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► **To cite this version:**

Guido van Wingen, Elbert Geuze, Eric Vermetten, Guillén Fernández. Perceived threat predicts the neural sequelae of combat stress. *Molecular Psychiatry*, 2011, 10.1038/mp.2010.132 . hal-00609049

HAL Id: hal-00609049

<https://hal.science/hal-00609049>

Submitted on 18 Jul 2011

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Perceived threat predicts the neural sequelae of combat stress

Running title: Neural sequelae of combat stress

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ABSTRACT

Exposure to severe stressors increases the risk for psychiatric disorders in vulnerable individuals, but can lead to positive outcomes for others. However, it remains unknown how severe stress affects neural functioning in humans and what factors mediate individual differences in the neural sequelae of stress. The amygdala is a key brain region involved in threat detection and fear regulation, and previous animal studies have suggested that stress sensitizes amygdala reactivity and reduces its regulation by the prefrontal cortex. Here, we used a prospective study design to investigate the consequences of severe stress in soldiers before and after deployment to a combat zone. We found that combat stress increased amygdala and insula reactivity to biologically salient stimuli across the group of combat exposed individuals. In contrast, its influence on amygdala coupling with the insula and dorsal anterior cingulate cortex (dACC) was dependent on perceived threat, rather than actual exposure, suggesting that threat appraisal affects interoceptive awareness and amygdala regulation. Our results demonstrate that combat stress has sustained consequences on neural reactivity, and suggest a key role for the appraisal of threat on an amygdala-centered neural network in the aftermath of severe stress.

Key words: Stress, combat, PTSD, fMRI, amygdala, ACC

INTRODUCTION

Highly stressful experiences such as natural disaster, terrorism, assault, or military combat are significant events in people's lives. Such stressful life events have large impact on the exposed individual, but its influence on subsequent psychological well-being is highly variable between individuals. Whereas traumatic experiences lead to positive changes for some^{1,2}, they increase the risk for posttraumatic stress disorder (PTSD) and depression for others^{3,4}, which suggests that the impact of stress is highly variable between individuals. However, the neural consequence of severe stress on neural functioning in humans remains unknown. Patients with PTSD show exaggerated amygdala responses and deficient prefrontal cortex function, in particular in the anterior cingulate cortex and ventromedial prefrontal cortex⁵⁻⁸. The amygdala and prefrontal cortex are key brain regions involved in threat detection and fear regulation⁹⁻¹¹. These neural alterations in PTSD presumably result from a combination of stress exposure and stress vulnerability¹²⁻¹⁴. Nevertheless, animal studies suggest that severe stress has lasting influences on neural functioning^{15,16}. Specifically, recent animal studies have shown that prolonged severe stress sensitizes amygdala responsivity and reduces its regulation by the prefrontal cortex^{17,18}. Furthermore, acute stress exposure increases amygdala reactivity in humans¹⁹. To investigate whether prolonged severe stress also affects amygdala functioning in humans, even in the absence of acute stress, we used a prospective study design. We assessed amygdala functioning in soldiers before and after deployment to a combat zone, which is typically associated with severe stress exposure. In addition, we

included a group of soldiers who were never deployed to control for repeated testing and unspecific time effects.

MATERIALS AND METHODS

Participants

The combat stress group consisted of thirty-three healthy soldiers who were deployed for 4 months to Afghanistan as part of the NATO International Security Assistance Force (ISAF) peacekeeping operation. We investigated them before and on average 1.5 (SD = 0.8) months after their first military deployment. They were recruited from a larger prospective study on the development of stress-related disorders following military deployment in the Dutch armed forces. Their duties included combat patrols, clearing or searching homes and buildings, participation in demining operations, and transportation across enemy territory. They were exposed to typical war-zone stressors such as enemy fire, armed combat, and seeing seriously injured fellow soldiers and civilians (including women and children). The control group consisted of twenty-six soldiers who were recruited from training bases and army divisions currently not involved in combat missions. One soldier of the control group scored above a clinical threshold for PTSD symptoms on a self-report questionnaire (see below) at baseline, and one soldier of the combat group scored above the threshold after deployment. Both participants were therefore excluded from the analyses. The groups did not differ significantly in sex ratio, age, and intelligence quotient (see Table 1). Furthermore, we investigated both groups at the same test-retest interval (mean \pm SD; 6.9 \pm 1.6 months; $t(55) = 0.5$, $P = 0.65$). Because the number of females in the study was small, we also conducted all analyses with males only, which did not alter the pattern of results (data not shown). The study

was in accordance with the declaration of Helsinki and institutional guidelines of the local ethics committee (CMO Regio Arnhem-Nijmegen, The Netherlands), and all participants provided written informed consent after written and oral description of the study.

