Diminished preparatory physiological responses in frontotemporal lobar degeneration syndromes

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Abstract

Researchers typically study physiological responses either after stimulus onset or when the emotional valence of an upcoming stimulus is revealed. Yet participants may also respond when they are told that an emotional stimulus is about to be presented even without knowing its valence. Increased physiological responding during this time may reflect a "preparation for action." The generation of such physiological responses may be supported by frontotemporal regions of the brain that are vulnerable to damage in frontotemporal lobar degeneration (FTLD).

We examined preparatory physiological responses and their structural and functional neural correlates in five FTLD clinical subtypes (behavioral variant frontotemporal dementia, $n = 67$; semantic variant primary progressive aphasia, $n = 35$; non-fluent variant primary progressive aphasia, $n = 30$; corticobasal syndrome, $n = 32$; progressive supranuclear palsy, $n = 30$). Comparison groups included patients with Alzheimer's disease ($n = 56$; AD) and healthy controls ($n = 35$, HC). Preparatory responses were quantified as cardiac interbeat interval decreases (i.e., heart rate increases) from baseline to an "instruction period," during which participants were told to watch the upcoming emotional film but not provided the film's valence. Patients' behavioral symptoms (apathy and disinhibition) were also evaluated via a caregiver-reported measure.

Compared to HC and AD, the FTLD group showed significantly smaller preparatory responses. When comparing each FTLD clinical subtype with HC and AD, significant group differences emerged for behavioral variant frontotemporal dementia and progressive supranuclear palsy. Behavioral analyses revealed that FTLD patients showed greater disinhibition and apathy compared to AD patients. Further, these group differences in disinhibition (but not apathy) were mediated by patients' smaller preparatory responses. Voxel-based morphometry and resting-state functional MRI analyses revealed that across

patients and HCs, smaller preparatory responses were associated with smaller volume and lower functional connectivity in a circuit that included the ventromedial prefrontal cortex (vmPFC) and cortical and subcortical regions of the salience network.

Diminished preparatory physiological responding in FTLD may reflect a lack of preparation for actions that are appropriate for an upcoming situation, such as approaching or withdrawing from emotional stimuli. The vmPFC and salience network are critical for evaluating stimuli, thinking about the future, triggering peripheral physiological responses, and processing and interpreting interoceptive signals. Damage to these circuits in FTLD may impair preparatory responses and help explain often-observed clinical symptoms such as disinhibition in these patients.

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Abbreviations: ACC = anterior cingulate cortex; AD = Alzheimer's disease; AI = anterior insula; Amy = amygdala; ANS = automatic nervous system; bvFTD = behavioral variant frontotemporal dementia; CBS = corticobasal syndrome; CDR-Box = Dementia Rating Scale sum of boxes; CDR-Total = Dementia Rating Scale total score; FTLD = frontotemporal lobar degeneration; HC = healthy control; Hyp = hypothalamus; IBI = cardiac inter-beat intervals; MMSE = Mini-Mental State Examination; MNI = Montreal Neurological Institute; nfvPPA = non-fluent variant primary progressive aphasia, PAG = periaqueductal gray; PNS = parasympathetic automatic nervous system; PSP = progressive supranuclear palsy; ROI = regions-of-interest; rs-fMRI = resting-state functional MRI; SMN = sensorimotor network; SN = salience network; SNS = sympathetic nervous system; svPPA = semantic variant primary progressive aphasia; Thal = thalamus; TIV = total intracranial volume; UCB = Berkeley Psychophysiology Laboratory at the University of California, Berkeley; UCSF = Memory and Aging Center at the University of California, San Francisco; VBM = voxel-based morphometry; vmPFC = ventromedial prefrontal cortex

Introduction

Increased physiological activity often occurs when something significant is about to happen, sometimes even when we have yet to determine the emotional valence of the upcoming stimulus. For example, our heart rate may increase when we are about to unbox a gift or try new food without knowing whether the gift will bring pleasure or the food will taste bad. Such "preparatory physiological responses" (also referred to as "preparatory responses") may reflect a general *preparation for action* that serves to facilitate the subsequent behavioral changes that are tied to emotional responses (e.g., feeling disgusted and displaying withdrawal/expulsion behaviors associated with spoiled food).

Brain Mechanisms for Preparatory Responses

Although the precise brain mechanisms underlying these preparatory responses remain undetermined, findings from previous research suggest that multiple brain regions and networks may play critical roles in this process (Fig. 1A). The ventromedial prefrontal cortex (vmPFC) may be involved in evaluating situations and generating predictions (e.g., based on previous experiences, food could be either rewarding or punishing).[1, 2] The vmPFC can also communicate with the salience network (SN)[3]—including cortical areas such as the posterior

region of the anterior cingulate cortex (ACC), and subcortical areas such as the amygdala (Amy), hypothalamus (Hyp), and periaqueductal gray (PAG)—resulting in adjustments in the autonomic and somatic nervous systems (e.g., increased heart rate to support possible approach or avoidance behaviors).[4] The anterior insula (AI) and thalamus (Thal) in the SN may also be involved by providing the ACC and vmPFC with information about current bodily states (e.g., levels of cardiovascular activity, muscle contraction/relaxation) through proprioceptive and interoceptive feedback.

Preparatory Responses in FTLD

Frontotemporal lobar degeneration syndromes (FTLD) provide an ideal model for studying the preparatory responses. FTLD consists of a group of clinically, genetically, and pathologically related clinical disorders, including behavioral variant frontotemporal dementia (bvFTD), semantic variant primary progressive aphasia (svPPA), non-fluent variant primary progressive aphasia (nfvPPA), corticobasal syndrome (CBS), and progressive supranuclear palsy (PSP). In FTLD, neurodegeneration commonly occurs in frontal and anterior temporal brain regions,[5] which overlap with the aforementioned brain regions that may be involved in generating preparatory responses. Patients with FTLD also commonly develop behavioral symptoms such as apathy and impulsivity/disinhibition,[6, 7] which could reflect altered preparatory responses that then contribute to inappropriate subsequent behaviors (or lack thereof). For instance, in the example above, patients may have difficulty activating avoidance behaviors to contaminated food because their heart rate has not increased enough to support the somatic adjustments needed for such behaviors.

Although preparatory responses have not been studied in FTLD (nor in healthy adults), numerous studies have demonstrated diminished physiological responses in patients with bvFTD either after stimulus onset or when the emotional valence of an upcoming stimulus is revealed. Compared to healthy controls (HCs), patients with bvFTD have shown diminished physiological responses to disgust-eliciting films[8] and unpleasant smells.[9] Orienting responses to emotional stimuli,[10-12] which are typically characterized by decreased heart rate, are also diminished in bvFTD.[13] Patients with bvFTD also showed smaller skin conductance responses when they were told that an unpleasant smell would be delivered in 15 seconds. Importantly, these impairments have been associated with structural degeneration in the vmPFC and SN.[14-16]

The Present Study

The present study examines preparatory responses in FTLD. We quantified preparatory responses as decreases in cardiac inter-beat intervals (IBI; or increases in heart rate) from a pre-trial *baseline* period to an *instruction* period when participants were told that they would be watching a film clip but had not yet been provided information about the emotional valence. We focused on changes in IBI because they serve an essential role in providing metabolic support for somatic motor activities[17] that are important for subsequent coping behaviors. Changes in IBI also happen more rapidly than changes in other physiological measures (e.g., electrodermal responses and skin temperature)[18], allowing us to observe preparatory responses that could be very transient before the stimulus onset. We also quantified orienting responses as IBI changes from the *instruction* period to the first six seconds of the *film* clip. This enabled us to determine whether these two different responses were similarly affected across diagnostic groups and whether they are associated with different neural correlates. To determine whether diminished preparatory responses helped explain often-observed clinical symptoms in FTLD, patients' behavioral symptoms of apathy and disinhibition[6, 7] were assessed using the Neuropsychiatric Inventory (NPI).[19]

We made three hypotheses. First, because FTLD targets frontal and temporal regions of the brain (particularly the vmPFC and SN in bvFTD),[5] we hypothesized that FTLD as a group would have impaired preparatory responses compared to HC and Alzheimer's disease (AD), which is characterized by different patterns of neurodegeneration and clinical symptoms [20, 21] and that this impairment would be strongest in bvFTD. Second, we hypothesized that FTLD would exhibit greater behavioral symptoms than AD,[6, 7] and that these group differences would be mediated by greater impairments in preparatory responses in FTLD. Third, consistent with the neural circuitry described above (Fig. 1A), we hypothesized that greater impairment in preparatory responses would be associated with smaller gray matter volume and lower resting functional connectivity within the vmPFC and regions of the SN (e.g., vmPFC-ACC, AI-ACC).

Materials and methods

Participants

Participants included 276 patients (76 bvFTD, 38 svPPA, 31 nfvPPA, 36 CBS, 33 PSP, and 62 AD) and 38 HCs. All patients were recruited from the Memory and Aging Center (MAC) at the University of California, San Francisco (UCSF) between 2006 and 2016 in a collaborative research project between the MAC and the Berkeley Psychophysiology Laboratory at the University of California, Berkeley (UCB). At UCSF, patient diagnoses were determined by a multidisciplinary team that consisted of neurologists, nurses, clinical psychologists, and neuroscientists (by reviewing clinical interviews and patients' neurological, neuropsychological, neuroimaging testing data) using current research criteria for bvFTD,[22] svPPA, nfvPPA,[23] CBS,[24] PSP,[25, 26] and AD.[20] HCs without a history of neurological or psychiatric disorders were recruited from the community via advertisements.

Procedure

All participants first visited UCSF, where they underwent detailed clinical interviews (with their caregivers), neurological examination, functional assessment, neuropsychological evaluation, structural MRI, and resting-state functional MRI (rs-fMRI). Following this UCSF visit (4 months for patients and 12 months for HCs), participants visited UCB for a comprehensive assessment of emotional functioning[27]. Informed consent was obtained upon arrival at both sites. Procedures were approved by the UCSF and UCB Institutional Review Boards.

The present study focused on a film-viewing task, which was the first task in the UCB assessment. Before the task, non-invasive physiological sensors were applied to the participants. The task consisted of three trials. Participants were informed that they would be watching several short films. Each trial began with a 60-second *baseline period* that started with participants being asked to watch an "X" on the center of the screen (Fig. 2A). Next, there was a six-second *instruction period* during which the screen displayed: *"Please watch the film. Say stop if you need the film stopped."* After the instruction period, there was a *film period* (86-106 seconds) in which participants watched a film selected to induce amusement (trial one), sadness (trial two), and disgust (trial three).[8, 28, 29] The order of the films was fixed across participants. For additional details about the procedure, Supplemental Procedure.

Later in the UCB assessment, participants also completed an acoustic startle task, where they sat for a 60-second baseline and heard a brief (100-ms) and loud (115-dB) burst

of white noise without warning. Our previous research[30] has demonstrated that this task produces marked physiological responses in HCs and patients with FTLD and AD. In the present study, IBI change in response to this simple, loud sensory stimulus was included as a covariate to adjust for individual differences in *overall physiological responding*.

Physiological Measures

Data acquisition and processing

Physiological data including electrocardiogram (ECG) and other physiological measures (e.g., electrodermal, somatic, respiration; data not presented here) were obtained using a BIOPAC MP150 system. For ECG, Beckman miniature electrodes with Redux paste were placed on opposite sides of the participant's chest, which were connected to a BIOPAC ECG100C amplifier, and a computer with analog-to-digital capability that sampled the signal at 300Hz. Using a program written by R.W.L., IBI was calculated as the interval between successive R-waves and then averaged every second. Trained research assistants examined the second-by-second data to identify and remove artifacts. Among the 314 participants enrolled in this study, 29 were excluded from analyses due to poor data quality (e.g., excessive movement artifact; Supplemental Physiological Methods describes details about data exclusion). The remaining 285 participants included 67 bvFTD, 35 svPPA, 30 nfvPPA, 32 CBS, 30 PSP, 56 AD, and 35 HC. Table 1 shows their sociodemographic and functional characteristics.

