity by physical/social pain, negative affect, or cognitive conflict, which are characterized by enhanced SN activity and augmented SN connectivity with other brain regions (Shackman et al., 2011; Kragel et al., 2018). Our findings suggest MS-specific modulations of SN activity that may help to understand prior findings of MS influences on human behavior (Burke et al., 2010; Juhl and Routledge, 2016).

More importantly, we showed evidence that MS-induced sequential modulations of SN activity underlie changes of the speed of learning reward-related objects. Our results first provide evidence for a link between the speed of reward learning and aMCC activity in response to loss feedback during the probabilistic learning task. In addition, our results highlight associations between MS modulations of SN activity across the three phases of our experimental procedure. While MS-priming induced feelings of closeness to death predicted variations in aMCC-centered connectivity during the resting state, this association was partially attributed to modulations of SN responses by MS priming. Furthermore, we showed evidence for associations between MS-modulations of SN activities to priming and aMCC responses to loss feedback during reward learning and this association was mediated by MS-modulations of resting-state aMCC-centered connectivity. These findings suggest a potential relationship between MS modulations of SN activity at two successive phases. Moreover, the sequential modulations of SN activity by MS priming might influence behavioral performance during the learning task by modulating aMCC responses to loss feedback, which played a key role in driving individuals' learning performance. Future research should further clarify the functional role of the MS-induced modulations of aMCC activity in modulating behavioral responses to other salient stimuli such as luxury brands (Audrin et al., 2018).

Our work also revealed significant individual differences in MS modulations of SN activity. Specifically, we showed that aMCC-centered connectivity during the resting state was stronger in those primed with MS with higher self-esteem. This result complements a recent finding that individuals with high (vs. low) self-esteem exhibited increased amygdala-ventrolateral prefrontal cortex connectivity during the processing of death-related stimuli (Yanagisawa et al., 2016), suggesting that enhanced functional brain activity during either the resting state or a cognitive task may serve as a neural mechanism by which self-esteem buffers MS effects on behavior and well-being (Greenberg et al., 1992; Harmon-Jones et al., 1997; Wisman et al., 2015; Guan et al., 2015). Other personality factors associated with well-being (e.g., optimism) (Scheier and Carver, 1992; Kleiman et al., 2017) and mental disorders (e.g., neuroticism) (Paulus et al., 2016) might also constrain the impact of MS, since our results showed that aMCC-centered functional connectivity during the resting state tended to vary across individuals along their self-reported optimism and neuroticism. It has been shown that trait optimism affects the cingulate activity during imagining positive future events (Sharot et al., 2007) and neuroticism influences the cingulate activity in response to negative affect such as pain (Coen et al., 2011). Our results suggest opposite patterns of optimism/neuroticism associations with MS-induced changes of aMCC-centered functional

connectivity.

During the priming phase, MS priming (vs. neutral statements) activated the frontal-parietal control network, similar to previous results (Han et al., 2010; Shi and Han, 2013). During the learning task, win (vs. loss) feedback activated the reward system consisting of the ventral striatum and vmPFC, replicating the previous findings (Holroyd et al., 2004; Varnum et al., 2014). Nevertheless, MS (vs. NA) priming failed to modulate either the frontal-parietal control network during priming or reward activities during reward learning, indicating dissociated effects of mortality threat on the SN and other neural networks. Under a mortality threat, decreased monetary reward, decreased loss aversion, or both, can inhibit the motives for quickly learning reward-related objects. Our results suggest that the SN may play a key role in mediating the MS effect on learning performance. MS might also influence the behavioral performance of reward learning by modulating SN connectivity with the reward system. This potential mechanism is implicated in our finding that the connectivity between the aMCC and the right ventral striatum was also able to predict the accuracy of choosing high reward fixed-mapping stimuli in the NA group but not in the MS group.

