Fear conditioning in frontotemporal lobar degeneration and Alzheimer’s disease

M. Hoefer,1,2 S. C. Allison,1,2 G. F. Schauer,1,2 J. M. Neuhaus,3 J. Hall,1,2 J. N. Dang,4 M. W. Weiner,1,5,6 B. L. Miller1,2 and H. J. Rosen1,2

1University of California at San Francisco Department of Neurology, 2USCF Memory and Aging Center, 3UCSF Department of Epidemiology and Biostatistics, 4UCSF School of Pharmacy, 5San Francisco Veterans Affairs Hospital Magnetic Resonance Imaging Unit and 6UCSF Department of Radiology, San Francisco, CA, USA

Correspondence to: Howard J. Rosen, UCSF Department of Neurology, Memory and Aging Center, 350 Parnassus Ave., Box 1207, Suite 706, San Francisco, CA 94143-1207, USA
E-mail: hrosen@memory.ucsf.edu

Emotional blunting and abnormal processing of rewards and punishments represent early features of frontotemporal lobar degeneration (FTLD). Better understanding of the physiological underpinnings of these emotional changes can be facilitated by the use of classical psychology approaches. Fear conditioning (FC) is an extensively used paradigm for studying emotional processing that has rarely been applied to the study of dementia. We studied FC in controls (n = 25), Alzheimer’s disease (n = 25) and FTLD (n = 25). A neutral stimulus (coloured square on a computer screen) was repeatedly paired with a 1 s burst of 100 db white noise. Change in skin conductance response to the neutral stimulus was used to measure conditioning. Physiological-anatomical correlations were examined using voxel-based morphometry (VBM). Both patient groups showed impaired acquisition of conditioned responses. However, the basis for this deficit appeared to differ between groups. In Alzheimer’s disease, impaired FC occurred despite normal electrodermal responses to the aversive stimulus. In contrast, FTLD patients showed reduced skin conductance responses to the aversive stimulus, which contributed significantly to their FC deficit. VBM identified correlations with physiological reactivity in the amygdala, anterior cingulate cortex, orbitofrontal cortex and insula. These data indicate that Alzheimer’s disease and FTLD both show abnormalities in emotional learning, but they suggest that in FTLD this is associated with a deficit in basic electrodermal response to aversive stimuli, consistent with the emotional blunting described with this disorder. Deficits in responses to aversive stimuli could contribute to both the behavioural and cognitive features of FTLD and Alzheimer’s disease. Further study of FC in humans and animal models of dementia could provide a valuable window into these symptoms.

Keywords: frontotemporal lobar degeneration; Alzheimer’s disease; emotion; fear conditioning

Abbreviations: ACC = anterior cingulate cortex; CS = conditioned stimulus; FC = fear conditioning; FTD = frontotemporal dementia; FTLD = frontotemporal lobar degeneration; GDS = Geriatric Depression Scale; OFC = orbitofrontal cortex; ROI = regions of interest; SCL = skin conductance level; SCR = skin conductance response; SD = semantic dementia; US = unconditioned stimulus; VBM = voxel-based morphometry


Introduction

Emotional problems are common in neurodegenerative disease, with symptoms ranging from depression, apathy and emotional blunting to anxiety, irritability and agitation (Cummings, 1997). In some disorders, such as frontotemporal dementia (FTD), social and emotional problems represent early signs and core features of the illness for diagnosis (Neary et al., 1998; McKhann et al., 2001). The diagnostic implications of emotional problems in neurodegenerative disease and their contribution to morbidity underscore the need for a deeper understanding of their origins.

Improved understanding of emotional dysfunction in dementia may come from the study of processes whose neurophysiological substrates are well-understood in animals, such as simple conditioning paradigms. The advantages of conditioning are two-fold. Unlike verbally mediated
neuropsychological testing, conditioning can be easily applied to animal models of dementia. Second, if abnormal in humans, conditioning paradigms can be studied and understood in animals at a physiological and molecular level.

Fear conditioning (FC) is one of the most extensively used models for studying emotional processing in animals. This paradigm, in which neutral stimuli are made to elicit fear responses through repetitive pairing with inherently aversive stimuli, represents an elemental process by which the brain’s emotional systems learn to anticipate threats in the environment. Successful FC is dependent on a discrete set of frontal and temporal regions, including the amygdala, insula and ventromedial pre-frontal cortex, and diencephalic and brainstem structures that mediate physiological and behavioural responses (LeDoux, 1992). Several fMRI and lesion studies have demonstrated that FC in humans is dependent on these same structures (Bechara et al., 1995, 1999; LaBar et al., 1995, 1998; Buchel and Dolan, 2000; Phelps et al., 2004). Although FC has been proposed as a model for understanding primary psychiatric disorders, particularly anxiety (Phillips et al., 2003b; Rauch et al., 2006), it has received little attention as means of studying dementia. Yet, the key FC structures are commonly affected in neurodegenerative disease. Frontotemporal lobar degeneration (FTLD) is associated with amygdala, insula and orbitofrontal tissue loss (Rosen et al., 2002; Liu et al., 2004; Boccardi et al., 2005). In Alzheimer’s disease, the amygdala is also a site of early involvement after the hippocampus and entorhinal cortex (Braak and Braak, 1991) and amygdala volumes are accordingly reduced (Barnes et al., 2006; Basso et al., 2006; Markesbery et al., 2006).

FTLD and Alzheimer’s disease differ clinically, with emotional blunting, loss of empathy, impaired social interactions and compulsive behaviours being early features in FTLD, while interpersonal behaviour is relatively well preserved in Alzheimer’s disease, although irritability and agitation often develop (Cummings, 1997; Liu et al., 2004). These clinical differences suggest different abnormalities in the emotional system, which might be revealed through direct assessment of emotional processing. FC depends on a number of component processes, including auditory and visual processing of incoming stimuli, and basic physiological reactivity to aversive stimuli-functions known to be altered in aging (LaBar et al., 2004). One prior study indicated that FC is impaired in Alzheimer’s disease patients who had no deficit in electrodermal response to aversive sounds (Hamann et al., 2002), but no studies have examined conditioning in FTLD. The emotional blunting in FTLD suggests that physiological reactivity to aversive stimuli might be altered in these patients. This would impact FC, and could also have implications for emotional processing in general because altered processing of aversive stimuli may change one’s behaviour towards them. The goal of the current study was to compare the effects of FTLD and Alzheimer’s disease on FC, taking into account the basic processing underlying FC.