Preparatory physiological responses

Preparatory responses were quantified as the *change* in the averaged IBI of the last three seconds of the baseline period and seconds 4-6 of the instruction period (Fig. 2B; time windows W2 - W1). Decreased IBI values correspond to increased heart rate, representing greater preparatory responses. The first three seconds of the instruction period were not included because preparatory responses may still be building at this time (Fig. 2B). In preliminary analyses, a repeated-measures ANOVA (3 trials x 7 diagnostic groups) did not reveal any significant effects for trial order ($F(2, 556) = 0.90$, $P = 0.41$) or trial x diagnostic group interactions ($F(12, 556) = 1.26$, $P = 0.24$). Therefore, responses from all trials were averaged.

Orienting responses

Preliminary analyses revealed an IBI increase in comparison groups (i.e., AD and HC) that generally started at the onset of the film and peaked approximately four to six seconds after film onset. Therefore, orienting responses were quantified as *changes* in average IBI from the last three seconds of the instruction period to seconds 4-6 of the film period (Fig. 2B; time windows W3 – W2). Increased IBI values correspond to decreased heart rate, representing greater orienting responses. Like preparatory responses, the first three seconds of the film were not included in the analyses because orienting responses were still building during this time. Responses from all trials were averaged.

Functional Measures

Dementia severity

The Clinical Dementia Rating Scale (CDR)[31] assessed dementia severity. CDR total score (CDR-Total; 0 = normal, 0.5 = very mild dementia; 1 = mild dementia, 2 = moderate dementia, 3 = severe dementia) and the sum of boxes score (CDR-Box; range: 0 to 18, with higher values indicating greater severity) were used. Using the same approach as previous studies,[32, 33] CDR-Total was used to determine participants' eligibility to be included in functional connectivity analyses (i.e., participants with CDR > 1 were ineligible due to severe loss of brain tissue). CDR-Box was added as a covariate because higher scores typically correlate with greater severity of neurodegeneration.[34]

Cognitive functioning

The Mini-Mental State Exam (MMSE)[35] was used to assess global cognitive functioning. MMSE scores range from 0 to 30, with higher values indicating greater cognitive functioning. Scores were added as a covariate to ensure our findings did not simply reflect patients' cognitive impairment.

Overall physiological responding

Overall physiological responding was quantified as *changes* in average IBI from the last three seconds of a baseline period to the first 6 seconds after the presentation of the loud noise in the acoustic startle task. We chose this 6-second time window because our preliminary analysis revealed an overall IBI decrease (or heart rate increase) during this period (Table 1 and Supplemental Fig. S1). IBI change scores were inverted so higher values

corresponded to larger responses to the loud noise. We used this as a covariate to ensure any preparatory response findings did not simply reflect changes in overall physiological responding.

Apathy and disinhibition

The NPI was administered by conducting a semi-structured interview with each patient's caregiver. The NPI included 12 neuropsychiatric symptoms that are frequently seen in neurodegenerative disorders.[7, 19] The present study focused on apathy and disinhibition—the two symptoms that are the most prominent in FTLD. [6, 7] Higher scores reflected more frequent or severe symptoms.

Neuroimaging Measures

Data acquisition and preprocessing

We obtained structural MRI data using 1.5T (n = 9), 3T (n = 176) or 4T (n = 37) research-quality scanners for 222 participants (43 bvFTD, 30 svPPA, 27 nfvPPA, 31 CBS, 28 PSP, 43 AD, 20 HC). MRIs were visually inspected for scan quality (e.g., no motion or metal artifact). We utilized statistical parametric mapping version 12 (SPM12) default parameters for preprocessing structural MRI data (for details, see Supplemental Neuroimaging Methods). We also characterized the areas of neurodegeneration for each patient group by examining structural differences in gray matter maps between each patient group and HC. These results are presented in Supplemental Fig. S2**.**

Task-free functional MRI images were also obtained in a subsample of 117 participants (17 bvFTD, 14 svPPA, 19 nfvPPA, 17 PSP, 22 CBS, 20 AD, 8 HC) who were scanned on the 3T scanner. Participants were instructed to relax with their eyes closed for 8 minutes. Rs-fMRI data were preprocessed using SPM12. Node-pair intrinsic connectivity analysis[36, 37] was applied to identify the functional connectivity between our hypothesized brain regions that support preparatory responses. Within each participant, pairwise correlation coefficients were calculated between a set of cortical and subcortical regions-of-interest (ROIs), including the vmPFC, ACC, amygdala, hypothalamus, PAG, thalamus, and AI. MARSBAR was used to create spherical ROIs centered on Montreal Neurological Institute (MNI) coordinates based on previous studies.[38-40] Supplemental Neuroimaging Methods describes parameters for data preprocessing including MNI coordinates for ROIs. To test our hypothesized neural circuit, we calculated regional summary scores by averaging each

participant's correlation coefficients (within and between hemispheres) within the following pairs of nodes: (a) vmPFC and ACC, (b) ACC and all subcortical regions combined—including the amygdala, hypothalamus, and PAG, (c) thalamus and AI, and (d) AI and ACC, and (e) AI and vmPFC. For each participant, we averaged these five correlation coefficients to obtain an *overall index* of functional connectivity for our hypothesized circuit.

Statistical Analysis

To test Hypothesis #1, we performed a one-way ANOVA to determine diagnostic group differences in preparatory responses. To compare, we performed the same analysis for orienting responses. To ensure our findings were robust, we repeated these analyses using ANCOVAs and included covariates that significantly differed between diagnostic groups (i.e., age, gender, dementia severity [CDR-Box], and cognitive functioning [MMSE]; Table 1). Significant group effects were followed by two-tailed *post-hoc* comparisons using the *Bonferroni* method to correct for multiple comparisons.

To test Hypothesis #2, we first performed bivariate correlations (two-tailed) to evaluate the associations between preparatory/orienting responses and apathy and disinhibition scores. We next performed independent-sample *t* tests to determine whether the previously reported group differences in apathy and disinhibition between FTLD and AD[6, 7] would be observed in our sample. We then conducted two mediation analyses (using SPSS PROCESS 3.4.1 default parameters)[41] to test whether group differences (FTLD = 1 vs. AD = 0) in disinhibition and/or apathy were mediated by levels of preparatory/orienting responses. To ensure findings were robust, we repeated these analyses and included overall physiological responding (i.e., IBI changes in response to the acoustic startle stimulus), which significantly correlated with preparatory responses (Supplemental Table S1).

To test Hypothesis #3, whole-brain voxel-based morphometry (VBM) analyses were performed, using a multivariate linear regression to examine areas of smaller volume associated with smaller preparatory/orienting responses. We examined statistical maps and reported findings at $P_{FWE} < 0.05$. The minimum cluster size reported was 350mm^3. We ran 5000 permutation analyses to derive a study-specific error distribution[42] using vlsm2[43] (see Supplemental Neuroimaging Data Analysis for more details). Analyses were adjusted for six diagnostic dummy variables (1 = patient diagnosis of interest; 0 = remaining groups) to ensure that our findings did not simply reflect diagnostic differences), two dummy variables for three different scanner types, total intracranial volume (TIV; to account for head size),

and two functional covariates that significantly correlated with preparatory responses (i.e., dementia severity and overall physiological responding; Supplemental Table S1). For functional connectivity analyses, bivariate correlations and linear regressions were performed to examine the associations between preparatory/orienting responses and overall and node-pair connectivity. All analyses were adjusted for six diagnostic dummy variables and two covariates that significantly correlated with preparatory responses (i.e., age and overall physiological responding; Supplemental Table S1). We also performed analyses without adjusting for these covariates and present these results in Supplemental Table S2.

For all analyses, effects with $P < 0.05$ were considered statistically significant.

Data Availability

Study data are available upon request from the corresponding author. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Results

Diagnostic Group Differences

Preparatory physiological response

When comparing FTLD (all syndromes combined), AD, and HC, an ANOVA revealed a group effect, $F(2, 282) = 11.80$, $P < 0.001$, Fig. 2C. Pair-wise *post hoc* comparisons indicated that the FTLD group had smaller preparatory responses (i.e., less pronounced IBI decreases or heart rate increases) than AD ($P < 0.001$) and HC ($P = 0.002$). No significant group differences emerged between HC and AD. When comparing each of the five FTLD syndromes to HC and AD, an ANOVA revealed syndrome group effects, $F(6, 278) = 5.17$, $P < 0.001$. *Post hoc* comparisons between each FTLD syndrome and AD or HC (total *n* of comparisons = 10; *Bonferonni* corrected) indicated smaller preparatory responses in bvFTD than in AD and HC (Ps < 0.001). PSP also had smaller responses than AD ($P = 0.036$). No other statistically significant comparisons emerged between FTLD syndromes and comparison groups.

Additional analyses were performed to examine the robustness of the findings above. To ensure these effects were not driven by demographic or functional differences between diagnostic groups, we repeated the analyses above with variables that significantly differed between groups as covariates. To ensure our findings from analyzing averaged IBI during seconds 4-6 of the instruction period were robust, we repeated our analyses using averaged IBI during the entire six seconds of the instruction period. To ensure our findings were not biased by increased knowledge about the task after the first trial, we repeated the above ANOVAs while replacing the averaged preparatory responses across all three trials with preparatory responses from only the first trial. To ensure our effects were not driven by participants' incorrect belief that the films would all be negatively valenced, we analyzed preparatory responses in the second trial only, which took place after participants watched the first trial's amusement film (and thus realized the films could also be positive). These tests of robustness supported the group effects reported above (*P*s < 0.05; Supplemental Fig. S3-S4).

Orienting response

ANOVAs did not reveal any group differences between FTLD, AD, and HC, $F(2, 272) = 1.22$, $P = 0.30$, or between each FTLD syndrome, AD, and HC, $F(6, 278) = 1.67$, $P = 0.13$, Fig. 2D.

Mediation Effects

Preparatory physiological response

Prior to data analyses, we inverted preparatory response scores, so that higher values corresponded to greater responses. Correlation analyses revealed an association between lower preparatory responses and greater disinhibition ($r = -0.18$, $P = 0.004$). A *t*-test revealed FTLD displayed more disinhibition than AD, $t(1, 239) = 4.92$, $p < 0.001$, Cohen's $d = 0.85$. Mediation analyses revealed that this group difference was mediated by lower preparatory responses (standardized indirect effect = 0.07, 95% CI [0.0030, 0.1591], accounting for 9.11% of the total effect), Fig. 3. The mediation effect remained marginally significant when the analyses adjusted for overall physiological responding (standardized indirect effect = 0.06, 90% CI [0.0010, 0.1331], accounting for 8.15% of the total effect, Supplemental Fig. S5).

Correlation analyses also revealed an association between lower preparatory responses and greater apathy (r = -0.13, P = 0.048). In addition, apathy was also greater in FTLD than AD, $t(1, 239)$= 2.92, p = 0.004, Cohen's d = 0.47. However, this group difference was not significantly mediated by preparatory responses (Supplemental Fig. S6).

Orienting response

Larger orienting responses were correlated with greater apathy (r = 0.18, P = 0.004) but not disinhibition (r = 0.09, P = 0.18). No mediation effects emerged for orienting responses (Supplemental Fig. S7).

Structural Neural Correlates

Preparatory physiological responses

A whole-brain VBM analysis revealed that smaller preparatory responses were associated with smaller gray matter volume in three large clusters (Table 2, Fig. 4): vmPFC, extending to the anterior ACC and bilateral caudate; right AI, extending to the right superior temporal pole, right rolandic operculum, and Heschl's gyrus; and left ventral AI, extending to the left inferior orbital frontal gyrus and left superior temporal pole.

Orienting response

No neural correlates emerged for orienting responses.