Questionnaires

To evaluate the influence of severe stress exposure on PTSD symptoms, mood, and anxiety, we used three questionnaires. The short version of a previously validated Dutch questionnaire, the Self-rating Inventory for PTSD, was used to assess self-reported PTSD symptoms. We excluded individuals with possible PTSD from all analyses, as defined by the cut-off score of 52.²⁰ The Dutch version of the Positive and Negative Affect Schedule (PANAS) was used to assess positive and negative mood²¹, and Dutch state version of the State Trait Anxiety Inventory (STAI) was administered to assess state anxiety²². To quantify individual differences in stress exposure, we measured combat exposure and perceived threat during deployment using the Combat experiences and Perceived threat scales of the Deployment Risk and Resilience Inventory²³. The scores on these scales were not significantly correlated ($r = 0.10$, $P = 0.58$), suggesting that the amount of perceived threat during the time of the military deployment was not directly related to the number of actual combat experiences.

Behavioral task

The experimental paradigm that was performed during functional MRI scanning consisted of a blocked design, including an emotion condition with angry and fearful face stimuli (<http://www.macbrain.org>) and a visuo-motor control condition with scrambled ellipse stimuli²⁴⁻²⁶. Two emotion blocks were interleaved with three control blocks, and each 30 s block consisted of six 5 s trials. Each trial consisted of three simultaneously presented stimuli, with the cue stimulus presented above the target and distractor. In the emotion condition, an angry or fearful face was presented on top as cue, and participants had to indicate by an appropriate button press which of the bottom two faces (one angry and one fearful) matched the cue in emotional expression. In the control condition, a horizontally or vertically oriented ellipse was presented as cue above two ellipses (one vertical and one horizontal), and participants had to select the identically oriented ellipse.

MRI data acquisition

MR data were acquired with a 1.5 T Siemens (Erlangen, Germany) Avanto MR scanner, equipped with a standard head coil. T2*-weighted BOLD images were acquired using EPI with an echo time of 35 ms to reduce signal dropout in the medial temporal lobes. Each image volume consisted of 32 axial slices (3.5 mm, 0.35 mm slice-gap, TR = 2.340 s, 64 × 64 matrix, FOV = 212 mm, FA = 90°). In addition, a high resolution T1-weighted structural MR image with optimized gray/white matter contrast was acquired (3D MP-RAGE, 1 × 1 × 1 mm³ voxels, TR = 2.730 s, TE = 2.95 ms, TI = 1000 ms, FOV = 256 mm, FA = 7°).

FMRI data analysis

Image analysis was performed with SPM5 (<http://www.fil.ion.ucl.ac.uk/spm>). The first five EPI-volumes were discarded and the remaining images were realigned to the first volume. Images were then coregistered to the anatomical scan, corrected for differences in slice acquisition time, spatially normalized to the MNI T1 template, resampled into $2 \times 2 \times 2 \text{ mm}^3$ voxels, and spatially smoothed (8 mm FWHM). Statistical analysis was performed within the framework of the general linear model²⁷. To assess the influence of combat stress on neural responsivity, the two experimental conditions were modelled as box-car regressors convolved with the canonical hemodynamic response function of SPM5. In addition, the realignment parameters were included to model potential movement artefacts, as well as a constant. Furthermore, a high-pass filter (cut-off 1/128 Hz) was included, and temporal autocorrelation was modelled with an AR(1) process. Contrast images subtracting the visuo-motor control condition from the emotion condition were obtained, and analyzed in subsequent random effects models.