A few limitations of the current work should be clarified in future research. For example, a 5-min scan was adopted for recording resting-state activity in our work, similar to previous studies of functional connectivity during the resting state (Greicius et al., 2007; Doucet et al., 2015; Creswell et al., 2016). A longer scan would help to obtain more stable and reliable results of functional connectivity during the resting state and would allow researchers to examine how functional connectivity in the resting state may vary dynamically when death-related thoughts due to MS priming dim from an explicit sate to an implicit state during a prolonged resting state. As the current work tested only Chinese participants, it is unclear how the findings of our work can be applied to participants in other cultures. This should be clarified in future work because cultural worldview provides a key buffer of existential anxiety (Greenberg et al., 1986; Rosenblatt et al., 1989; Pyszczynski et al., 1999) and cultural beliefs influence brain activities underlying multiple cognitive and affective processes (Han and Northoff, 2008; Han et al., 2013; Han and Ma, 2015; Han, 2017). Our work focused on the effects of MS on SN activity even though thoughts of death influence multiple cognitive and affective processes related to social behavior. For instance, there is evidence that MS priming increases financial expectations for the future (Kasser and Sheldon, 2000) and increases risky decisions in a gambling task (Hart et al., 2010). Mental processes underlying these tasks are related to other neural networks such as the nucleus accumbens and mesial prefrontal cortex underlying anticipation of monetary gains (Knutson et al., 2005) and the ventral medial prefrontal and orbital frontal cortices involved in decision making during the gambling task (Lawrence et al., 2008). These findings together suggest that MS priming may affect the activity of neural networks involved in risk-taking and monetary expectation, which needs to be tested in future work. Finally, to disentangle the exact effects of MS priming on brain activity involved in cognition and behavior, future research should estimate and control for possible differences in cognitive and affective ability (e.g., emotion regulation) between MS and NA groups.

Taken together, our fMRI results suggest a architecture of multistage neural mechanisms underlying mortality threats to human motives and behavior, which, however, may vary across individuals' personality traits. Given the key function of the SN in detecting stimuli or events that are meaningful or emotional provocative (Seeley et al., 2007; Uddin, 2015), our findings suggest that awareness of mortality reduces the ecological significance of encoding of the salience of stimuli or events and suggest a neural mechanism underlying MS influences on our mind and behavior (Audrin et al., 2018; Lee et al., 2017). The subregions of the cingulate cortex serve different cognitive and affective functions such that the aMCC represents physical pain, the dorsal region of the MCC is engaged in cognitive control, and the ventral region of the MCC responds to negative emotion (Kragel et al., 2018). Our results that all the subregions of the cingulate cortex were modulated by verbal reminders of mortality suggest the broad influences of mortality threat on cognition and emotion. Our findings suggest that the deactivation of the SN should be taken into consideration for understanding the effects of mortality threats on social attitudes, decision making, and well-being (Juhl and Routledge, 2016; Jain and Ellithorpe, 2016; Leippe et al., 2017; Pan et al., 2017).

Acknowledgement

This work was supported by the National Natural Science Foundation of China (Projects 31871134, 31661143039, 31421003, 31800916), the Natural Science Foundation of Guangdong Province (Project 2017A030310553) and the Science Foundation of Ministry of Education of China (Project 17YJCZH121). The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2019.116068.