Functional Connectivity Neural Correlates

Preparatory physiological responses

Correlation analyses revealed that smaller preparatory responses were associated with weaker connectivity between (a) the vmPFC and ACC (r = 0.22, P = 0.022), (b) ACC and subcortical SN regions (r = 0.21, P = 0.032), and (c) AI and ACC (r = 0.29, P = 0.002). We also observed an association between smaller preparatory responses and weaker overall connectivity within the vmPFC-SN circuit (r = 0.30, P = 0.001; Fig. 1B and Supplemental Table S2).

To ensure these findings were specific to our hypothesized vmPFC-SN circuit, we included a "control" brain network—the sensorimotor network (SMN; Supplemental Neuroimaging Data Preprocessing describes methods for computing SMN's overall connectivity). A correlation analysis did not reveal a relationship between preparatory responses and SMN connectivity ($r = 0.05$, $P = 0.576$; also see Supplemental Table S2). A linear regression including both the vmPFC-SN and SMN overall connectivity in the same model revealed that only the vmPFC-SN's connectivity predicted levels of preparatory responses ($\beta = 0.33$, $P = 0.001$; Table 3, model 1).

Next, within our hypothesized vmPFC-SN circuit, we determined which pair(s) of node-to-node connectivity were specifically critical for preparatory responses. When the SMN overall connectivity was adjusted, a linear regression with all five node-pairs entered (Table 3, model 2) revealed an association between lower AI-ACC connectivity and smaller preparatory responses ($\beta = 0.23$, $P = 0.043$). No other significant effects were found.

Orienting response

We performed the same correlation and linear regression analyses for orienting responses but did not find any significant effects (Supplemental Table S2 and Table 3).

Discussion

We found that FTLD, specifically bvFTD, exhibited smaller preparatory responses than AD and HCs. We also observed similar but somewhat weaker effects for PSP. No group differences were found for CBS, svPPA, or nfvPPA when compared to AD and HCs. Mediation analyses revealed that smaller preparatory responses mediated the effect of greater disinhibition (but not apathy) in FTLD but not in AD. Findings from both structural neuroimaging and functional connectivity analyses suggest that preparatory responses may be served by a neural circuit involving the vmPFC and the SN. Smaller gray matter volumes and lower functional connectivity within this circuit (particularly between the AI and ACC) were both associated with smaller preparatory responses. We also examined orienting responses but did not find any group differences, mediation effects, or neural correlates.

Diminished Preparatory Physiological Responses in FTLD

Diagnostic group differences

Supporting our first hypothesis, FTLD as a group was associated with an impairment in preparatory responses, with findings being most significant for bvFTD and PSP groups. Our hypothesis that all FTLD syndromes would demonstrate impairment was not supported, although preparatory responses in svPPA and CBS were in the hypothesized direction. These physiological findings parallel recent studies reporting different degrees of behavioral changes in different FTLD syndromes. Importantly, across studies, bvFTD is typically found to show the greatest impairment, followed by svPPA, PSP or CBS; nfvPPA typically shows the least impairment compared to the other FTLD syndromes.[6, 44-46] Taken together, these findings expand upon the FTLD literature by providing further evidence of a *spectrum* of impairment in physiological and behavioral functioning across FTLD syndromes.[6, 44, 45]

Supporting our second hypothesis, preparatory response impairments mediated the effect of greater disinhibition in FTLD as compared to AD. The activation of the automatic nervous system (ANS), and the cardiovascular system in particular, supports changes in muscle activity that are critically involved in behavioral adjustments (e.g., fighting, fleeing, freezing, approaching).[17, 47] In FTLD, reduced ANS activation before a stimulus onsets may hinder the subsequent behavioral adjustments needed to address positive and negative emotional challenges. Therefore, patients may be less prepared physiologically to mount subsequent withdrawal behaviors when negative stimuli make them feel distressed,[48] mount subsequent approach behaviors when positive stimuli make them feel pleasant, or inhibit initial responses that are inappropriate to the current situation.[6, 49] Interestingly, we did not find similar mediation effects on apathy. We suspect this may be due to the smaller group differences in apathy (Cohen's $d = 0.47$) than disinhibition (Cohen's $d = 0.85$). In addition, the NPI apathy score only reflected the overall severity of apathetic behaviors. Future studies are needed to systematically investigate the specific aspects of apathy (e.g., loss of interest in activities vs. low motivation) that are affected by impaired preparatory responses.

Our findings let us reject several alternative hypotheses concerning preparatory responses. The first alternative hypothesis was that our results simply reflected patients' inattention or lack of orientation to the computer monitor during the instruction period. This hypothesis is unlikely because orienting responses to external stimuli are associated with a

rapid increase in IBI.[12, 50] During the instruction period of our study, the comparison groups exhibited decreased IBI, indicating preparatory responses, rather than the increased IBI that would have been consistent with the orienting response. Notably, bvFTD patients did show IBI changes consistent with the orienting responses (i.e., increased IBI) during the film period. This suggests that their ability to orient remained intact. A second alternative hypothesis is that our findings may reflect a general lowering of ANS functioning associated with older age or FTLD.[51, 52] However, we did not find significant group differences in patients' overall physiological responding (which is consistent with the literature[30, 53] indicating that physiological responding to simple stimuli remains relatively intact in early stage of FTLD). Importantly, our main findings remained robust after adjusting for individual differences in age and overall physiological responses. These findings together undercut the likelihood of these alternative hypotheses accounting for our findings.

Neural correlates

The neuroimaging findings support our hypothesis that preparatory responses would be influenced by a circuit that involves the vmPFC and the SN. First, we found that smaller preparatory responses were associated with smaller gray matter volumes in the bilateral vmPFC and weaker connectivity between the vmPFC and the ACC. In the current experimental context, we believe the vmPFC is involved in stimulus evaluation (based on past experiences, social norms, etc.) and generating predictions for the future, particularly during uncertainty.[2, 54, 55] The vmPFC is strongly connected to the ACC—a cortical area critical for response preparation, initiation, and monitoring—including controlling the ANS via activating subcortical regions such as the PAG, which is critical for the propagation and modulation of sympathetic (SNS) and parasympathetic (PNS) activities.[56-58] Co-activation of the vmPFC and ACC is often found in decision-making tasks that involve anticipation with uncertainty.[59, 60] In our study, smaller vmPFC volumes may make patients less attentive to the cues indicating that a film will start soon (e.g., instructions "*Please watch the film*"). It may also impair patients' ability to retrieve semantic knowledge or similar memories from the past (e.g., from prior trials) and compare them with the current situation to predict the salience of upcoming emotional stimuli. The loss of functional connectivity between the vmPFC and ACC may lead to the ACC receiving partial or inappropriate signals from the vmPFC, leading to reduced ANS activation that can compromise preparation for coping with the upcoming emotional stimulus.

Second, we found smaller preparatory responses were associated with smaller gray matter volumes in the bilateral AI and weaker functional connectivity between AI and ACC—above and beyond all other node pairs examined in this study. The AI receives interoceptive signals from the body—including those reflecting cardiac activity—via relays in the thalamus and posterior insula.[61] It has been argued that the AI integrates such interoceptive signals with input from other brain regions, interprets the meaning of these signals, and generates representation into conscious awareness. The outputs of AI go to the ACC for simultaneous monitoring of current responses, detection of errors, and preparation for future actions including changes in the ANS.[57, 62, 63] Co-activation of AI and ACC has often been noted in studies with emotional tasks.[64, 65] In our study, the AI-ACC connection may be particularly important during physiological preparation. Input from the AI (and vmPFC) may enable the ACC to compute the predicted requirements of the body (i.e., homeostatic and coping behavior needs) relative to its current status, which in turn can activate or deactivate the ANS.[66] In FTLD, declines in the AI structure and its connectivity to the ACC may result in partial and inaccurate interoceptive information to the ACC, leading the ACC to underestimate the amount of ANS changes needed for the body to prepare for upcoming emotional stimuli.

Third, functional connectivity analyses also revealed that weaker *overall connectivity* among nodes in the vmPFC-SN circuit was associated with smaller preparatory responses. This relationship was not found between preparatory responses and the SMN, which underscores the specific contribution of the vmPFC-SN circuit to preparatory responding. Interestingly, several nodes in the functional connectivity analyses (e.g., posterior ACC) did not emerge in our VBM analyses. In neurodegenerative diseases like FTLD, functional decline of brain tissue typically precedes permanent structural loss.[67, 68] Additionally, disproportionate progression in gray matter tissue (e.g., ACC) versus white matter tract loss (e.g., AI to ACC) may also occur, especially in CBS and PSP.[69] Thus, differences between our structural and functional findings may reflect the pathological complexity in neurodegenerative diseases, highlighting the need for deploying a multi-imaging-method approach in research and clinical practice.

The VBM analyses also revealed that smaller preparatory responses were associated with smaller volumes in the anterior temporal lobe and dorsal striatum (i.e., caudate). The anterior temporal lobe is strongly involved in social cognition including processing social concepts.[70-72] In our study, patients with volume loss to this region may have encountered

difficulties in accessing the meanings of the social context (i.e., participating in a study of emotion and being asked to watch films). Such information may be necessary for the vmPFC to predict the salience level of the upcoming emotional stimuli. The dorsal striatum implements motor planning,[73, 74] thus volume loss to this region may impair patients' ability to strategize the sequence of motor actions needed for the next moment and the amount of ANS changes required for these actions. Importantly, both the anterior temporal lobe and dorsal striatum have strong connections to the vmPFC to form a "semantic appraisal network" (along with other brain regions such as the orbital gyrus)[33]. Therefore, our VBM findings raise the possibility that, in addition to our hypothesized vmPFC-SN circuit, other brain regions/networks might also contribute to diminished preparatory responding. Interestingly, past research has often reported the ventral striatum and amygdala as being involved in the anticipation of positive and negative emotional stimuli, respectively. Nevertheless, our VBM analyses did not reveal any significant effects in these regions. While there are many factors that might account for these non-significant findings, including study design (e.g., monetary rewards vs. emotional films), patient disease severity (i.e., structural declines in these regions may occur in later disease stages), and statistical thresholds, these findings also indicate that generating preparatory responses may not require evaluating the valence of an upcoming emotional stimulus.

Orienting Responses in FTLD

Interestingly, our analyses did not reveal any diagnostic group differences, mediation effects, or neural correlates for orienting responses, which is not consistent with the existing literature.[13-16] One factor that may contribute to these disparate findings is that these previous studies typically did not include an "instruction period" that preceded the stimulus. The instruction period in our study may have attenuated the magnitude of orienting responses by making the timing of the stimulus onset more predictable. In addition, in our study, orienting and preparatory responses occurred in proximity, which could have obscured effects for both responses—particularly for orienting responses, which may have overlapped with the late phase of preparatory responses. Future studies will benefit from including trials with and without the instruction period to systematically compare preparatory versus orienting responses in FTLD and their neural correlates.

Implications

Findings of our study have several important implications. First, our findings advance clinical characterizations of emotional and physiological responding in FTLD. Importantly, behavioral disinhibition is one prominent characteristic in FTLD; it is also a diagnostic criterion for bvFTD and PSP.[22, 26] This is consistent with our findings that impairment in preparatory responses are (a) found most prominently in bvFTD and PSP, and (b) mediate the effect of greater disinhibition in FTLD than in AD. Given the strong association between cardiovascular ANS responding and somatic muscle activity,[47] our findings suggest that impaired preparatory responses may be one source for the behavioral symptoms in FTLD. Second, contemporary neuroscience models argue the brain is a "predictive machine," which constantly integrates exteroceptive and interoceptive information from current and past events in order to make predictions about what the brain and body will need in the next moment.[66, 75] Most of these models speculate AI-ACC-vmPFC interactions are critical for making such predictions. Our neuroimaging findings provide empirical support for these models and highlight the importance of investigating whether other brain regions are also involved (e.g., dorsal striatum, anterior temporal regions) or less important in (e.g., ventral striatum, SMN) in making these predictions. Third, methodologically, prior research has typically treated responses prior to stimuli onsets as the baseline and either excluded or adjusted for these responses in data analyses. Our findings suggest that responses during this pre-stimulus period may reflect important psychological processes. While between-group differences may emerge before stimulus onset, future research may benefit from carefully evaluating the dynamic change of responses over time.