Two variants of FTLD were included: FTD and semantic dementia (SD). Both of these variants are associated with significant behavioural abnormalities and tissue loss in orbitofrontal cortex (OFC), cingulate and insular cortex (Bozeat et al., 2000; Snowden et al., 2001; Liu et al., 2004; Rosen et al., 2006).

Methods
Participants
Seventy-five consecutively recruited individuals, including 25 patients with probable Alzheimer’s disease [13 men, 12 women, mean age = 61.8 years (range = 50–77)], 25 with FTLD [17 men, 8 women, mean age = 63 years (range = 49–83)] and 25 healthy older individuals [normal controls, 10 men, 15 women, mean age = 66.8 years (range = 51–79)] participated. There were no significant differences across groups for age or sex (Table 1).

Patients were diagnosed using published criteria (McKhann et al., 1984; Neary et al., 1998) after a comprehensive evaluation at the UCSF Memory and Aging Center including neurological history and examination, nursing evaluation, laboratory evaluation and neuropsychological assessment of memory, executive function, language and mood (see subsequently). Twenty-one of the normal controls underwent the same evaluation, and the other five were spouses of patients or other individuals who did not undergo formal evaluation but had no cognitive complaints and no history of neurological or psychiatric disease.

FTLD is comprised of three clinical syndromes: FTD, SD and progressive non-fluent aphasia (Neary et al., 1998). For this experiment, patients with FTD (n = 15) and SD (n = 10) were included because they also share many social/emotional behavioural abnormalities (Bozeat et al., 2000; Snowden et al., 2001; Liu et al., 2004) whereas progressive non-fluent aphasia patients show a less prominent behavioural disturbance (Rosen et al., 2006).

Briefly, the neuropsychological evaluation consists of the Mini-Mental State Examination (Folstein et al., 1975), and tests of working memory (digit span backwards), verbal episodic memory [California Verbal Learning Test (Delis et al., 2000)], visual episodic memory (memory for details of a modified Rey-Osterrieth figure), visual-spatial function (copy of a modified Rey-Osterrieth figure), confrontation naming [15 items from the Boston Naming Test (Kaplan et al., 1983)], phonemic (words beginning with the letter ‘D’), semantic (animals) and non-verbal fluency [novel designs (Delis et al., 2001)] and visual-motor sequencing [a modified version of the ‘Trails B’ test (Reitan, 1958)]. Other variables of interest collected at our clinical visits were the Clinical Dementia Rating Scale score (Morris, 1997) that measures functional impairment and is often used as a surrogate of disease severity, the Geriatric Depression Scale [GDS (Yesavage et al., 1983)] and the Neuropsychiatric Inventory, which assesses behavioural dysfunction (Cummings, 1997).

The research protocol was approved by the UCSF committee on human research and all subjects gave informed consent before participating.

Psychophysiological testing
Overview
During the FC paradigm, two alternating neutral stimuli (coloured squares on a computer monitor) were repetitively presented every
16 s over an ~15-min experiment. The experiment proceeded in three phases: habituation, where each of the stimuli were initially presented four times, acquisition, where one of the neutral stimuli (the conditioned stimulus, or CS+), was immediately followed by an aversive sound presented through headphones (the unconditioned stimulus, or US) and the other (the CS−) was not and extinction, where the CS+ and CS− were again repetitively presented unaccompanied by the US. Figure 1 illustrates the organization of the experiment and the creation of variables for analysis.

**Stimuli**

The CS+ and CS− were usually blue and orange rectangles, of equal luminance that occupied the entire computer screen for 2 s. For a few subjects, green and red stimuli were used because these subjects were also participating in a related experiment. The colours used for the CS+ and CS− were counterbalanced across participants (within diagnostic group). The US was a 1-s burst of 100 db white noise presented through headphones.