References

Aiken, L.S., West, S.G., Reno, R.R., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage.
Arnow, B.A., Desmond, J.E., Banner, L.L., Glover, G.H., Solomon, A., et al., 2002. Brain activation and sexual arousal in healthy, heterosexual males. Brain 125, 1014–1023.
Audrin, C., Cheval, B., Chanal, J., 2018. Materialism moderates the impact of mortality salience on impulsive tendencies toward luxury brands. Death Stud. 42, 115–122.
Baron, R.M., Kenny, D.A., 1986. The moderator–mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J. Personal. Soc. Psychol. 51, 1173–1182.
Bullmore, E., Sporns, O., 2009. Complex brain networks: graph theoretical analysis of structural and functional systems. Nat. Rev. Neurosci. 10, 186–198.
Burke, B.L., Martens, A., Faucher, E.H., 2010. Two decades of terror management theory: a meta-analysis of mortality salience research. Personal. Soc. Psychol. Rev. 14, 155–195.
Coen, S.J., Kano, M., Farmer, A.D., Kumari, V., Giampietro, V., et al., 2011. Neuroticism influences brain activity during the experience of visceral pain. Gastroenterology 141, 909–917.
Creswell, J.D., Taren, A.A., Lindsay, E.K., Greco, C.M., Gianaros, P.J., et al., 2016. Alterations in resting-state functional connectivity link mindfulness meditation with reduced interleukin-6: a randomized controlled trial. Biol. Psychiatry 80, 53–61.
Dai, Z., Yan, C., Wang, Z., Wang, J., Xia, M., et al., 2012. Discriminative analysis of early Alzheimer's disease using multi-modal imaging and multi-level characterization with multi-classifier (M3). Neuroimage 59, 2187–2195.
Daniel, R., Stefan, P., 2014. A universal role of the ventral striatum in reward-based learning: evidence from human studies. Neurobiol. Learn. Mem. 114, 90–100.
Doucet, G.E., Rider, R., Taylor, N., Skidmore, C., Sharan, A., et al., 2015. Presurgery resting-state local graph-theory measures predict neurocognitive outcomes after brain surgery in temporal lobe epilepsy. Epilepsia 56, 517–526.
Droutman, V., Read, S.J., Bechara, A., 2015. Revisiting the role of the insula in addiction. Trends Cogn. Sci. 19, 414–420.
Eysenck, S.B., Eysenck, H.J., 1975. Manual of the Eysenck Personality Questionnaire. Hougter & Soughton, London, UK.
Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191.
Feng, C., Azarian, B., Ma, Y., Feng, X., Wang, L., Luo, Y., Krueger, F., 2017. Mortality salience reduces the discrimination between in-group and out-group interactions: afunctional MRI investigation using multivoxel pattern analysis. Hum. Brain Mapp. 38, 1281–1298.
Greenberg, J., Pyszczynski, T., Solomon, S., 1986. The causes and consequences of a need for self-esteem: a terror management theory. In: Baumeister, R.R. (Ed.), Public Self and Private Self. Springer-Verlag, New York, pp. 189–212.
Greenberg, J., Pyszczynski, T., Solomon, S., Rosenblatt, A., Veeder, M., et al., 1990. Evidence for terror management theory II: the effects of mortality salience on reactions to those who threaten or bolster the cultural worldview. J. Personal. Soc. Psychol. 58, 308–318.
Greenberg, J., Solomon, S., Pyszczynski, T., Rosenblatt, T., Burling, A., et al., 1992. Why do people need self-esteem? Converging evidence that self-esteem serves an anxiety-buffering function. J. Personal. Soc. Psychol. 63, 913–922.
Greenberg, J., Arndt, J., Simon, L., Pyszczynski, T., Solomon, S., 2000. Proximal and distal defenses in response to reminders of one's mortality: evidence of a temporal sequence. Personal. Soc. Psychol. Bull. 26, 91–99.
Greicius, M.D., Flores, B.H., Menon, V., Glover, G.H., Solvason, H.B., et al., 2007. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biol. Psychiatry 62, 429–437.