Strength and Limitations

Our study had several strengths, including: (a) examining physiological processes during a preparatory time period that have been largely overlooked; (b) using a large sample across the full spectrum of FTLD and AD and including HC, thus enabling us to evaluate diagnostic specificity/generalizability, maximize neuroanatomical and behavioral heterogeneity, and increase statistical power; (c) utilizing both VBM and functional connectivity analyses, allowing us to examine structural and functional changes associated with diminished preparatory responses; (d) examining physiological responding preceding a range of emotional films, which increases the generalizability of findings; (e) testing a

number of alternative hypotheses (e.g., orienting responses, SMN connectivity) and covariates (e.g., overall physiological responding, disease severity), which helped rule out the possibility that our findings simply reflected confounding influences.

Our study also had limitations: (a) we did not include a control trial in which participants were told to wait for an emotionally neutral film to start; thus, it remains undetermined whether preparatory responses only occur preceding emotional stimuli; (b) seeing the sentence "say stop if you need the film stopped" during the instruction period may have made some participants falsely believe that all films were negatively valenced. Although our additional findings (Supplemental Fig. S4) suggest that this might not affect our results, future studies without this sentence are needed to determine whether preparatory responding occurs similarly before positive and negative stimuli; (c) our node-pair connectivity analyses only focused on the pairs driven from our hypothesized model but not those outside the model; (d) although our hypothesized model suggests neural processes to be sequential and directional, our analyses only tested simultaneous covariations between nodes.

Conclusion

This is the first study to examine preparatory responses that occur prior to the onset of emotional stimuli and their neural correlates. We report (a) FTLD, particularly bvFTD and PSP, had impaired preparatory responses; (b) impairment in preparatory responses explained greater disinhibition—an often-observed behavioral symptom in FTLD; (c) smaller preparatory responses were associated with smaller volumes and lower functional connectivity in a brain circuit that involves the vmPFC and SN. These findings advance our knowledge of how FTLD can negatively impact patients' emotional and physiological responding and produce behavioral symptoms. These findings also shed light on how predictions and preparations are made in the brain to help our bodies physiologically prepare for everyday challenges and opportunities.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1. A brain circuit for preparatory physiological responses. **(1A)** A hypothesized circuit. The dashed black box represents the entire circuit. The five solid black boxes represent cortical and subcortical regions involved in this process. The back arrows represent signal flows. **(1B)** Functional connectivity results. The blue font by the yellow lines indicates the correlation coefficients between each node-to-node connectivity (e.g., AI-ACC, AI-vmPFC) and preparatory physiological responses; the blue line indicates the correlation coefficient between preparatory physiological responses and the vmPFC-SN circuit's overall functional connectivity. Note that prior to data analyses, connectivity between the three subcortical efferent regions (i.e., amygdala, hypothalamus, PAG) and ACC were averaged together. In addition, connectivity between and within each hemisphere were also averaged for each pair of brain regions (nodes) of interests. ACC = anterior cingulate cortex; AI = anterior insula; Amy = amygdala; Hyp = hypothalamus; PAG = periaqueductal gray; Thal = thalamus; vmPFC = ventromedial prefrontal cortex. $^{\dagger}P < 0.10$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Figure 2. Task procedures and the quantification of preparatory and orienting physiological responses across diagnostic groups. **(2A)** Task procedure. The film watching task consisted of three trials. In each trial, participants sat for a 60-second baseline period and then were presented with instructions for 6 seconds, which informed them the film was about to start. Immediately following the instructions, participants watched a film clip that lasted between 87-106 seconds. **(2B)** Averaged time series of cardiac inter-beat intervals (IBI) across all three film trials for the seven diagnostic groups. Preparatory physiological responses were quantified as IBI change from the last 3 seconds of the rest period to the last 3 seconds of the instruction period (i.e., periods B-A). Orienting responses were quantified as IBI changes from the last 3 seconds of the instruction period to the second 3 seconds of the film period (i.e., period C-B). **(2C)-(2D)** Averaged preparatory and orienting responses by diagnostic group, *Mean* ± 95% confidence intervals. Annotations indicate significant or trending effects as compared to the two comparison groups (i.e., AD and HC) revealed by ANOVA and *post-hoc* comparisons. FTLD = frontotemporal lobar degeneration; bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal

syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy control. $^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Figure 3. Results of mediation analysis. Preparatory physiological responses as a mediator for the effects of greater disinhibition in FTLD (vs. AD). Standardized indirect effect = 0.07, 95% CI [0.0030, 0.1591], accounting for 9.11% of the total effect. FTLD = frontotemporal lobar degeneration; AD = Alzheimer's disease.

Figure 4. Results of full-brain voxel-based morphometry (VBM) analyses. *T*-score map of brain areas for which smaller gray matter volume was associated with smaller preparatory physiological responses after adjusting for diagnostic group, scanner type (two scanner type variables), total intracranial volume, overall physiological functioning, and disease severity (CDR-Box). Three large clusters emerged in the (a) bilateral ventromedial prefrontal cortex (vmPFC) and caudate; (b) right anterior insula (AI), right superior temporal pole, right Rolandic operculum, and right Heschl's gyrus; and (c) in the left ventral AI, left orbitofrontal frontal gyrus, and left superior temporal pole ($P_{FWE} < 0.05$).

Table 1 Sociodemographic and functional characteristics of participants in main data analyses (N = 285).

		FTLD syndromes								
	Total Sample	**bvFTD**	**svPPA**	**nfvPPA**	**PSP**	**CBS**	**AD**	**HC**	***F/X²***	***P***
n	**285**	67	35	30	30	32	56	35		
Gender									14.91	0.02
Men	**150**	46	21	13	17	15	26	12		
Women	**135**	21	14	17	13	17	30	23		
Handedness									9.22	0.16
Right	**241**	60	34	26	22	29	46	24		
Left/Ambidextrous	**32**	6	1	4	7	3	9	2		
N/A	**12**	1	0	0	1	0	1	9		
Race									23.94	0.77
White/European American	**254**	60	31	26	24	29	52	32		
Black/African-American	**2**	0	0	0	0	1	1	0		
Latinx/Chicanx American	**10**	1	1	2	3	0	2	1		
Asian American	**16**	5	2	2	2	2	1	2		
Multi-racial/ prefer to self-describe	**2**	0	1	0	1	0	0	0		
N/A	**1**	1	0	0	0	0	0	0		
Age	**64.69 (7.82)**	62.19 (8.14)*	63.85 (5.85)	68.61 (7.02)	67.34 (6.75)	66.10 (5.61)	62.55 (8.75)*	66.85 (8.24)	4.69	<0.001
Education	**16.54 (3.10)**	16.12 (3.10)	16.60 (2.76)	16.47 (3.83)	17.41 (3.45)	16.26 (3.64)	16.38 (2.77)	17.21 (2.11)	0.88	0.51
Dementia Severity (CDR-Total)	**0.73 (0.56)**	1.157 (0.62)***	0.66 (0.42)***	0.48 (0.43)***	0.87 (0.39)***	0.63 (0.46)***	0.83 (0.38)***	0 (0)	28.75	<0.001
Dementia Severity (CDR-Box)	**4.04 (3.14)**	6.49 (3.18)***	3.87 (2.43)***	1.88 (1.94)**	5.60 (2.56)***	3.47 (2.39)***	4.40 (2.16)***	0 (0)	34.87	<0.001
Cognitive functioning (MMSE)	**23.93 (6.27)**	23.76 (6.87)***	24.14 (4.89)**	24.35 (6.06)**	25.60 (3.84)†	23.27 (7.16)***	20.66 (6.43)***	29.64 (0.57)	7.30	<0.001
Overall physiological responding (IBI changes in response to a loud white noise)	**-44.41 (72.63)**	-55.85 (88.53)	-51.75 (62.30)	-35.73 (60.39)	-17.90 (67.76)	-49.37 (69.12)	-30.91 (65.80)	-66.51(72.62)	1.85	0.09

Note. *MEAN* (*SD*). F/X^2 = Main effects of diagnostic groups revealed by one-way ANOVAs or chi-squared tests. Annotations indicate significant or trending effects (post hoc) as compared to the HC group. bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy control. †$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2 Structural neural correlates of preparatory physiological responses.

Anatomical Region	Volume mm^3	x	y	z	Max *T*	Corrected *P*
Left vmPFC	10969	-4	24	-9	4.67	0.0056
Right vmPFC	†					
Bilateral caudate	†					
Bilateral anterior ACC	†					
Right AI	7607	44	10	-12	4.31	0.0108
Right superior temporal pole	†					
Right rolandic operculum	†					
Right Heschl's gyrus	†					
Left ventral AI	3213	-36	20	-8	3.88	0.0284
Left inferior orbital frontal gyrus	†					
Left superior temporal pole	†					

Analyses adjusting for six diagnostic variables, scanner type, TIV, overall physiological responding (IBI change in response to a loud white noise), and disease severity (CDR-Box). Results considered significant at $P_{FWE} < 0.05$. ACC = anterior cingulate cortex; AI = anterior insula; vmPFC = ventromedial prefrontal cortex. †Signifies that these regions were included in the cluster above.

Table 3. Functional connectivity (linear regression model 1: overall connectivity; model 2: node-pair connectivity) correlates of preparatory physiological responses.

	Preparatory Physiological Responses				Orienting Responses			
	Model 1		Model 2		Model 1		Model 2	
	Beta	***P***	***Beta***	***P***	***Beta***	***P***	***Beta***	***P***
Diagnostic Covariates								
bvFTD	-0.02	0.906	-0.01	0.961	0.30	0.061	0.31	0.052
svPPA	-0.04	0.791	-0.01	0.926	0.06	0.667	0.06	0.717
nfvPPA	0.09	0.553	0.11	0.460	0.07	0.658	0.06	0.732
CBS	0.04	0.818	0.05	0.771	0.02	0.889	-0.01	0.945
PSP	0.03	0.816	0.04	0.793	0.00	0.980	-0.03	0.842
AD	0.17	0.255	0.18	0.231	0.00	0.999	-0.02	0.893
Demographic & Functional Covariates								
Age	**-0.18**	**0.048**	**-0.20**	**0.034**	-0.14	0.150	-0.13	0.200
Overall physiological responding	**0.29**	**0.001**	**0.31**	**0.001**	-0.12	0.201	-0.13	0.195
Functional networks								
SMN	-0.09	0.368	-0.14	0.195	-0.09	0.400	-0.07	0.541
vmPFC-SN	**0.33**	**0.001**			0.06	0.610		
vmPFC-ACC			0.15	0.152			0.00	0.979
ACC-Amy/Hyp/PAG			0.08	0.454			0.04	0.726
Thal-AI			-0.04	0.713			0.05	0.674
AI-ACC			**0.23**	**0.043**			0.06	0.659
AI-vmPFC			0.06	0.594			-0.10	0.401

Note. For preparatory physiological responses, higher values indicate larger responses (i.e., greater IBI decrease). For functional networks, analyses included our hypothesized vmPFC-SN circuit and a control SMN network. Italic font indicates node-pair connectivity within the vmPFC-SN network. bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; SMN = sensorimotor network; SN = salience network; ACC = anterior cingulate cortex; AI = anterior insula; Amy = amygdala; Hyp = hypothalamus; PAG = periaqueductal gray; Thal = thalamus; vmPFC = ventromedial prefrontal cortex. Bolded font indicates significant effects at the threshold of $P < 0.05$.