**Table 1** Demographics and neuropsychological test results in controls, Alzheimer’s disease, FTD and SD

<table>
<thead>
<tr>
<th></th>
<th>Overall ANOVA</th>
<th>Controls Mean (SD)</th>
<th>AD Mean (SD)</th>
<th>FTD Mean (SD)</th>
<th>SD Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>F(3, 73) = 1.51</td>
<td>66.7 (8.6)</td>
<td>62.0 (9.2)</td>
<td>62.3 (8.7)</td>
<td>64.6 (7.9)</td>
</tr>
<tr>
<td>Males/females</td>
<td>NS (x²)</td>
<td>10/15</td>
<td>13/12</td>
<td>11/4</td>
<td>6/4</td>
</tr>
<tr>
<td>Clinical Dementia Rating Scale (sum of boxes)</td>
<td>F(3, 56) = 23.2</td>
<td>0.04 (0.1)</td>
<td>6.9 (3.0)</td>
<td>7.7 (3.9)</td>
<td>4.9 (1.8)</td>
</tr>
<tr>
<td>Mini-Mental State Examination (max = 30)</td>
<td>F(3, 73) = 11.1</td>
<td>29.7 (0.5)</td>
<td>21.4 (6.4)</td>
<td>20.3 (9.6)</td>
<td>22.1 (6.0)</td>
</tr>
<tr>
<td>CVLT 10 free recall (max = 9)</td>
<td>F(3, 43) = 11.6</td>
<td>7.3 (1.0)</td>
<td>0.8 (1.2)</td>
<td>3.5 (2.8)</td>
<td>2.0 (2.9)</td>
</tr>
<tr>
<td>Mod. Rey-O Delay (max = 17)</td>
<td>F(3, 52) = 13.1</td>
<td>13.0 (3.1)</td>
<td>3.0 (4.2)</td>
<td>7.5 (5.9)</td>
<td>6.2 (6.2)</td>
</tr>
<tr>
<td>Digit span backwards</td>
<td>F(3, 56) = 5.6</td>
<td>5.2 (1.5)</td>
<td>3.0 (1.3)</td>
<td>3.9 (2.2)</td>
<td>4.3 (1.4)</td>
</tr>
<tr>
<td>Modified Trails #/lines/min</td>
<td>F(3, 50) = 4.25</td>
<td>28.8 (78)</td>
<td>9.5 (14.8)</td>
<td>16.6 (19.7)</td>
<td>18.2 (11.5)</td>
</tr>
<tr>
<td>Stroop # correct/min</td>
<td>F(3, 44) = 14.9</td>
<td>48.2 (16.3)</td>
<td>12.6 (11.2)</td>
<td>32.9 (16.4)</td>
<td>30.0 (15.4)</td>
</tr>
<tr>
<td>Phonemic fluency</td>
<td>F(3, 54) = 19.4</td>
<td>16.6 (3.2)</td>
<td>8.1 (5.0)</td>
<td>7.2 (3.6)</td>
<td>4.8 (4.2)</td>
</tr>
<tr>
<td>Semantic fluency</td>
<td>F(3, 54) = 47.8</td>
<td>24.1 (5.7)</td>
<td>7.2 (3.6)</td>
<td>11.0 (5.8)</td>
<td>4.3 (4.0)</td>
</tr>
<tr>
<td>Abbreviated BNT (max = 15)</td>
<td>F(3, 54) = 23.0</td>
<td>14.5 (3.2)</td>
<td>11.9 (3.6)</td>
<td>10.0 (5.0)</td>
<td>3.0 (2.4)</td>
</tr>
<tr>
<td>Mod. Rey-O copy (max = 17)</td>
<td>F(3, 54) = 8.4</td>
<td>16.1 (1.3)</td>
<td>9.6 (6.6)</td>
<td>13.8 (3.5)</td>
<td>15.8 (9.9)</td>
</tr>
<tr>
<td>Calculations (max = 5)</td>
<td>F(3, 56) = 5.4</td>
<td>4.6 (0.6)</td>
<td>2.7 (0.8)</td>
<td>3.5 (1.5)</td>
<td>3.8 (1.5)</td>
</tr>
<tr>
<td>GDS (max = 30, lower better)</td>
<td>F(3, 48) = 2.9</td>
<td>3.3 (2.9)</td>
<td>6.1 (4.8)</td>
<td>3.1 (2.7)</td>
<td>6.9 (4.3)</td>
</tr>
</tbody>
</table>

SD = standard deviation, NS = not significant. *P <0.05 across groups (ANOVA). †P <0.05 versus Controls (post hoc using Tukey HSD). ‡P <0.05 versus Alzheimer’s disease (post hoc using Tukey HSD). §P <0.05 versus FvFTD (post hoc using Tukey HSD).

---

**Fig. 1** Graphic illustration of the experiment and creation of physiological variables. SCRdiff_hab, SCRdiff_acq and SCRdiff_ext are graphed in Fig. 2. Note that no trials with a US are used to examine conditioning. Responses to trials containing a US are analysed separately and graphed in Fig. 3 (for each presentation prior to and during the experiment) and Fig. 4 (averaged across the experiment).
**Apparatus**

Visual stimuli were presented on a 17in. CRT computer monitor attached to a custom-built PC running the STIM software package (James Long Co., Caroga Lake, NY, www.jameslong.com). Acoustic stimuli were presented via Telephonics high-impedance headphones (Farmingdale, NY).

Skin conductance level (SCL) was measured in microSiemens (μS) by attaching two 1081 FG-DIN Ag/AgCl sensors prepared with Biogel electrode gel (UFI inc., Morro Bay, CA) to the ventral surface of the middle phalanges on the middle and index fingers of the participant’s non-dominant hand. The sensors were connected to an SAI bioamplifier (0.5 V constant voltage with a sensitivity of 600 pS, SAI Inc., Hauppauge, NY), which fed the signal to a DI-720-P data acquisition unit (DataQ, Akron, OH), and the digitized data were transmitted to a laptop computer running Snapmaster software (version 3.5.8, HEM Data Corporation, Southfield, MI). The physiological software acquired skin conductance data on two channels: one with the SCL and the other with SCR. SCL is derived by applying excitation voltage to the sensors attached to the hand. The resultant current flow is presented, after root-mean square conversion and signal conditioning, as a DC conductance level. SCL bandpass is 10 Hz, and range is 0–25 μS. SCR is derived by sampling the SCL channel and directing it through high gain signal conditioning circuits, which also remove the DC signal, resulting in a magnified display of skin conductance change. The bandpass is 0.01–10 Hz, and the range was −2.5 to +2.5 μS.

**Experimental procedure**

After consent, subjects washed their hands and had the sensors attached. They were then seated about 50 cm from the computer monitor and instructed to breathe normally and to refrain from moving or talking while a 60 s baseline reading of SCL/SCR was taken.

Participants were then presented with the conditioned stimuli and asked to name the colours, as a test of whether they could appreciate differences between them. Headphones were then placed over the subject’s ears and a hearing test was administered using a series of 1 and 2 kHz tones. Participants were asked to indicate the onset and offset of the tones by raising and lowering their hands. The hearing screening revealed several patients (two controls, three FTLD and one Alzheimer’s disease) with some hearing difficulties (unilateral hearing loss or bilateral high frequency hearing loss). Inspection of the data in these patients did not suggest that the hearing problems impacted their reactivity, and all patients reported hearing the aversive sound loudly and clearly. Hearing problems are a normal accompaniment of aging, and not specifically associated with any of the disorders studied here. Thus, in order to increase the generalizability of the results, these subjects were included in our analyses, with the presence of these abnormalities entered as a covariate.