Guan, L., Chen, Y., Xu, X., Qiao, L., Wei, J., Han, S., Yang, J., 2015. Self-esteem buffers the mortality salience effect on the implicit self-face processing. Personal. Individ. Differ. 85, 77–85.
Han, S., 2017. The Social Cultural Brain: Cultural Neuroscience Approach to Human Nature. Oxford University Press, Oxford, UK.
Han, S., Ma, Y., 2015. In: A Culture-Behavior-Brain Loop Model of Human development. Trends Cogn. Sci, vol. 9, pp. 666–676.
Han, S., Northoff, G., 2008. Culture-sensitive neural substrates of human cognition: a transcultural neuroimaging approach. Nat. Rev. Neurosci. 9, 646–654.
Han, S., Qin, J., Ma, Y., 2010. Neurocognitive processes of linguistic cues related to death. Neuropsychologia 48, 3436–3442.
Han, S., Northoff, G., Vogeley, K., Wexler, B.E., Kitayama, S., Varnum, M.E.W., 2013. A cultural neuroscience approach to the biosocial nature of the human brain. Annu. Rev. Psychol. 64, 335–359.
Harmon-Jones, E., Simon, L., Greenberg, J., Pyszczynski, J., Solomon, T., et al., 1997. Terror management theory and self-esteem: evidence that increased self-esteem reduced mortality salience effects. J. Personal. Soc. Psychol. 72, 24–36.
Hart, J., Schwabach, J.A., Solomon, S., 2010. Going for broke: mortality salience increases risky decision making on the Iowa gambling task. Br. J. Soc. Psychol. 49, 425–432.
Holroyd, C.B., Nieuwenhuis, S., Yeung, N., Nystrom, L., Mars, R.B., Coles, M.G., Cohen, J.D., 2004. Dorsal anterior cingulate cortex shows fMRI response to internal and external error signals. Nat. Neurosci. 7, 497–498.
Jain, P., Ellithorpe, M.E., 2016. Mortality salience influences attitudes and information-seeking behavior related to organ donation. J. Commun. Healthc. 9, 126–134.
Juhl, J., Routledge, C., 2016. Putting the terror in terror management theory: evidence that the awareness of death does cause anxiety and undermine psychological well-being. Curr. Dir. Psychol. Sci. 25, 99–103.
Kasser, T., Sheldon, K.M., 2000. Of wealth and death: materialism, mortality salience, and consumption behavior. Psychol. Sci. 11, 348–351.
Kenyon, B.L., 2001. Current research in children's conceptions of death: a critical review. Omega J. Death Dying 43, 63–91.
Klackl, J., Jonas, E., Kronbichler, M., 2013. Existential neuroscience: self-esteem moderates neuronal responses to mortality-related stimuli. Soc. Cogn. Affect. Neurosci. 9, 1754–1761.
Kleiman, E.M., Chiara, A.M., Liu, R.T., Jager-Hyman, S.G., Choi, J.Y., Alloy, L.B., 2017. Optimism and well-being: a prospective multi-method and multi-dimensional examination of optimism as a resilience factor following the occurrence of stressful life events. Cognit. Emot. 31, 269–283.
Knutson, B., Taylor, J., Kaufman, M., Peterson, R., Glover, G., 2005. Distributed neural representation of expected value. J. Neurosci. 2519, 4806–4812.
Kragel, P.A., Kragel, P.A., Kano, M., Van Oudenhove, L., HG, L., Dupont, P., et al., 2018. Generalizable representations of pain, cognitive control, and negative emotion in medial frontal cortex. Nat. Neurosci. 21, 283–289.
Lawrence, N.S., Jollant, F., O'daly, O., Zelaya, F., Phillips, M.L., 2008. Distinct roles of prefrontal cortical subregions in the Iowa Gambling Task. Cerebr. Cortex 19, 1134–1143.
Lee, S.M., Nathan, A.H., Park, J.W., Kim, H., Koo, J., Chun, S., 2017. When sex doesn't sell to men: mortality salience, disgust and the appeal of products and advertisements featuring sexualized women. Motiv. Emot. 4, 478–491.
Leippe, M.R., Bergold, A.N., Eisenstadt, D., 2017. Prejudice and terror management at trial: effects of defendant race/ethnicity and mortality salience on mock-jurors' verdict judgments. J. Soc. Psychol. 157, 279–294.
Luo, S., Shi, Z., Yang, X., Wang, X., Han, S., 2014. Reminders of mortality decrease midcingulate activity in response to others' suffering. Soc. Cogn. Affect. Neurosci. 9, 477–486.
Luo, S., Yu, D., Han, S., 2017. 5-HTTLPR moderates the association between interdependence and brain responses to mortality threats. Hum. Brain Mapp. 38, 6157–6171.
Mandel, N., Heine, S.J., 1999. Terror Management and Marketing: He who dies with the most toys wins. Adv. Consum. Res. 26, 527–532.
Menon, V., 2011. Large-scale brain networks and psychopathology: a unifying triple network model. Trends Cogn. Sci. 15, 483–506.