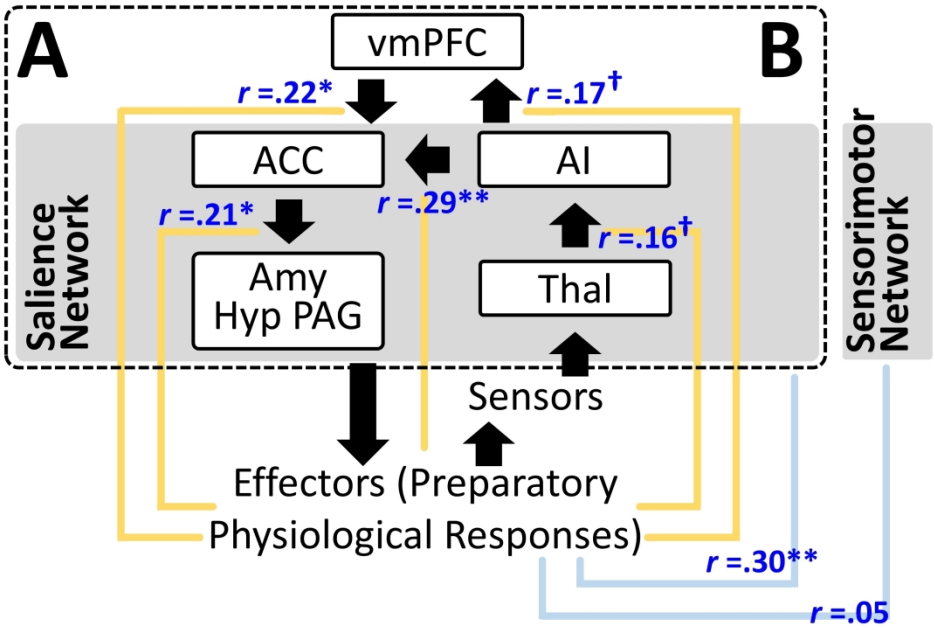

Figure 1. A brain circuit for preparatory physiological responses. (1A) A hypothesized circuit. The dashed black box represents the entire circuit. The five solid black boxes represent cortical and subcortical regions involved in this process. The back arrows represent signal flows. (1B) Functional connectivity results. The blue font by the yellow lines indicates the correlation coefficients between each node-to-node connectivity (e.g., AI-ACC, AI-vmPFC) and preparatory physiological responses; the blue line indicates the correlation coefficient between preparatory physiological responses and the vmPFC-SN circuit's overall functional connectivity. Note that prior to data analyses, connectivity between the three subcortical efferent regions (i.e., amygdala, hypothalamus, PAG) and ACC were averaged together. In addition, connectivity between and within each hemisphere were also averaged for each pair of brain regions (nodes) of interests. ACC = anterior cingulate cortex; AI = anterior insula; Amy = amygdala; Hyp = hypothalamus; PAG = periaqueductal gray; Thal = thalamus; vmPFC = ventromedial prefrontal cortex. †P < 0.10; *P < 0.05; **P < 0.01; ***P < 0.001.

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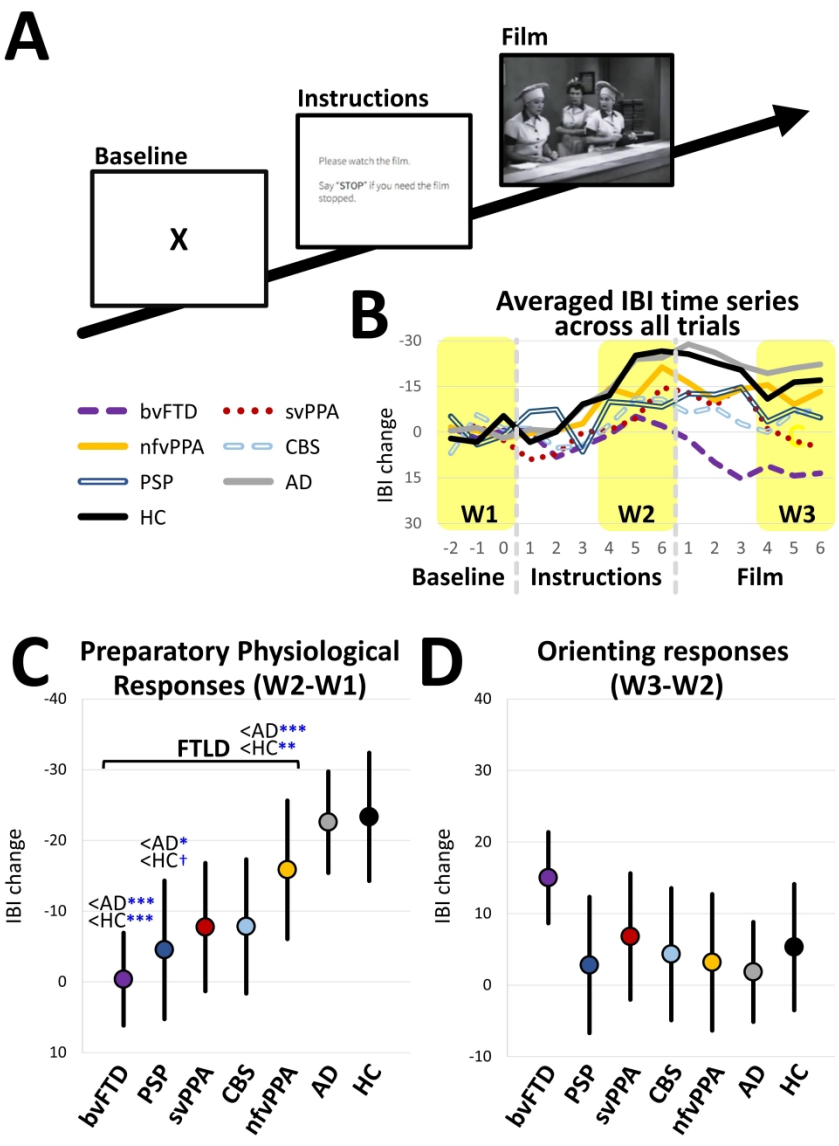

Figure 2. Task procedures and the quantification of preparatory and orienting physiological responses across diagnostic groups. (2A) Task procedure. The film watching task consisted of three trials. In each trial, participants sat for a 60-second baseline period and then were presented with instructions for 6 seconds, which informed them the film was about to start. Immediately following the instructions, participants watched a film clip that lasted between 87-106 seconds. (2B) Averaged time series of cardiac inter-beat intervals (IBI) across all three film trials for the seven diagnostic groups. Preparatory physiological responses were quantified as IBI change from the last 3 seconds of the rest period to the last 3 seconds of the instruction period (i.e., periods B-A). Orienting responses were quantified as IBI changes from the last 3 seconds of the instruction period to the second 3 seconds of the film period (i.e., period C-B). (2C)-(2D) Averaged preparatory and orienting responses by diagnostic group, Mean ± 95% confidence intervals. Annotations indicate significant or trending effects as compared to the two comparison groups (i.e., AD and HC) revealed by ANOVA and post-hoc comparisons. FTLD = frontotemporal lobar degeneration; bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive

supranuclear palsy; AD = Alzheimer's disease; HC = healthy control. †P < 0.10; *P < 0.05; **P < 0.01; ***P < 0.001.

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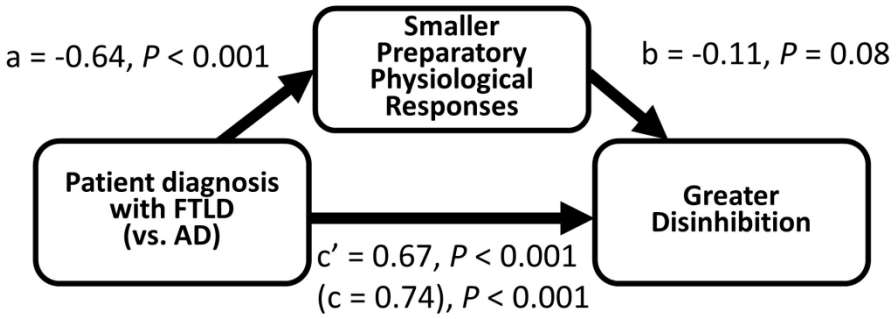

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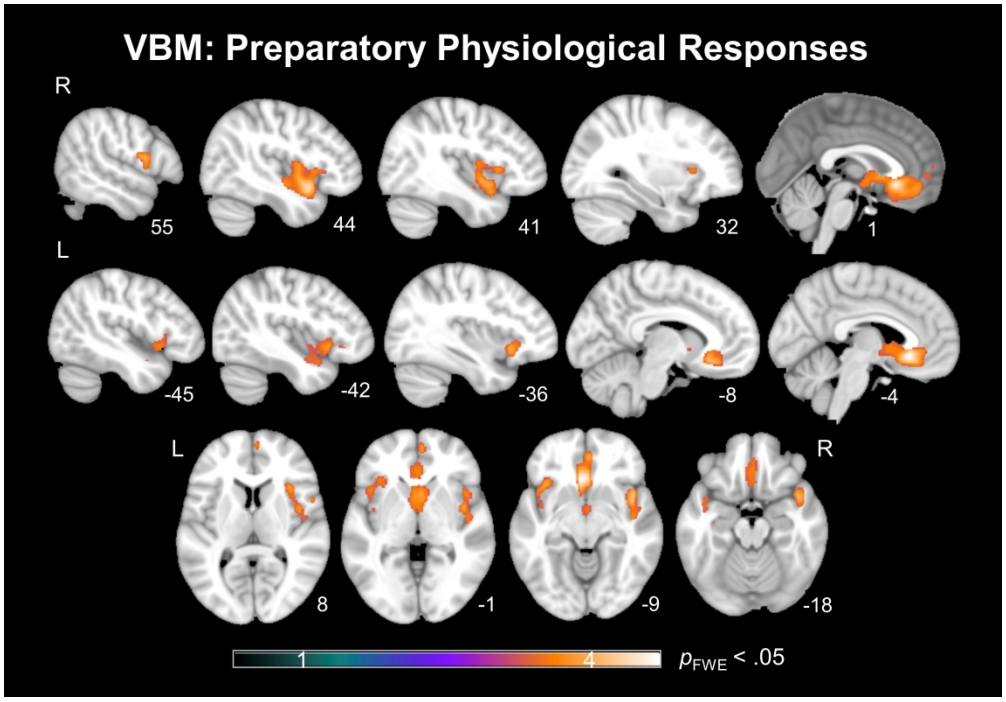


Figure 4. Results of full-brain voxel-based morphometry (VBM) analyses. T-score map of brain areas for which smaller gray matter volume was associated with smaller preparatory physiological responses after adjusting for diagnostic group, scanner type (two scanner type variables), total intracranial volume, overall physiological functioning, and disease severity (CDR-Box). Three large clusters emerged in the (a) bilateral ventromedial prefrontal cortex (vmPFC) and caudate; (b) right anterior insula (AI), right superior temporal pole, right Rolandic operculum, and right Heschl's gyrus; and (c) in the left ventral AI, left orbitofrontal frontal gyrus, and left superior temporal pole (PFWE < 0.05).

275x190mm (300 x 300 DPI)

SUPPLEMENTAL MATERIALS

Supplemental Procedure

Data were collected in a 3 m x 6 m room. Instructions and film clips were presented on a 21-in. monitor at a distance of 1.75 m from the participant. The first film clip (amusement) was a scene from the TV sitcom I Love Lucy (1951–1957) depicting two female workers wrapping chocolate candy. The second film clip (sadness) was a scene from the movie The Champ (1979) depicting a boy crying after his father dies. The third film clip (disgust) was a scene from the TV show Fear Factor (2001 - 2006) depicting a man sucking fluids out of cow intestines and subsequently drinking the fluid. These film clips lasted between 87 seconds to 106 seconds.