Next, the subject was given a warned presentation of the US without concomitant presentation of the CS+ in order to acquaint them with the stimulus. After this presentation, the subject was given an opportunity to discontinue the experiment if the US was deemed too aversive (no participants discontinued at this point). Participants were then informed that they would see a series of colours and occasionally hear the loud sound. They were instructed to press a button each time they saw a colour (this was done to ensure that they were attending to the stimulus).

During the conditioning paradigm, stimuli were presented in pseudorandom order to diminish anticipatory confounds. During inter-trial intervals the computer screen was dark. During habituation, the subject was presented with four CS+ trials and four CS− trials without presentation of the US. Acquisition was broken into two parts: (i) constant acquisition, where the subject was presented with four CS+ trials each paired with the US and four CS− trials and (ii) variable acquisition, where the subject was presented with 6 CS+/US trials, 16 CS− trials and 8 CS+ trials without a paired US. The variable acquisition phase allowed us to measure the SCR to the CS+ after learning, but without the confounding effect of the US being presented immediately after the CS+ and in a phase where the US/CS+ association was still being intermittently reinforced. During extinction the subject was presented with 16 unpaired CS+ and 16 CS− trials.

At the end of the experiment, a second 1 min 60 s assessment of resting SCL/SCR was taken.

**Image acquisition**

T1-weighted images were available in 53 of the 75 subjects (17 controls, 21 FTLD, 16 Alzheimer’s disease). Structural MR imaging was accomplished using a 1.5-T Magnetom VISION system (Siemens Inc., Iselin, NJ), a standard quadrature head coil and previously described sequences (Rosen et al., 2002) to obtain (i) scout views of the brain for positioning subsequent MRI slices, (ii) proton density and T2-weighted MRIs and (iii) T1-weighted (MP-RAGE) images of the entire brain. MP-RAGE images were used for analysis.

**Analysis**

For all analyses except baseline assessment of reactivity (see subsequently), we used the maximum SCR during the period running from 0.5 s to 4.5 s after stimulus offset.

**Conditioning and extinction**

Conditioning was represented as the change in maximum SCR to the CS+, as compared with the CS−, after acquisition. Relative response to the CS+ was measured by taking the mean maximum SCR to the CS− for all trials representing each phase and subtracting this from the mean maximum SCR to the CS+ across targeted trials for the same phase. If conditioning occurs, this difference (SCRdiff) should be zero for habituation, but positive after acquisition. In order to create one number representing conditioning, this SCRdiff in habituation was subtracted from the SCRdiff after acquisition. Target trials were as follows: all trials during habituation, and those trials occurring in the latter half of acquisition that were not followed by the US (variable acquisition phase). A single value for extinction was also created by subtracting the SCRdiff in the latter half of extinction from the SCRdiff in the latter half of acquisition.

**Physiological response to US**

The SCR response to the US was examined for each of the 10 times it was presented during acquisition. We used the average response across all these trials as a covariate for some statistical analysis.
Baseline assessment of reactivity

Various factors, including skin thickness, aging and medications might affect the ability to measure electrodermal reactivity. To assess this, we examined the maximum SCR during the 60 s rest periods that occurred at the beginning or end of the session. During these rest periods, the experimenter monitored participants’ activity and noted excessive subject movement or talking. Problems with the initial rest period were noted in 6 of the 75 subjects (four FTLD, two Alzheimer’s disease). In three of these, the rest period at the end of the experiment was considered valid, and was used instead. In the other three (two FTLD and one Alzheimer’s disease), both rest periods had problems. For these subjects, the first rest period was used. Baseline reactivity in Alzheimer’s disease appeared to be higher than in controls and FTLD, which had similar baseline reactivity, but the difference was not significant across groups [one way ANOVA; $F(2,72) = 1.28, P = 0.29$]. Baseline SCL also did not differ across groups. Baseline reactivity (SCR) was included as a covariate in all statistical analyses.

Medications

SCR is a sympathetic response mediated by cholinergic fibres in the periphery. While there is not extensive data examining the effects of medications on FC, the bulk of the available literature indicates that agents with substantial anticholinergic effects can alter FC, but there is little evidence that agents with effects on the sympathetic nervous system have much impact (Fryer and Lukas, 1999; Mueck-Weymann et al., 2001; Grillon et al., 2004; Siepmann et al., 2004; Praharaj and Arora, 2006). To address this issue, we reviewed participants’ medication lists and identified agents likely to have effects on SCR or conditioning. Targets included antipsychotics (both first and second generation), selective serotonin reuptake inhibitors (including fluoxetine, paroxetine, citalopram and escitalopram, sertraline, duloxetine), other antidepressants (tricyclics, trazadone, venlafaxine), antihistamines and benzodiazepines [which have been shown to affect FC (Brignell and Curran, 2006)]. Most patients were on one of these agents. A few were on two or three. Patients were coded dichotomously as to whether they were, or were not on these agents.

Cholinesterase inhibitors are a standard of care for patients with Alzheimer’s disease and are sometimes prescribed in other dementia syndromes. Because they enhance cholinergic function, they could conceivably enhance SCRs. Thus, we also coded each patient according to whether they were on such agents. The presence or absence of anticholinergic medications and cholinesterase inhibitors were included in all statistical analyses.

Correlations between physiology and other clinical features

Abnormalities in conditioning or physiologic responding could be mediated by other cognitive or clinical factors, including severity of disease. To explore this, we conducted bivariate regression analyses between physiological variables and the Clinical Dementia Rating Scale, Neuropsychiatric Inventory and GDS, and all the neuropsychological variables described earlier.

Voxel-based morphometry (VBM)

VBM is a technique that allows voxel-wise analysis of the relationship between changes in brain tissue content and independent variables of interest (Ashburner and Friston, 2000). A detailed description of the VBM pre-processing and analysis steps used in this study has been published (Rosen et al., 2005). Briefly, our approach included the use of a study specific template composed of the average of all study participants (Good et al., 2001; Testa et al., 2004), optimized spatial normalization of grey matter images (Good et al., 2002) and multiplication of the gray matter images by the Jacobian determinants used in normalization to preserve the original volume. Normalized gray matter images were smoothed using a 12 mm full width at half-maximum isotropic Gaussian kernel.