Opsahl, T., Agneessens, F., Skvoretz, J., 2010. Node centrality in weighted networks: generalizing degree and shortest paths. Soc. Netw. 32, 245–251.
Pan, P.L., Zhou, S., Thompson Hayes, M., 2017. Immigrant perpetrators in the news: a terror management approach to resultant hostility, perceived vulnerability, and immigration issue judgment. J. Int. Intercult. Commun. 3, 219–236.
Paulus, D.J., Vanwoerden, S., Norton, P.J., Sharp, C., 2016. Emotion dysregulation, psychological inflexibility, and shame as explanatory factors between neuroticism and depression. J. Affect. Disord. 190, 376–385.
Power, J.D., Cohen, A.L., Nelson, S.M., Wig, G.S., Barnes, K.A., et al., 2011. Functional network organization of the human brain. Neuron 72, 665–678.
Preacher, K.J., Hayes, A.F., 2008. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Methods 40, 879–891.
Pyszczynski, T., Greenberg, J., Solomon, S., 1999. A dual-process model of defense against conscious and unconscious death-related thoughts: an extension of terror management theory. Psychol. Rev. 106, 835–845.
Rosenberg, M., 1965. Rosenberg Self-Esteem Scale (RSE). Acceptance and Commitment Therapy. Measures Package, vol. 61. University of Wollongong, Woolongong, Australia.
Rosenblatt, A., Greenberg, J., Solomon, S., Pyszczynski, T., Lyon, D., 1989. Evidence for terror management theory: I. The effects of mortality salience on reactions to those who violate or uphold cultural values. J. Personal. Soc. Psychol. 57, 681–690.
Rubinov, M., Sporns, O., 2010. Complex network measures of brain connectivity: uses and interpretations. Neuroimage 52, 1059–1069.
Schaefer, M., Rotte, M., 2007. Thinking on luxury or pragmatic brand products: brain responses to different categories of culturally based brands. Brain Res. 1165, 98–104.
Scheier, M.F., Carver, C.S., 1985. Optimism, coping, and health: assessment and implications of generalized outcome expectancies. Health Psychol. 4, 219–247.
Scheier, M.F., Carver, C.S., 1992. Effects of optimism on psychological and physical well-being: theoretical overview and empirical update. Cogn. Ther. Res. 16, 201–228.
Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., Glover, G.H., Kenna, H., Reiss, A.L., Greicius, M.D., 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27, 2349–2356.
Shackman, A.J., Salomons, T.V., Slagter, H.A., Fox, A.S., Winter, J.J., Davidson, R.J., 2011. The integration of negative affect, pain and cognitive control in the cingulate cortex. Nat. Rev. Neurosci. 12, 154–167.
Sharot, T., Riccardi, A.M., Raio, C.M., Phelps, E.A., 2007. Neural mechanisms mediating optimism bias. Nature 450, 102–105.
Shi, Z., Han, S., 2013. Transient and sustained neural responses to death-related linguistic cues. Soc. Cogn. Affect. Neurosci. 8, 573–578.
Sporns, O., Chialvo, D.R., Kaiser, M., Hilgetag, C.C., 2004. Organization, development and function of complex brain networks. Trends Cogn. Sci. 8, 418–425.
Templer, D.I., 1970. The construction and validation of a death anxiety scale. J. Gen. Psychol. 82, 165–177.
Templer, D.I., Harville, M., Hutton, S., Underwood, R., Tomeo, M., et al., 2002. Death depression scale-revised. Omega J. Death Dying 44, 105–112.
Uddin, L.Q., 2015. Salience processing and insular cortical function and dysfunction. Nat. Rev. Neurosci. 16, 55–61.
Van Den Heuvel, M.P., Sporns, O., 2011. Rich-club organization of the human connectome. J. Neurosci. 31, 15775–15786.
Varnum, M.E.W., Shi, Z., Chen, A., Qiu, J., Han, S., 2014. When "our" reward is the same as "my" reward: self-construal priming shifts neural responses to own vs. friends' rewards. Neuroimage 87, 164–169.
Wang, J., Zuo, X., He, Y., 2010. Graph-based network analysis of resting-state functional MRI. Front. Syst. Neurosci. 4, 16.
Wisman, A., Heflick, N., Goldenberg, J.L., 2015. The great escape: the role of self-esteem and self-related cognition in terror management. J. Exp. Soc. Psychol. 60, 121–132.
Yan, C., Zang, Y., 2010. DPARSF: a MATLAB toolbox for" pipeline" data analysis of resting-state fMRI. Front. Syst. Neurosci. 4, 13.
Yanagisawa, K., Abe, N., Kashima, E.S., Nomura, M., 2016. Self-esteem modulates amygdala-ventrolateral prefrontal cortex connectivity in response to mortality threats. J. Exp. Psychol. Gen. 145, 273–283.