Functional connectivity

To assess the influence of severe stress on the amygdala network, we performed an additional functional connectivity (psychophysiological interaction) analysis using SPM5. The time-course of amygdala activity was obtained for each scanning session. The first eigenvariate of a sphere with 5 mm radius around the peak group \times time interaction in the right amygdala (24, -4, -18) was extracted and entered as an additional regressor to

the original fMRI model, as well as the interaction between the experimental conditions and amygdala time-course²⁸. Psychophysiological interaction (time-course \times condition) images were obtained, and analyzed in subsequent random effects models.

Voxel-based morphometry

To assess the influence of severe stress on regional gray matter volume, we performed voxel-based morphometry using SPM5 with standard procedures²⁹ and default parameters of the VBM5 toolbox (<http://dbm.neuro.uni-jena.de/vbm.html>)³⁰. Analyses were performed on gray matter segmentations, which were multiplied by the nonlinear components derived from the normalization matrix in order to preserve local gray matter volumes (i.e., the modulated images) and spatially smoothed (12 mm FWHM).

Statistical testing

Mixed model ANOVAs with the factors group and time were used to test whether changes in brain structure and function over time were different between the combat stress and control groups. To test whether stress-induced changes were related to individual differences in combat exposure and perceived threat, additional correlation analyses were performed. Statistical tests were family-wise error (FWE) rate corrected ($P < 0.05$) for multiple comparisons across the entire brain, or for the search volume for regions of interest using a small volume correction (SVC)³¹. The search volumes for the amygdala, insula, and anterior cingulate cortex (Brodmann areas 24 and 32) were anatomically defined using the WFU Pickatlas toolbox implemented in SPM5³².

RESULTS

Questionnaires

We observed no significant differences in self-reported PTSD symptoms, state anxiety, positive affect, and negative affect scores at baseline, nor different changes in these variables between the combat stress group and control group (see Table 1), indicating that changes in neural activity did not reflect increases in symptomatology. In addition, we measured combat exposure and perceived threat during deployment to quantify the individual differences in stress exposure. The average score for combat exposure (mean \pm SD; 5.0 ± 2.5) was higher than that of a previously reported reference population of Gulf War veterans (4.0 ± 3.2)²³, confirming that the combat group was exposed to typical combat zone stressors such as armed combat, combat patrols, and exposure to enemy fire, as well as modern warfare with a risk of exposure to improvised explosive devices (IEDs). To explore whether there was a delayed onset of PTSD symptoms, we contacted the deployed individuals again six months after their deployment, but observed no significant changes in PTSD symptoms over time in those individuals that completed all three questionnaires ($n = 16$; $F(2,14) = 1.3$, $P = 0.31$).

Behavioral performance

Across both groups and measurements, task accuracy was higher in the emotion than control condition ($Z = -2.0$, $P = 0.04$; median \pm IQR; emotion condition: $100.0 \pm 4.2\%$, control condition: $97.2 \pm 5.6\%$) but reaction times were slower ($F(1,55) = 171.1$, $P <$

0.001; mean \pm SD; emotion condition: 1863 ± 346 ms, control condition: 1141 ± 391 ms). However, no significant changes in accuracy (emotion condition: $U = 362.0$, $Z = -0.73$, $P = 0.47$; control condition: $U = 387.0$, $Z = -0.22$, $P = 0.83$) or reaction times ($F < 1$) over time between the combat stress and control groups were observed, which suggests that the imaging results are unlikely explained by differences in behavioral performance.

Neural responsivity

To verify that the task indeed activated the amygdala, we compared the emotion condition with the control condition across both groups and measurements. As expected, the task increased activity in the amygdala, as well as in the insula, dorsal anterior cingulate cortex (dACC), occipitotemporal cortex, hippocampus, orbitofrontal cortex, inferior and middle frontal gyri, thalamus, precuneus, angular gyrus, and cerebellum (all $P_{\text{cor}} < 0.05$). These activation patterns were not significantly different between groups at baseline ($P_{\text{cor}} > 0.05$), suggesting that neural functioning was comparable before stress exposure.