VBM analyses were constructed to compare tissue content across groups before examining the correlates of the physiological variables. Physiological correlates were examined by entering the physiological variables into ‘covariates only’ design matrices to examine their anatomical correlates. The data were then reanalysed with ‘conditions and covariates’ matrices to look for group-specific effects. Age, sex and total intracranial volume, calculated as the sum of the modulated gray, white and CSF volumes, used to control for head size) were always used as covariates.

Statistical significance was evaluated at $P < 0.05$ corrected for multiple comparisons using family-wise error correction (Friston et al., 1996). The human and animal literature indicates that particular aspects of FC are linked to specific brain regions, providing a basis for selection of regions of interest (ROI) to limit the number of multiple comparisons. Acquisition is most closely associated with the amygdala (Bechara et al., 1995, 1999; LaBar et al., 1995, 1998; Buchel and Dolan, 2000). Extinction is associated with ventromedial frontal cortex ( Phelps et al., 2004; Milad et al., 2005; Quirk and Beer, 2006) and related processes of reversal learning with posterior OFC (Kringelbach and Rolls, 2004). More generally, emotional processing is linked with a diverse set of frontal and temporal regions including the anterior and mid-cingulate regions, medial superior frontal gyrus, ventromedial frontal and OFC and amygdala (Phan et al., 2002; Phillips et al., 2003a). These regions were used as the ROI for reactivity to the US, as the literature does not provide a basis for constraining the ROI further. ROIs were constructed using WFU Pickatlas software (version 2.0 for Linux, Wake Forest University, Raleigh, NC), (Maldjian et al., 2003) and the AAL brain atlas (Tzourio-Mazoyer et al., 2002). For each physiological function, potential effects within the appropriate ROI were explored down to a voxel-level $P$-value of 0.01. All VBM statistical analyses were conducted using SPM (version 2, University College, London, UK) operating in a MATLAB environment (version 7.1, Mathworks, Inc., Natick, MA).

Statistical analyses

Our chief aim was to see how FC is affected in each of the clinical dementia syndromes. We examined this with regression analysis using our conditioning variable (see Conditioning and extinction in the analysis section above) as the dependent variable and diagnosis (control, Alzheimer’s disease or FTLD) and other covariates as independent variables. Regressions were conducted in two blocks, with all covariates entered in the first block and diagnosis in the second block. If the contribution of diagnosis was significant, as indicated by a $P < 0.05$ for the $\Delta R^2$ after adding diagnosis into the model, differences between each patient group and controls were evaluated at $P < 0.05$ threshold using a Fischer’s least significant difference approach (Miller, 1981).
We also compared the basic reactivity to the US across groups. Because the US was delivered 10 times during the experiment, each subject contributes multiple SCR measurements representing reactivity to the US. We analysed these repeated measures using linear mixed effects models (McCulloch and Searle, 2001). Examination of the correlations between individual US responses across time did not suggest that these responses departed from the assumption of compound symmetry.

Because of the known effects of age on FC (Labar et al., 2004), age was included in all of the analyses in addition to baseline physiological reactivity, medication use and the presence or absence of mild hearing impairment. Statistical analyses were performed using SPSS version 14 (SPSS inc., http://www.spss.com).

Results

FC

Figure 2 shows that the SCRdiff for CS+ versus CS− increased for controls in the acquisition phase, consistent with conditioning. In contrast, there is no evidence of learning in FTLD or Alzheimer’s disease. The overall effect for diagnosis was significant \((P=0.01)\), and pairwise comparisons were significant for FTLD versus controls \((B=-0.042, P<0.01)\) and for Alzheimer’s disease versus controls \((B=-0.049, P<0.01)\).

Reactivity to the US

We examined the response to the US across the experiment to assess whether this might be abnormal in either patient group. Linear mixed effects analysis revealed a significant effect for group overall \((P<0.04)\). Pairwise comparisons were significant for FTLD versus control (mean difference 0.09 μS, 95% CI 0.02–0.16, \(P<0.02\)) but not for Alzheimer’s disease versus controls (mean difference 0.06 μS, 95% CI −0.13 to 0.12, \(P<0.45\)). The difference between FTLD and Alzheimer’s disease approached significance (mean difference 0.09 μS, 95% CI −0.01 to 0.13, \(P<0.09\)). Response to the US is depicted in Fig. 3, which also includes the first presentation of the US prior to the experiment. This first response was higher than subsequent responses in all groups, but still lowest in FTLD.

We also analysed the reactivity to the US in terms of how many participants had significant SCRs in response to the US. Participants were scored as having a significant SCR if any of their responses were >0.5 μS, a commonly used threshold for SCRs (Ohman et al., 2000). Using this threshold, 40% of the controls and 48% of the Alzheimer’s disease patients had significant SCRs in response to the US, but only 12% of the FTLD subjects had significant SCRs \((\chi^2 P<0.05\) overall, and for FTLD versus controls and FTLD versus Alzheimer’s disease).

The FTLD group was composed of two syndromes: FTD and SD. To explore whether either of these groups was disproportionately showing reduced reactivity, we examined the response to the US averaged across all 10 learning trials separately for FTD and SD. As shown in Fig. 4, the US response was low in both SD and FTD, compared with the other groups. A post hoc statistical comparison confirmed that there was no significant difference in average response to the US between SD and FTD (two-sample t-test, \(P=0.326\)).
Reactivity to the US and conditioning

The low reactivity to the US in FTLD suggested that this may be an important factor determining their apparent lack of conditioning. Thus, the contribution of the US response to conditioning was assessed by repeating the original regression model with conditioning as the dependent variable and US response as an additional independent variable. For this analysis, response to the US was averaged across all 10 presentations to create a single covariate. With this change, the overall effect of adding diagnosis to the model was still significant ($P < 0.04$). However, the difference between FTLD and controls for conditioning was reduced and was only marginally significant ($B = -0.028$, $P = 0.067$), while the impairment in Alzheimer’s disease remained significant ($B = -0.041$, $P < 0.01$).