Supplemental Physiological Methods

Data Processing

Among the 314 participants enrolled in this study, 17 were excluded from analyses because at least 25% of their data (based on the length of full trial that include the resting, instruction, and film periods combined for the film-watching task) were removed due to outliers or errors. We further excluded 12 participants who had extremely high IBI variability during the resting period (i.e., SD of IBI over the last 40 seconds of the resting period > 2 SD of the research sample; note that we did not include the first 20 seconds of the baseline because IBI during this time period may reflect the recovery from interactions with the experimenter before the first trial and answering questions about emotions experienced after each film). A total of 285 participants remained in the main data analyses.

Supplemental Neuroimaging Methods

Data Acquisition

176 MRIs (79%) were acquired on a 3T Siemens (Siemens, Iselin, NJ) TIM Trio scanner equipped with a 12-channel head coil located at the UCSF Neuroscience Imaging Center using a volumetric MPRAGE sequence (160 sagittal slices; slice thickness, 1.0 mm; FOV, 256×230mm; matrix, 256×230; voxel size, 1.0×1.0×1.0mm; TR, 2,300 ms; TE, 2.98 ms; flip angle, 9°). 37 MRIs (17%) were acquired on a 4T Bruker MedSpec system at the San Francisco Veterans Administration Hospital with an 8-channel head coil controlled by a Siemens Trio console, using an MPRAGE sequence (192 sagittal slices; slice thickness, 1 mm; FOV, 256×224 mm; matrix, 256×224; voxel size, 1.0×1.0×1.0mm; TR,

2,840 ms; TE, 3 ms; flip angle, 7°). 9 MRIs (4%) were acquired on a 1.5T Siemens Magnetom VISION system (Siemens, Iselin, NJ) at the San Francisco Veterans Administration Hospital, equipped with a standard quadrature head coil, using a magnetization prepared rapid gradient echo (MPRAGE) sequence (164 coronal slices; slice thickness, 1.5 mm; field of view [FOV], 256×256mm; matrix, 256×256; voxel size, 1.0×1.5×1.0mm; repetition time [TR], 10 ms; echo time [TE], 4 ms; flip angle, 15°).

Note that there were no diagnostic differences (six patient diagnostic groups) in the proportion of MRI scans acquired through the three different scanners (X^2 (12, 222) = 12.03, P = 0.44); also see Supplemental Table S3. Although neuroimaging analyses that include images collected across different types of scanners have robust effects and are unlikely to cause artifacts at strict statistical thresholds[1], we included two variables for scanner types (dummy coded 1 for the scanner of interest or 0 for the remaining scanners) as covariates in all VBM analyses to account for different scanner types used for data collection.

In a subsample of 117 participants scanned on the 3 T Siemens scanner at the UCSF Neuroscience Imaging Center, task-free functional MRI images were obtained over 8 minutes on the same scanner. During data acquisition, participants were instructed to relax with their eyes closed, using a T2*-weighted gradient echo planar imaging sequence (2000 ms repetition time; 27 ms echo time; 80° flip angle; 230 × 230 mm^2 field of view; 2.5 mm^2 inplane voxel size; 92×92 matrix size). The sequence was acquired with an online gradient adjustment to compensate for head motion.

Structural MRI Data Preprocessing

For structural MRI data, we utilized statistical parametric mapping version 12 (SPM12) default parameters (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) for preprocessing with the light clean-up procedure in the morphological filtering step. We then corrected structural T1 images for bias field and segmented images into gray matter, white matter, and cerebrospinal fluid, and spatially normalized into Montreal Neurological Institute (MNI) space[2]. We used default tissue probability priors (voxel size, 2.0 × 2.0 × 2.0 mm) of the International Consortium for Brain Mapping. Segmented images were then visually inspected for adequate gray matter segmentation. Smoothing was then performed on these images with an 8mm full-width at half-maximum Gaussian kernel.

Functional MRI Data Preprocessing

Rs-fMRI data were analyzed also using SPM12. After discarding the first 5 volumes to allow for magnetic field stabilization, functional images were spatially realigned, unwarped (reduction of artifacts due to movement-by-deformation interactions), co-registered to each subject's structural T1-weighted image, normalized to the MNI T1 template, re-sampled at a voxel size of $2mm^3$, and smoothed with a 6mm full-width at half maximum Gaussian kernel. To reduce the effect of low frequency drift and high-frequency noise (Lowe et al., 1998), a low pass band filter ranging between 0.0083 and 0.15 was applied. Because head motion can induce systematic but spurious correlations particularly in older and clinical populations (Power et al., 2012), all 117 participants fulfilled all of the following criteria: translational movement ≤ 3 mm, rotational movement ≤ 3°, maximum displacement ≤ 3 mm, and spikes (=max displacement > 1 mm) occurring in < 10% of the 235 volumes. Mean root-mean-square of volume-to-volume changes in translational (in mm) and rotational (mean Euler angle) movement was calculated because these metrics can be associated with ICN strength (Van Dijk et al., 2012). GLMs showed no statistical differences in translational and rotational movements between diagnostic groups (Supplemental Table S3).

For node-pair intrinsically connectivity analysis[3, 4], each participants' pairwise correlation coefficients were calculated between a set of cortical and subcortical regions-of-interest (ROIs), including the mPFC (±10, 11, -9), ACC (±2, 10, 40), amygdala (±20, -8, -12), hypothalamus (±4, -6, 10), PAG (dorsolateral: ±2, -32, -5; lateral: ±4, -31, -8; ventrolateral : ±3, -32, -12), thalamus (±4, -16, 8), and AI (±42, 17, -10). MARSBAR was used to create spherical ROIs centered on MNI coordinates. The MNI coordinates for the AI nodes were selected based on Seeley et al.[5]. MNI for other nodes selected based on two recent neuroimaging meta-analyses[6, 7]. Four mm spherical ROIs were centered on the peak MNI coordinates of the ventral vmPFC, AI, ACC, thalamus, and amygdala. To avoid an overlap of the ROIs centered on the right and left hypothalamus and on the PAG subregions, a ROI size of 3mm was chosen for the hypothalamus, and a ROI size of 2mm for the different PAG subregions.

A CSF mask in the central portion of the lateral ventricles and a white matter (WM) mask based on the highest probability in the FMRIB Software Library (FSL) tissue probability mask were used to extract mean CSF and WM time series. Each ROI's mean voxel-wise BOLD signal time series was used to calculate correlations with all other node-pairs, controlling for CSF, white matter, and motion regressors as described above[8]. To test our hypothesized neural circuit for preparatory physiological

responses, we calculated regional summary scores by averaging each participant's correlation coefficients within each pair of nodes below: (a) vmPFC and ACC (e.g., we averaged correlation coefficients between right vmPFC to right ACC, right vmPFC to left ACC, left vmPFC to right ACC, and left vmPFC to left ACC), (b) ACC and all subcortical regions (amygdala, hypothalamus, PAG) critical for physiological activation, (c) thalamus and AI, and (d) AI and ACC, and (e) AI and vmPFC, which resulted in four correlation coefficients for each participant. Finally, for each participant we averaged these five correlation coefficients to obtain an overall index of functional connectivity for our hypothesized circuit.

To ensure our connectivity findings were specific to our ROIs or the circuit that we hypothesized, rather than reflecting a general decline in functional connectivity across other regions of the brain, we included a "control" intrinsic connected networks (ICN), the sensorimotor network (SMN). Consistent with previous studies[9, 10], ROI-based ICN analysis was applied to identify the SMN. The MARSBAR toolbox for SPM (Brett, Anton, Valabreque, & Poline, 2002) was used to create 4 mm radius spheres centered on the right precentral gyrus (28, -16, 66), which is the hub region of the SMN according to previous evidence from healthy participants[11]. MARSBAR was also used to extract the average blood oxygen level-dependent (BOLD) signal time series of all voxels at each of the 235 volumes within right precentral gyrus (see supplementary material and methods for details). The average BOLD signal time series was then used as covariate of interest in a whole brain regression model to derive each participant's SMN t-map. Controlling for the same CSF, white matter, and motion regressors as described above, mean ICN connectivity was calculated separately for each participant's SMN t-map by computing the mean beta value across all voxels within an ICN specific mask that was height and extent thresholded at $P_{FWE} < 0.001$. The ICN's specific mask was created from an independent sample of healthy older participants (n = 30). The mask was derived by the same ROI-based ICN approach as described above, with the exception that it was created by combining the ROI-based maps seeded in the right and left hemisphere to ensure full bi-hemispheric coverage.

Supplemental Neuroimaging Data Analysis

Permutation analysis

Permutation analysis is a resampling approach for significance testing through which a test statistic is compared with the null distribution derived from the present study's data set and is an accurate representation of Type 1 error at $P < 0.05$ across the entire mask. The combined peak and

extent thresholds were used to determine the one-tailed *T*-threshold for multiple comparisons correction at $P_{FWE} < 0.05$. This approach has been used in similar research in this patient population[12-14]. Images were overlaid with mricron on an MNI average brain based on the gray matter templates used for preprocessing.

Supplemental Tables

Supplemental Table S1. Determining the covariate variables for the mediation, VBM, and functional connectivity analyses.

Covariates for mediation analyses (conducted based on participants with NPI apathy and disinhibition scores available)

	Preparatory physiological responses (*n* = 247)		
	r	***P***	Note
Age	-0.13	0.059	
Gender	-0.003	0.960	
Handedness	0.01	0.905	
Education	0.01	0.890	
Dementia severity	-0.09	0.201	
Cognitive functioning	0.04	0.590	
Overall physiological responding	**0.17**	**0.012**	Included as covariate in mediation analyses

Covariates for VBM analyses (conducted based on participants with structural MRI data available)

	Preparatory physiological responses (*n* = 222)		
	r	***p***	Note
Age	-0.12	0.067	
Gender	0.02	0.720	
Handedness	0.00	0.994	
Education	0.03	0.713	
Dementia severity	-0.13	0.058	Included as covariate in VBM analyses*
Cognitive functioning	0.04	0.599	
Overall physiological responding	**0.18**	**0.007**	Included as covariate in VBM analyses

Note. Although the association between disease severity (indexed by CDR-Box) and preparatory physiological responses was only significantly trending, we still included it as a covariate in the VBM analyses because it is typically positively correlated with the severity of neurodegeneration[15].

Covariates for functional connectivity analyses (conducted based on participants with rs-fMRI data available)

	Preparatory physiological responses (*n* = 117)		
	r	***P***	Note
Age	**-0.27**	**0.003**	Included as covariate in functional connectivity analyses
Gender	-0.01	0.910	
Handedness	0.00	0.976	
Education	0.01	0.942	
Dementia severity	0.03	0.737	
Cognitive functioning	-0.03	0.726	
Overall physiological responding	**0.32**	**< 0.001**	Included as covariate in functional connectivity analyses

Supplemental Table S2. Correlations between preparatory/orienting responses and functional connectivity (including our hypothesized vmPFC-SN circuit (overall and node-pair) and a control SMN network). Top: Raw scores. Bottom: Adjusted scores.

Raw Score

	Preparatory Responses		Orienting Responses	
	r	***P***	***r***	***P***
vmPFC-SN	**0.35**	**< 0.001**	0.01	0.886
vmPFC-ACC	**0.24**	**0.009**	-0.02	0.848
ACC-Amy/Hyp/PAG	**0.28**	**0.002**	-0.01	0.939
Thal-AI	**0.26**	**0.005**	0.05	0.620
AI-ACC	**0.29**	**0.001**	0.01	0.884
AI-vmPFC	**0.19**	**0.045**	0.01	0.938
SMN	0.10	0.282	-0.10	0.274

Note. Italic font indicates node-pair connectivity within the vmPFC-SN network. Bolded font indicates significant effects at the threshold of $P < 0.05$. SMN = sensorimotor network; SN = salience network; ACC = anterior cingulate cortex; AI = anterior insula; Amy = amygdala; Hyp = hypothalamus; PAG = periaqueductal gray; Thal = thalamus; vmPFC = ventromedial prefrontal cortex.