Next, we compared the change in amygdala reactivity over time between groups. The increase in amygdala reactivity was significantly larger in the combat stress group than the control group (peak Montreal Neurological Institute coordinate (x, y, z); (24, -4, -18), $Z = 3.1$, $P_{\text{cor}} = 0.037$). Subsequent tests showed that combat stress exposure increased amygdala reactivity ((30, 0, -18), $Z = 3.1$, $P_{\text{cor}} = 0.044$; see Fig. 1a), whereas no significant change in activity was observed in the control group ($P_{\text{cor}} > 0.05$). A similar group \times time interaction was also observed in the anterior insula ((32, 24, 2), $Z = 3.8$, P_{cor}

= 0.037; see Fig. 1b), which is a brain region involved in interoceptive awareness and thought to signal internal body states^{33,34}. Subsequent tests showed that combat stress exposure also increased insula reactivity ($P = 0.002$), whereas insula reactivity decreased with repeated testing in the control group ($P < 0.001$), but these additional tests did not remain significant after correction for multiple comparisons. These results demonstrate that severe and long-term stress exposure sensitized amygdala and insula reactivity to biologically salient stimuli in humans. Next, to evaluate whether these activity changes over time were related to individual differences in stress exposure, we performed additional correlation analyses. However, we observed no significant correlations between activity changes and combat experiences or perceived threat ($P_{\text{cor}} > 0.05$).

Amygdala connectivity

To investigate whether the influence of severe stress on amygdala reactivity also affected the neural network that is centered around the amygdala^{11,35}, we performed a functional connectivity analysis. Across groups and investigations, the amygdala was negatively coupled to cingulate cortex areas (see Fig. 2a) including the dorsal anterior cingulate cortex (dACC; (6, 8, 44), $Z = 4.1$, $P_{\text{cor}} = 0.017$), pregenual ACC ((6, 48, 2), $Z = 3.8$, $P_{\text{cor}} = 0.040$), middle cingulate cortex ((4, -20, 42), $Z = 4.3$, $P_{\text{cor}} = 0.006$), and posterior cingulate cortex (-2, -36, 26), $Z = 5.9$, $P_{\text{cor}} < 0.001$). These connectivity patterns were not significantly different between groups at baseline ($P_{\text{cor}} > 0.05$), suggesting that amygdala coupling was comparable before stress exposure. Changes in functional connectivity over time were not significantly different between the groups ($P_{\text{cor}} > 0.05$), but were related to

the amount of perceived threat in stress exposed individuals. Importantly, the strength of amygdala coupling with the dACC was positively related to perceived threat ((-10, 0, 42), $Z = 4.5$, $P_{\text{cor}} = 0.003$; see Fig. 2b-c). Thus, negative coupling of the amygdala with the dACC was enhanced in individuals that perceived little threat, but reduced in individuals that perceived much threat. In addition, perceived threat also enhanced amygdala coupling with the insula ((42, 16, -2), $Z = 3.8$, $P_{\text{cor}} = 0.039$; see Fig. 3). In contrast, no significant correlations between actual combat exposure and functional connectivity changes were observed ($P_{\text{cor}} > 0.05$). Moreover, the associations with perceived threat remained significant after correcting for combat exposure, suggesting that perceived threat rather than actual exposure influenced amygdala coupling.

Brain structure

To investigate whether the stress induced changes in neural activity and connectivity were related to changes in brain structure, we compared regional brain volumes using voxel-based morphometry. We observed no significant volume differences at baseline, no significant changes over time between the combat stress group and control group, and no significant correlations between combat experiences or perceived threat and regional volume changes ($P_{\text{cor}} > 0.05$).

DISCUSSION

To study the neural consequences of severe stress exposure, we investigated soldiers before and after deployment to a combat zone. Our results demonstrate that combat stress increases amygdala and insula reactivity to biologically salient stimuli. These neural changes were not observed in the control group and occurred in the absence of self-reported changes in posttraumatic stress symptoms. This suggests that sustained enhanced reactivity to biologically salient stimuli is a common adaptive response to prolonged environmental threat. The amygdala is a crucial brain region for the detection of threat and the enhancement of vigilance, and both the amygdala and insula are part of a larger salience network^{9,36}. These adaptations may therefore be beneficial to maintain sustained vigilance in continuously dangerous situations, such as the combat zone the soldiers were deployed to. But as heightened amygdala and insula reactivity is thought to increase the risk for mood and anxiety disorders^{37,38}, these alterations may not be adaptive for safe environments. Although our second follow-up assessment suggests that the combat group did not develop PTSD symptoms within half a year after deployment, it remains possible that stress symptoms do evolve later on. Furthermore, the amygdala and insula sensitization may also increase the vulnerability to future stressors.