Neuropsychological and behavioural data and clinical-physiology correlations

Tables 1 and 2 summarize the neuropsychological and behavioural findings in controls and in the three patient groups: Alzheimer’s disease, FTD and SD. There were no differences in age or sex distribution across groups. All patient groups showed increased Clinical Dementia Rating Scales and decreased Mini-Mental State Examinations compared with controls, but there were no differences across patient groups. As would be expected, Alzheimer’s disease patients showed the worst performance in memory and figure copying, and they also showed poor calculation abilities and low performance on many measures sensitive to frontal lobe disease. SD patients showed the worst picture naming. In the behavioural domain (Table 2), both FTD and SD showed high levels of apathy, disinhibition and eating disorders, none of which were significantly different between FTD and SD, consistent with prior reports demonstrating behavioural overlap between FTD and SD (Liu et al., 2004).

Bivariate correlation analyses were performed examining the relationship between the degree of conditioning, reactivity to the US and all the variables listed in Tables 1 and 2 across patients. GDS score was correlated with reactivity to the US ($r = 0.383$, $P < 0.02$, uncorrected for multiple comparisons). This analysis was repeated as a partial correlation, factoring out diagnosis and the relationship between the degree of conditioning, reactivity to the US and all the variables listed in Tables 1 and 2 across patients. GDS score was correlated with reactivity to the US and all the variables listed in Tables 1 and 2 across patients. GDS score was correlated with reactivity to the US ($r = 0.383$, $P < 0.02$, uncorrected for multiple comparisons). This analysis was repeated as a partial correlation, factoring out diagnosis and the relationship was still significant. No other variables were correlated with the physiology data.

VBM

FTLD and Alzheimer’s disease both showed regions of significant volume loss ($P < 0.05$, corrected for multiple comparisons) compared with controls (Fig. 5). In FTLD, these regions included the left posterior orbitofrontal region, extending into the left insula, and the right insula. In Alzheimer’s disease, the regions were in the temporoparietal cortex bilaterally.

Anatomical correlates of physiological variables are summarized in Table 3 and illustrated in Fig. 6. Conditioning was examined using reactivity to the US as a covariate, in addition to age, sex and total intracranial volume. No regions correlated with conditioning were identified across the experimental group at a whole-brain corrected level of significance, or within the amygdala to a level of $P < 0.01$. However, in FTLD, conditioning was correlated with tissue content in the right amygdala (voxel-level $P = 0.004$, $P = 0.062$ corrected for volume of the bilateral amygdala ROI). No effects could be found in controls or Alzheimer’s disease.

Table 2 Neuropsychiatric Inventory frequency $\times$ severity product (and per cent with that feature) across diagnostic groups

<table>
<thead>
<tr>
<th>Feature</th>
<th>Controls</th>
<th>Alzheimer's disease</th>
<th>FTD</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delusions</td>
<td>0</td>
<td>0.6 (29)</td>
<td>0.8 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0</td>
<td>0.3 (24)</td>
<td>0.4 (16)</td>
<td>0.2 (10)</td>
</tr>
<tr>
<td>Agitation</td>
<td>0</td>
<td>1.2 (29)</td>
<td>2.8 (50)</td>
<td>2.9 (60)</td>
</tr>
<tr>
<td>Depression</td>
<td>0</td>
<td>2.2 (52)</td>
<td>1.7 (17)</td>
<td>1.0 (40)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0</td>
<td>2.0 (52)</td>
<td>2.6 (50)</td>
<td>1.8 (40)</td>
</tr>
<tr>
<td>Elation</td>
<td>0</td>
<td>0.4 (10)</td>
<td>0.8 (33)</td>
<td>1.3 (30)</td>
</tr>
<tr>
<td>Apathy</td>
<td>0</td>
<td>3.8 (57)</td>
<td>6.3 (92) $^*$</td>
<td>4.9 (70)</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0</td>
<td>0.8 (38)</td>
<td>4.1 (67) $^{*}¥$</td>
<td>5.1 (80) $^{*}¥$</td>
</tr>
<tr>
<td>Irritability</td>
<td>0</td>
<td>0.8 (43)</td>
<td>1.4 (33)</td>
<td>2.6 (40)</td>
</tr>
<tr>
<td>Aberrant motor</td>
<td>0</td>
<td>2.0 (33)</td>
<td>3.8 (58)</td>
<td>2.8 (50)</td>
</tr>
<tr>
<td>Sleep Disorders</td>
<td>0</td>
<td>1.1 (38)</td>
<td>2.3 (50)</td>
<td>0.8 (30)</td>
</tr>
<tr>
<td>Eating Disorders</td>
<td>0</td>
<td>1.6 (33)</td>
<td>5.9 (75) $^{*}¥$</td>
<td>4.7 (80) $^{*}¥$</td>
</tr>
</tbody>
</table>

$^*P<0.05$ across groups (ANOVA for frequency $\times$ severity product). $^*P<0.05$ versus Controls (post hoc using Tukey HSD).

$^{*}¥P<0.05$ versus Alzheimer’s disease (post hoc using Tukey HSD).

Fig. 4 Magnitude of the SCR averaged in each subject across all 10 presentations of the US during the experiment. Means for controls, FTD, SD and Alzheimer’s disease.
Reactivity to the US (average across the 10 presentations) was correlated with volume in dorsal anterior cingulate cortex (ACC) region (voxel-level \( P = 0.01 \)) and left insula across the entire group (voxel-level \( P = 0.001 \)) but these effects were not significant after multiple comparison correction. However, in controls, US reactivity was more significantly correlated with dorsal ACC volume (voxel-level \( P < 0.001, \ P = 0.028 \) after multiple comparisons correction within the frontal-temporal ROI). In FTLD, reactivity to the US appeared to be correlated with volume in the left insula (voxel-level \( P = 0.004 \), not significant after multiple comparisons correction). No correlations were detected in Alzheimer’s disease.