Adjusted Score

	Preparatory Responses		Orienting Responses	
	r	***P***	***r***	***P***
vmPFC-SN	**0.30**	**0.001**	0.02	0.868
vmPFC-ACC	**0.22**	**0.022**	-0.02	0.806
ACC-Amy/Hyp/PAG	**0.21**	**0.032**	0.05	0.639
Thal-AI	0.16	0.093	0.06	0.510
AI-ACC	**0.29**	**0.002**	0.04	0.694
AI-vmPFC	0.17	0.070	-0.07	0.470
SMN	0.05	0.576	-0.07	0.488

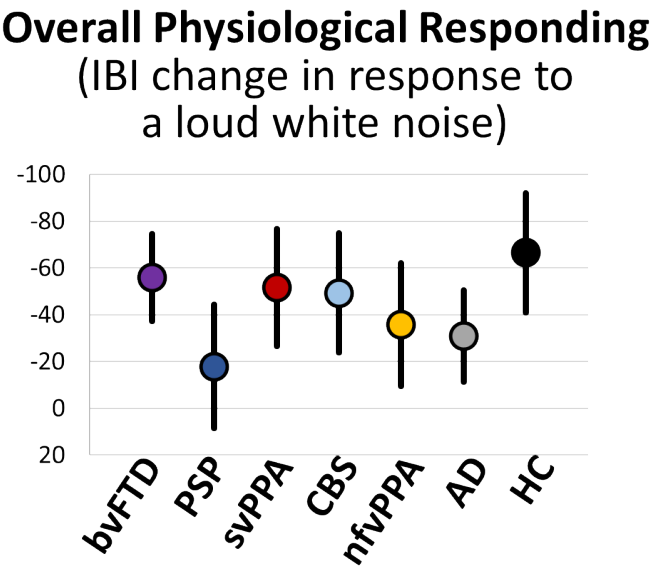
Note. Analyses adjusted for diagnostic groups, age, and overall physiological responding. Italic font indicates node-pair connectivity within the vmPFC-SN network. Bolded font indicates significant effects at the threshold of $P < 0.05$. SMN = sensorimotor network; SN = salience network; ACC = anterior cingulate cortex; AI = anterior insula; Amy = amygdala; Hyp = hypothalamus; PAG = periaqueductal gray; Thal = thalamus; vmPFC = ventromedial prefrontal cortex.

Supplemental Table S3. *Chi-squared* tests and one-way ANOVAs did not reveal any diagnostic differences for proportion of MRI scans acquired through the three different scanners nor translational and rotational movements during rs-fMRI data acquisition.

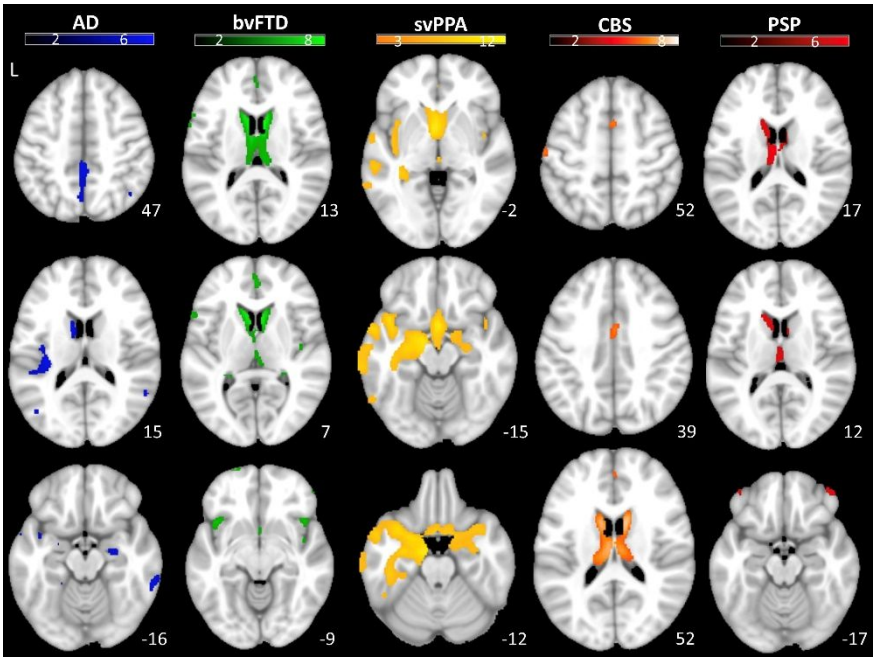
		FTLD syndromes					Comparisons			
	Total	**bvFTD**	**svPPA**	**nfvPPA**	**PSP**	**CBS**	**AD**	**HC**	***F/X²***	***P***
MRI Scanner									12.03	0.44
NIC 3T	**176**	31	23	26	21	27	31	17		
SFVA 1.5T	**9**	1	2	0	2	1	2	1		
SFVA 4T	**37**	11	5	1	5	3	10	2		
Movements during fMRI										
Translational		0.89 (0.11)	0.76 (0.12)	0.88 (0.10)	0.82 (0.11)	0.11 (0.09)	0.87 (0.10)	0.81 (0.15)	0.91	0.49
Rotational		0.64 (0.11)	0.76 (0.13)	0.56 (0.11)	0.65 (0.12)	0.72 (0.10)	0.75 (0.11)	0.43 (0.17)	0.72	0.63

Note. bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy control.

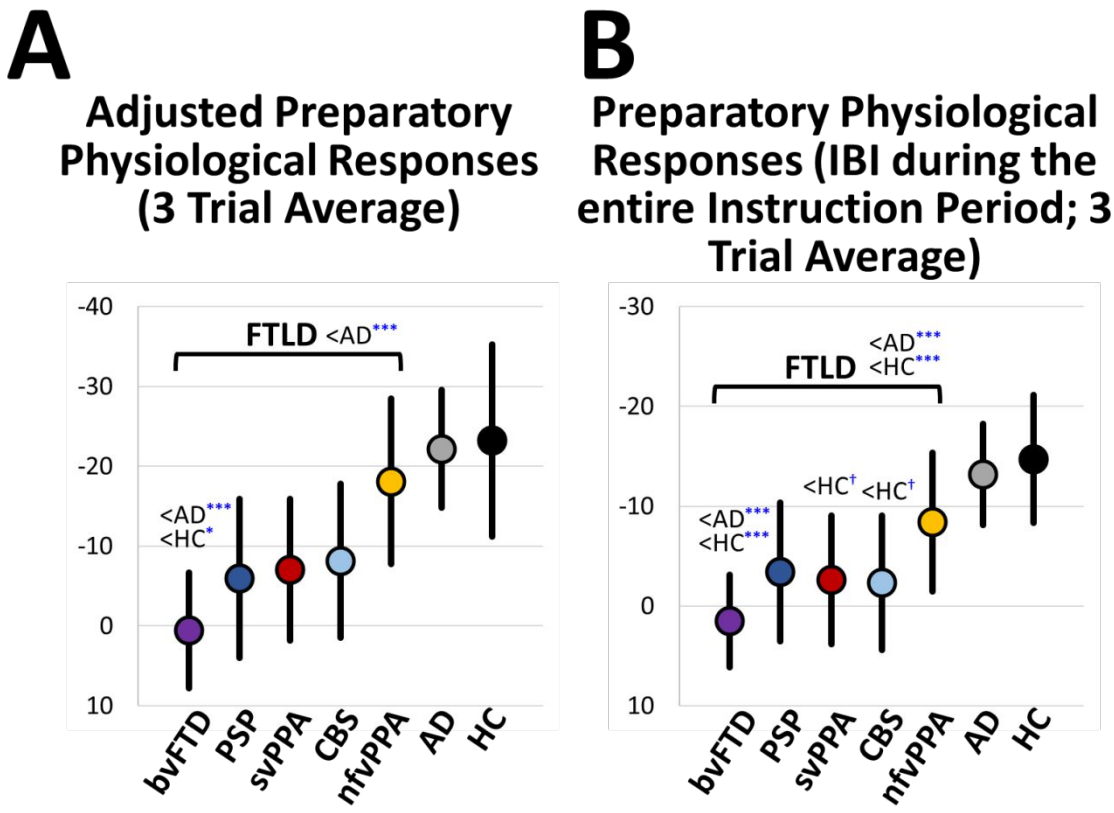
Supplemental Figures



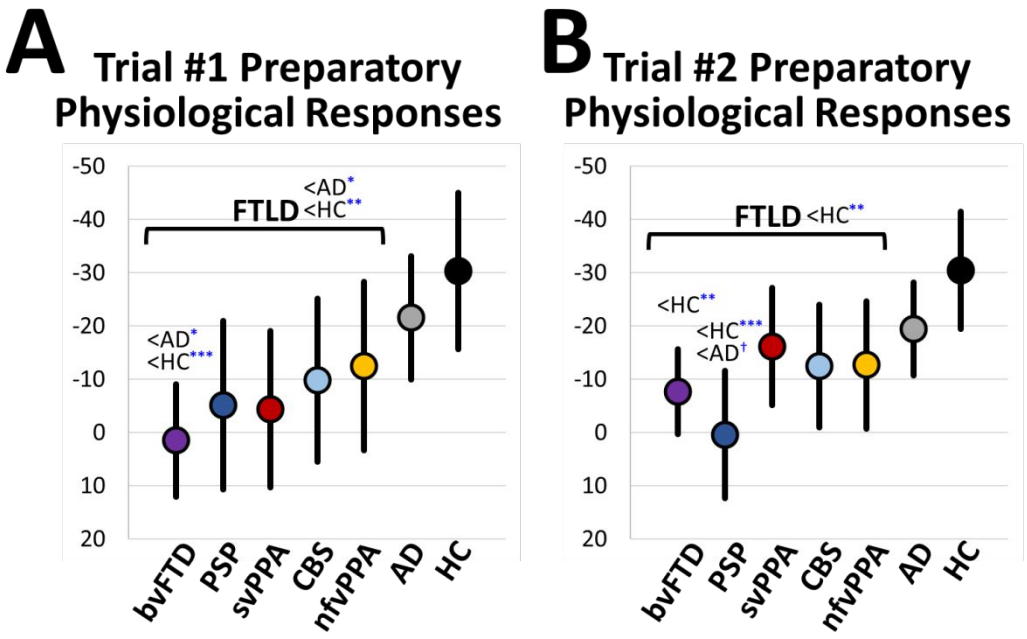
Supplemental Fig. S1. Averaged IBI change to a loud white noise by diagnostic groups, which was measured as a proxy for overall physiological responding. An ANOVA analyses did not reveal any significant differences between diagnostic groups ($F = 1.85$, $P = 0.09$; also see Table 1). bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy control.



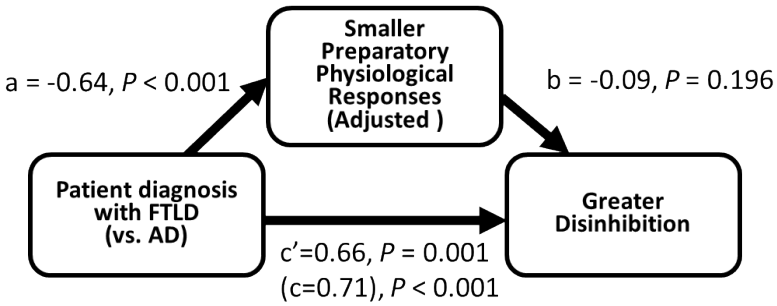
Supplemental Fig. S2. Distribution of neurodegeneration. Color bars represent *T*-scores for regions with smaller gray matter volume in patient groups compared to HCs after adjusting for age, sex, scanner type, and total intracranial volume ($P_{FWE} < 0.05$). Results are overlaid on an MNI template brain. The nfvPPA group did not have areas of significant volume loss compared to controls. As expected, the AD group had smaller volumes in the precuneus, hippocampus, and posterior temporal regions; the bvFTD group had smaller volumes in medial frontal, cingulate, insula, and striatum regions; the svPPA group had smaller volumes in predominantly left anterior temporal, insula, amygdala, and striatum regions; the CBS group had smaller volumes in supplementary motor area, medial frontal, cingulate, and striatum regions; and the PSP group had smaller volumes in the orbitofrontal, caudate, and thalamus regions[9, 17]. Presumably due to being in earlier stages of the disease (i.e., as indexed by lower CDR scores), the nfvPPA group did not show significant volume loss as compared to HCs ($P_{FWE} > 0.05$). AD = Alzheimer's disease; bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; HC = healthy control; nfvPPA = non-fluent variant primary progressive aphasia.