To investigate whether the influence of severe stress on amygdala reactivity also affected the neural network that is centered around the amygdala, we performed an additional functional connectivity analysis. Our results show that the influence of severe stress on amygdala coupling with the dACC and insula was dependent on individual

differences in perceived threat. Individuals that perceived little threat during deployment showed enhanced negative amygdala-dACC coupling, whereas individuals that perceived much threat showed reduced coupling. Given that the dACC is thought to regulate amygdala activity^{10,11}, this finding suggests that the perception of threat during severe stress exposure alters amygdala regulation thereafter. In addition, perceived threat also enhanced amygdala-insula coupling. The insula is involved in interoceptive awareness and is thought to signal internal body states^{33,34}, which suggests that this may reflect increased bodily awareness in those individuals that perceive the most threat.

The impact of perceived threat rather than actual combat exposure on the amygdala network is in line with appraisal theory, which posits that the cognitive appraisal of threat rather than the actual environmental stressor determines the impact of stress exposure^{39,40}. Thus, our results suggest that cognitive appraisal also has a critical role in determining the impact of severe stress on the amygdala network. Interestingly, perceived threat also appears a better predictor for PTSD symptoms than actual combat exposure²³. Even though previous studies have demonstrated a close relation between combat experiences and the prevalence of PTSD⁴, path analyses suggest that the influences of combat exposure on PTSD symptoms is mediated by its influence on perceived threat⁴¹.

The divergent influences of severe stress on the amygdala network may help to explain why some individuals are vulnerable to stress whereas others are stress resilient. Our results show opposite effects on amygdala-dACC and amygdala-insula coupling depending on how the stressful experience is perceived. In turn, this may lead to opposite

effects on amygdala regulation and interoceptive awareness, with better emotion regulation in those individuals that perceive little threat but worse emotion regulation in those that experience much threat. These individual differences may explain in part why we did not observe consistent changes in symptomatology across the group of combat exposed individuals, even though their amygdala and insula reactivity had increased, and suggest a neural mechanism by which traumatic experiences could lead to highly variable outcomes¹⁻⁴.

The stress-induced changes in the amygdala network did not lead to consistent changes in mood and anxiety as assessed here. We have measured the changes in neural reactivity during affective stimulation. But in addition to increased reactivity to biologically salient stimuli, combat exposure may also have altered the manner in which the brain restores after stress exposure. For example, the release of cortisol after stress exposure normalizes amygdala reactivity⁴², and adaptive changes in this process may prevent the development of symptoms. Interestingly, patients with PTSD have abnormalities in the hypothalamic-pituitary-adrenal axis⁴³, and soldiers that develop PTSD symptoms after combat exposure have a high preexisting number of glucocorticoid receptors⁴⁴. This suggests that PTSD symptoms could result from unsuccessful normalization after exposure to consecutive stressors^{45,46}. Therefore, we propose that whereas the stress-induced changes in neural reactivity appear a common adaptive response to prolonged environmental threat, maladaptive normalization of neural hyperactivity may lead to the development of stress symptoms, which could be addressed in future studies.

The neural sequelae of severe stress exposure were remarkably similar to the neural alterations in PTSD, which include amygdala and insula hyperactivity and dACC hypoactivity^{8,47}. This is in line with the idea that PTSD reflects the upper end of a stress response continuum⁴⁸. However, the neural basis of PTSD presumably results from a combination of stress exposure and stress vulnerability^{12,49}. Previous studies have shown that high amygdala reactivity to masked stimuli (which prevents conscious awareness) and dACC metabolism prior to stress exposure increase the risk for subsequent stress symptoms^{13,14}. Our results now suggest that sustained increases in amygdala and insula reactivity to biologically salient stimuli reflect a consequence of stress exposure, which may be additive to preexisting vulnerability factors. In contrast, the individual differences in altered amygdala coupling suggest that the impact of stress on the amygdala network is a consequence of the interaction between stress vulnerability and stress exposure.