Extinction was examined using the magnitude of conditioning as a covariate, and was correlated with volume in the posterior OFC, with a more significant effect on the right (voxel-level \( P < 0.001, \ P = 0.03 \) after multiple comparisons correction) within the frontal-temporal ROI. No significant correlations were detected in FTLD.

### Table 3 Regions of correlation between gray matter volume and physiological variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conditioning</th>
<th>US reactivity</th>
<th>Extinction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI</td>
<td>B/L amygdala</td>
<td>B/L ACC/OFC/amygdala</td>
<td>B/L posterior OFC</td>
</tr>
<tr>
<td></td>
<td>Coordinate(^a)</td>
<td>Coordinate(^b)</td>
<td>Coordinate(^a)</td>
</tr>
<tr>
<td></td>
<td>Region(^b)</td>
<td>Uncorrected/corrected</td>
<td>Uncorrected/corrected</td>
</tr>
<tr>
<td>Across Groups</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FTLD</td>
<td>26, 2, −24</td>
<td>Ramygdala</td>
<td>0.004/0.062</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\)Coordinate in the standardized space based on the Montreal Neurological Institute (Evans et al., 1994) brain.

\(^b\)Anatomical region of the coordinate, based on overlaying statistical map on the study specific template. L = left, R = right.
multiple comparisons correction within the posterior OFC bilaterally) The same region appeared to be correlated with extinction in controls (voxel-level \( P = 0.003 \), not significant after multiple comparisons correction), and at a less significant level in Alzheimer’s disease (voxel-level \( P = 0.01 \), not significant after multiple comparisons correction). No effects with a \( P \)-value <0.01 were seen in this region in FTLD.

**Discussion**

Our study suggests that both Alzheimer’s disease and FTLD are associated with abnormalities emotional reactivity in a classical fear FC paradigm, but that the nature of the deficit differs between the two disorders. In Alzheimer’s disease, the deficit is isolated to conditioning, with normal reactivity to an inherently aversive stimulus, a replication of a prior finding in Alzheimer’s disease (Hamann et al., 2002). In FTLD, the deficit appears to be different, with reduced physiological reactivity to an aversive stimulus. Physiological deficits were not accounted for by medication use or hearing deficits, and the deficit in reactivity to the US was present in both SD and FTD—two variants of FTLD that show a large degree of behavioural overlap (Bozeat et al., 2000; Snowden et al., 2001; Liu et al., 2004; Rosen et al., 2006), again seen in this study. Physiological reactivity did not correlate with any neuropsychological variables, indicating that these deficits were not mediated by cognitive impairment. Thus, this FC paradigm appears to pinpoint functional deficits in FTLD and Alzheimer’s disease that are not addressed by traditional neuropsychological testing and do not correlate with any particular cognitive test. While the numbers in this study are relatively small for this type of paradigm and the findings for FTLD should be replicated, the distinctive types of abnormalities in FTLD versus Alzheimer’s disease suggested here offer new insights into the emotional and cognitive abnormalities seen in these two disorders.

Our findings highlight the contribution of several cortical regions to emotional learning. The amygdala, which is the primary site for the establishment of CS–US association (LeDoux, 1992), is strongly interconnected with the anterior cingulate, insula and OFC (Amaral and Price, 1984). These paralimbic regions function as transition zones between evolutionarily older limbic cortex and newer neocortical structures subserving higher-level multimodal processing, providing a link between visceral processing and other sensory information (Mesulam and Mufson, 1982a, b; Mufson and Mesulam, 1982). Prior studies have shown that injury to some of these regions, including the ventromedial frontal cortex and ACC, as well as right parietal regions, causes reduced electrodermal reactivity to aversive stimuli similar to those used in our study (Tranel and Damasio, 1994). FTLD consistently affects these paralimbic regions (Rosen et al., 2002; Diehl et al., 2004; Ibach et al., 2004; Liu et al., 2004; Boccardi et al., 2005), which was highlighted by our VBM data showing bilateral insula and left OFC tissue loss in FTLD. Thus, we propose that injury to the OFC and/or insula causes reduced reactivity to the US in FTLD. The VBM analysis suggested that insula, in particular, may account for this deficit in FTLD, although the statistical significance of this association was low, possibly because FTLD patients were uniformly low in both insular tissue content and US reactivity, limiting the variance needed to estimate the relationship. The insula is well-established as a site of visceral processing (Cechetto and Saper, 1987; King et al., 1999). Insular activation correlates with SCR magnitude in fMRI studies (Critchley et al., 2000), and the insula has been proposed as one route for transmission of aversive stimulus information to the amygdala in FC paradigms (Shi and Davis, 1999).

The VBM analysis also highlighted a relationship between cerebral changes in normal aging and physiological reactivity. Normal aging is known to be associated with reductions in reactivity to USs (Labar et al., 2004) and this was correlated with tissue loss in the ACC in our analysis. This association was statistically significant, after correction using a large ROI encompassing the amygdala and paralimbic regions mentioned earlier. Notably, this association was not detected in Alzheimer’s disease, suggesting that other factors may affect US reactivity in this group.