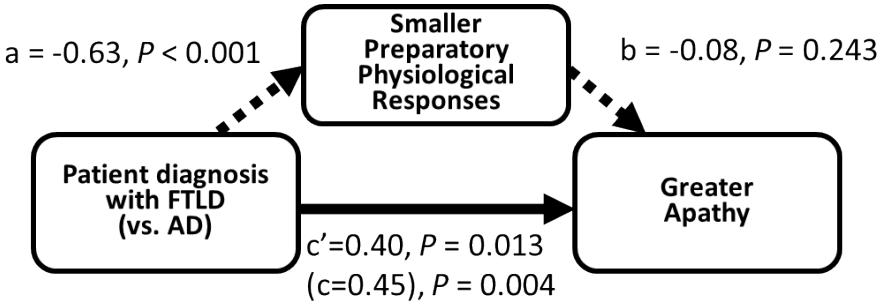
Supplemental Fig. S3. (**S3A**) Three-trial average of preparatory physiological responses adjusted for age, gender, dementia severity (CDR-Box), and cognitive functioning (MMSE), by diagnostic groups. *Mean* ± 95% confidence intervals. To ensure these effects were not driven by demographic or functional differences between diagnostic groups (Table 1), we repeated the main ANOVA analyses with an ANCOVA, including variables that significantly differed between groups. Results were very similar to the primary data analyses: when comparing FTLD, AD, and HC, $F(2, 264) = 7.99$, $P < 0.001$; FTLD < AD, $P < 0.001$; FTLD < HC, $P = 0.125$; when comparing FTLD syndromes with AD or HC, $F(6, 260) = 3.95$, $P < 0.001$; bvFTD < AD, $P < 0.001$; bvFTD < HC, $P < 0.05$. (**S3B**) In our study, preparatory physiological responses were quantified as the change in the averaged IBI of the last three seconds of the baseline period and seconds 4-6 of the instruction period (Fig. 2B; time windows W2 - W1). To ensure our primary findings using this approach were robust, we repeated our analyses using the change in the averaged IBI of the last three seconds of the baseline period and the entire six seconds of the instruction period. ANAOVs and *post hoc* tests revealed very similar results to the primary findings: when comparing FTLD, AD, and HC, $F(2, 282) = 11.16$, $P < 0.001$; FTLD < AD, $P < 0.001$; FTLD < HC, $P < 0.001$; when comparing FTLD syndromes with AD or HC, $F(6, 278) = 4.69$, $P < 0.001$; bvFTD < AD, $P < 0.001$; bvFTD < HC, $P < 0.001$. FTLD = frontotemporal lobar degeneration; bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy control. $^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.



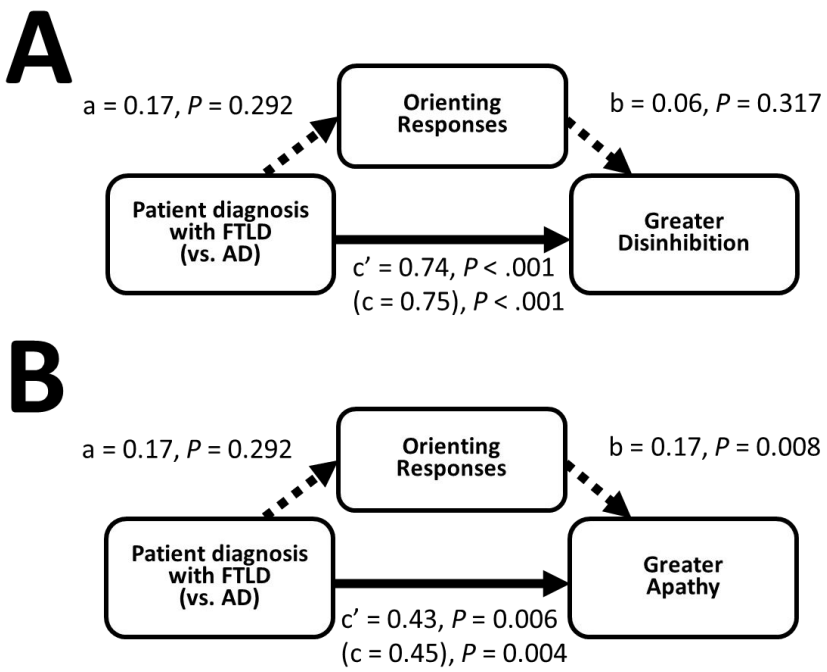
Supplemental Fig. S4. Preparatory physiological responses raw score for first (amusement film) and second (sadness film) trials by diagnostic groups. *Mean* ± 95% confidence intervals. **(S4A)** To ensure our findings were not biased by increased knowledge about the task after the first trial, we completed analyses using only preparatory physiological responses from the first trial. ANOVAs revealed very similar group effects as in the main analyses: when comparing FTLD, AD, and HC, $F(2, 272) = 7.05$, $P = 0.001$; FTLD < AD or HC (Ps < 0.05); when comparing each FTLD syndrome against AD or HC, $F(6, 278) = 2.79$, $P = 0.012$; bvFTD < AD: $P = 0.04$; bvFTD < HC, $P < 0.001$. **(S4B)** To ensure our effects did not result from participants' incorrect beliefs that the films would be always negative, we performed additional analyses focusing on preparatory physiological responses in the second trial only, which took place after participants watched an amusement film clip in the first trial (thus participants realized the films could also be positive). Again, ANOVAs (Fs > 3.07, $P < 0.006$) and *post hoc* analyses revealed very similar group effects as reported in the main analyses, FTLD < HC: $P = 0.002$; bvFTD < HC: $P = 0.006$; nfvPPA < HC, $P < 0.001$; nfvPPA < AD, $P = 0.095$. FTLD = frontotemporal lobar degeneration; bvFTD = behavioral variant frontotemporal dementia; svPPA = semantic variant primary progressive aphasia; nfvPPA = non-fluent variant primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy control. $^{\dagger}P < 0.10$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.



Supplemental Fig. S5. When overall physiological responding was adjusted, we observed a marginally significant effect that preparatory physiological responses mediated diagnostic group differences between FTLD and AD in disinhibition (standardized indirect effect = 0.06, 90% CI [0.0010, 0.1331], accounting for 8.15% of the total effect). FTLD = frontotemporal lobar degeneration; AD = Alzheimer's disease.



Supplemental Fig. S6. Preparatory physiological responses did not significantly mediate diagnostic group differences between FTLD and AD in apathy (standardized indirect effect = 0.0487, 95% CI [-0.0650, 0.1597], accounting for 10.74% of the total effect). FTLD = frontotemporal lobar degeneration; AD = Alzheimer's disease.



Supplemental Fig. S7. Orienting responses did not significantly mediate diagnostic group differences between FTLD and AD in **(S7A)** disinhibition (standardized indirect effect = 0.0103, 95% CI [-0.0164, 0.0766], accounting for 1.40 % of the total effect) or **(S7B)** apathy (standardized indirect effect = 0.0279, 95% CI [-0.0381, 0.1152], accounting for 6.15 % of the total effect). FTLD = frontotemporal lobar degeneration; AD = Alzheimer's disease.

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50-WORD Abbreviated summary

Chen et al. reported that frontotemporal lobar degeneration syndromes impair patients' ability to generate increased physiological activity to upcoming emotional stimuli. This effect was associated with functional and structural damage to the patients' ventromedial prefrontal cortex and salience network. This physiological alteration helps explain patients' often-observed clinical symptoms such as disinhibition.

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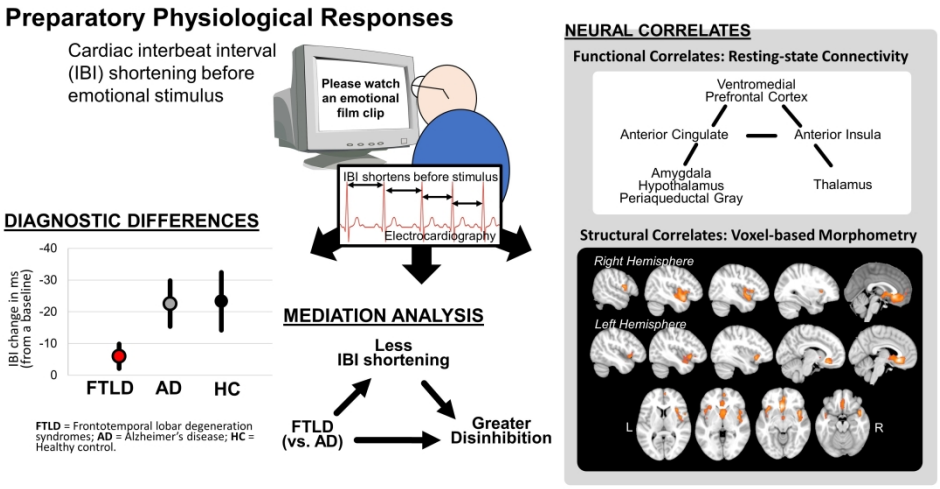

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STROBE statement: Reporting guidelines checklist for cohort, case-control and cross-sectional studies

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
TITLE AND ABSTRACT			
	1a	Indicate the study's design with a commonly used term in the title or the abstract	1
	1b	Provide in the abstract an informative and balanced summary of what was done and what was found	1
INTRODUCTION			
Background and objectives	2	Explain the scientific background and rationale for the investigation being reported	3-5
	3	State specific objectives, including any pre-specified hypotheses	5
METHODS			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6a	Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	6 - 7
	6b	Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case Variables	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect	6-8

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
		modifiers. Give diagnostic criteria, if applicable	
Data sources/measurements	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group.	6-8
Bias	9	Describe any efforts to address potential sources of bias.	10
Study size	10	Explain how the study size was arrived at	6-7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.	10
Statistical methods	12a	Describe all statistical methods, including those used to control for confounding	10
	12b	Describe any methods used to examine subgroups and interactions	10
	12c	Explain how missing data were addressed	7, 37
	12d	Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	N/A
	12e	Describe any sensitivity analyses	N/A
RESULTS			
Participants	13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 7, 9, 42
	13b	Give reasons for non-participation at each stage	6, 7, 9
	13c	Consider use of a flow diagram	N/A
Descriptive Data	14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	34
	14b	Indicate number of participants with missing data for each variable of interest	6, 7, 9, 42
	14c	Cohort study—Summarise follow-up time (eg, average and total amount)	N/A

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
Outcome Data	15*	Cohort study—Report numbers of outcome events or summary measures over time Case-control study—Report numbers in each exposure category, or summary measures of exposure Cross-sectional study—Report numbers of outcome events or summary measures	7, 9, 10
Main Results	16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11-14
	16b	Report category boundaries when continuous variables were categorized	N/A
	16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
	16d	Report results of any adjustments for multiple comparisons	10, 11, 40
Other Analyses	17a	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	42-51
	17b	If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken	N/A
	17c	If detailed results are available elsewhere, state how they can be accessed	N/A
DISCUSSION			
Key Results	18	Summarise key results with reference to study objectives	14, 20
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	19-20
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results Other information	19
FUNDING			
	22	Give the source of funding and the role of the funders for the present study and, if applicable,	21

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
		for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.