The similarity between the neural consequences of stress exposure and PTSD pathophysiology has important implications for studies investigating the neural basis of PTSD. Previous neuroimaging studies with PTSD patients have included a control group of trauma exposed individuals that did not develop PTSD, to control for trauma exposure (for example, see ref 5). Our results underscore the importance for this procedure, by demonstrating that trauma exposure by itself also influences brain functioning. Comparing PTSD patients to non-trauma exposed individuals may result in misattribution of group differences to PTSD pathophysiology.

One of the thirty-three soldiers (3%) scored above the clinical threshold for PTSD symptoms on a self-report questionnaire after their four month deployment to

Afghanistan. This is comparable to PTSD prevalence rates for Dutch and UK soldiers that have been deployed to Iraq or Afghanistan^{50,51}, but lower than the estimate for US soldiers⁴. Differences in these prevalence rates may have various causes, including differences in study populations, level of combat exposure, and screening methods⁵². Although our sample appears representative for the military population, it may not be representative for the general population. For example, the process of self-selection to serve in the military may lead to a relatively resilient population. To address this issue, future studies may investigate the consequences of severe and traumatic stress in non-military samples.

In conclusion, our results demonstrate that severe stress exposure sensitizes amygdala and insula reactivity. In addition, individual differences in threat perception predicted divergent influences on the amygdala network, which may explain why some individuals are vulnerable to stress whereas others are stress resilient. Long-term follow-up studies are required to determine whether these stress-induced neural changes indeed represent resilience or vulnerability factors.

ACKNOWLEDGEMENTS

We thank A. Beekman and M. Groenewald for assistance with data collection and P. Gaalman for technical support. This work was supported by a grant (918.66.613) from the Netherlands Organization for Scientific Research (NWO) and the Dutch Ministry of Defense. The sponsors had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The authors have no potential conflicts of interest.

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FIGURE LEGENDS

Figure 1. Severe stress exposure increases amygdala and insula reactivity to biologically salient stimuli. *a)* Combat exposure increased amygdala reactivity in military soldiers, whereas no significant change in amygdala reactivity was observed in soldiers that were never deployed. *b)* Combat exposure also increased insula reactivity in soldiers relative to response habituation over time in the control group. The figures show statistical parametric maps illustrating the significant effects ($P < 0.05$, corrected) at $P < 0.005$, uncorrected.

Figure 2. Individual differences in perceived threat during military deployment affects functional coupling of the amygdala with the dorsal anterior cingulate cortex (dACC). *a)* The dACC and other midline structures were in general negatively coupled to the amygdala when analyzed across stress exposure groups and testing sessions. *b)* The change in amygdala coupling with the dACC after stress exposure was positively correlated to perceived threat during military deployment. *c)* The scatter plot illustrates the correlation in panel b at the peak voxel. Panels a and b show statistical parametric maps illustrating the significant effects ($P < 0.05$, corrected) at $P < 0.005$, uncorrected.

Figure 3. Individual differences in perceived threat during military deployment affects functional coupling of the amygdala with the insula. *a)* The change in amygdala coupling with the insula after stress exposure was positively correlated to perceived threat during military deployment. The statistical parametric map illustrates the significant effect ($P < 0.05$, corrected) at $P < 0.005$, uncorrected. *b)* The scatter plot illustrates the correlation in panel a at the peak voxel.

TABLES

Table 1. Demographic and questionnaire data (mean \pm SD).

	Combat stress group (n = 32)				Control group (n = 25)				Statistics	
	Baseline		Follow-up		Baseline		Follow-up		Baseline ^a	Group \times Time ^b
	Mean	SD	mean	SD	mean	SD	mean	SD	P	P
Age	24.3	8.0			23.9	6.7			0.82	
IQ	89.4	10.6			92.1	13.3			0.40	
Sex (M/F)	31/1				23/2				0.41 ^c	
Questionnaires										
PTSD symptoms	27.6	4.6	27.6	5.9	26.5	3.8	26.8	4.6	0.35	0.77
Positive affect	32.0	5.5	33.1	5.6	32.6	5.0	31.4	6.7	0.64	0.10
Negative affect	12.9	4.8	11.6	2.3	11.5	2.6	11.2	2.0	0.21	0.26
State anxiety	31.6	7.6	30.7	6.7	30.2	6.8	28.2	5.3	0.54	0.50

^a Two-sample t-test, ^b Group \times Time ANOVA, ^c Pearson's χ^2