Although electrodermal activity was reduced in FTLD, many normal controls had low electrodermal activity using this method as well, as has been shown in previous work (Labar et al., 2004). Thus, electrodermal responses to this specific stimulus are not a sensitive diagnostic marker of FTLD, but they do suggest that other measures probing the emotional reactivity system in the brain, such as fMRI, may provide sensitive measures of disease. In addition, although electrodermal activity did not correlate with specific behaviours on the Neuropsychiatric Inventory, reduced physiological reactivity has potential implications for behaviour in FTLD. Our image analysis and prior studies referenced above suggest that electrodermal reactivity to aversive stimuli depends on the integrity of several structures, including the ACC and VMPC/insula, which are important structures for emotional processing. Reduced physiological responsiveness to aversive stimuli may be a marker of underactivation of these structures in response to punishments, which may cause events that would be considered aversive to normal individuals be considered less fearsome by FTLD patients. If this were the case, FTLD patients may be less likely to alter their choices based on the fear that their actions could generate potentially aversive consequences. In support of the idea that FTLD patients are less sensitive to aversive consequences, previous studies have indicated that FTLD patients have reduced sensitivity to pain (Snowden et al., 2001). The specific physical sensations associated with physiological activation have also been hypothesized to play an important role in cognitive and social decision making (Bechara et al., 2000),
Conditioning in FTLD and Alzheimer’s disease

Brain (2008), 131, 1646–1657

1655

an idea sometimes referred to as the somatic marker hypothesis (Damasio, 1996). Based on this hypothesis the absence of the visceral sensations associated with peripheral physiological activation itself may be a factor contributing to a change in behaviour in FTLD, such that these sensations deprives FTLD patients of important information for cognitive and social decision making. Whether the deficit in sensory input due to impaired physiological activation could be, in and of itself, an important contributor to abnormal behaviour in FTLD, as opposed to altered physiology just being a marker of altered cortical processing, is unclear, and cannot be resolved from our data. Because some normal older individuals showed low physiological reactivity, but this was more prevalent in FTLD, we would favour the idea that our findings represent a marker of more pervasive impairment in activation of cortical emotional processing systems.

Even after correction for reactivity to the US, the FC impairment in FTLD was marginally significant, suggesting a true conditioning deficit in addition to their deficit in reactivity. The residual FC ability was correlated with amygdala volume. This is consistent with studies demonstrating FC impairment due to amygdala damage (Bechara et al., 1995; LaBar et al., 1995), and with prior studies showing volumetric changes in the amygdala in FTLD (Chan et al., 2001; Liu et al., 2004). Whereas reduced electrodermal reactivity appeared to be characteristic of FTLD, conditioning may have occurred in patients with relatively little amygdala loss.

Abnormal electrodermal responding in FTLD does not necessarily imply that all emotional reactivity is impaired. A recent study of emotional reactivity in FTLD demonstrated impaired activation of self-conscious emotions, but found that basic reactivity to a startle stimulus was intact (Sturm et al., 2006). The stimulus used in that study was presented without warning and was three times louder (115 db) than the stimulus used in this study, which does not typically elicit a full defensive startle response. The preservation of the defensive response to the more powerful startle stimulus suggests that the basic physiological mechanisms for autonomic and behavioural responding, generated in the brainstem, hypothalamus and spinal cord, remain intact in FTLD. It is notable that FTLD patients in the current study appeared to show short-term habituation, decreasing their reactivity to the US after the first exposure prior to the experiment. Short-term habituation of the acoustic startle reflex has been demonstrated in decerebrate rats, indicating that it can occur purely through brainstem mechanisms (Leaton et al., 1985). This further supports the idea that basic subcortical mechanisms of behavioural and physiological responding to aversive stimuli are intact in FTLD. What the current study suggests is that cortical influences on these basic reactions are altered, so that responses to less dramatic stimuli can be impaired.

Our data also demonstrated impaired FC in Alzheimer’s disease, where the electrodermal response to the US was normal. These data confirm a previous study (Hamann et al., 2002) that demonstrated impaired FC in Alzheimer’s disease. Although this abnormality could relate to amygdala damage, we could not identify this relationship with VBM, even after placing a ROI in the amygdala. This relationship may emerge with a larger sample, or the deficit may be determined by functional changes in the amygdala that are not correlated with volume loss. The behavioural implications of the FC deficit in Alzheimer’s disease are not established. One potential effect is an impact on episodic memory, because of the known effects of emotional processing in enhancing episodic memory (Cahill and McGaugh, 1998). A previous study demonstrated a blunted impact of emotional processing on memory in Alzheimer’s disease (Hamann et al., 2000). There may also be effects in the emotional realm. Alzheimer’s disease frequently causes behavioural abnormalities including apathy, irritability and agitation (Cummings, 1997). If deficits in emotional learning prevent learning new associations between neutral events and aversive outcomes, patients could become confused about what is threatening in their environment, leading to irritability and agitation. It is also notable that reactivity to the US was correlated across all patients with the GDS score, indicating this type of physiological reactivity may influence mood, both in Alzheimer’s disease and FTLD.

We also found that right OFC volume is associated with extinction. Although this finding was significant across the whole group of subjects (after multiple comparison correction for an OFC ROI), the data looking at this relationship in individual groups suggested that most of the effect was driven by the normal control subjects, who were able to generate conditioned responses. This finding is consistent with previous studies relating posterior OFC to extinction in FC paradigms, and more generally to modification of previously established cue-reward associations (Kringelbach and Rolls, 2004; Phelps et al., 2004; Milad et al., 2005; Quirk and Beer, 2006).

The finding of abnormal FC in dementia may have broad implications. FC is a unique behavioural probe for studying neurodegenerative disease. Although it represents a very low-level emotional response that cannot easily be linked to more complex behaviour, this paradigm has several attractive features. The behavioural component is simple, and requires relatively little comprehension, allowing patients of varying severity to be studied. It does not seem to correlate with standard measures of cognitive function, suggesting that it provides a novel window into brain function relative to these measures. In addition, our data suggest that these processes are disrupted early in neurodegenerative disease. Most importantly, FC has been extensively studied in humans and animals, and appears to depend on the same brain structures across species, allowing parallel studies in humans and animals to be designed. These features suggest that FC can be a powerful tool for linking molecular changes in neurodegenerative disease with human behaviour.
References
Brigell CM, Curran HV. Drugs, sweat, and fears: a comparison of the effects of diazepam and methylphenidate on fear conditioning. Psychopharmacology (Berl) 2006; 186: 504–16.