Cerebral Cortex June 2011;21:1435–1442
doi:10.1093/cercor/bhq230
Advance Access publication November 11, 2010

Performance-Related Increases in Hippocampal *N*-acetylaspartate (NAA) Induced by Spatial Navigation Training Are Restricted to BDNF Val Homozygotes

Martin Lövdén[1,2,3], Sabine Schaefer[1], Hannes Noack[1], Martin Kanowski[4], Jörn Kaufmann[4], Claus Tempelmann[4], Nils Christian Bodammer[1], Simone Kühn[5,6], Hans-Jochen Heinze[4,7], Ulman Lindenberger[1], Emrah Düzel[4,7,8] and Lars Bäckman[1,3]

[1]Center for Lifespan Psychology, Max Planck Institute for Human Development, 14195 Berlin, Germany, [2]Department of Psychology, Lund University, 223 50 Lund, Sweden, [3]Aging Research Center, Karolinska Institutet & Stockholm University, 113 30 Stockholm, Sweden, [4]Department of Neurology, Otto-von-Guericke University of Magdeburg, 39120 Magdeburg, Germany, [5]Department of Psychology, Max Planck Institute for Human Cognitive and Brain Sciences, 04103 Leipzig, Germany, [6]Department of Experimental Psychology, Ghent University, 9000 Ghent, Belgium, [7]German Center for Neurodegenerative Disorder (DZNE), 39120 Magdeburg, Germany and [8]Institute of Cognitive Neuroscience, University College, WC1N 3AR London, UK

Address correspondence to Martin Lövdén, Center for Lifespan Psychology, Max Planck Institute for Human Development, Lentzeallee 94, 14195 Berlin, Germany. Email: loevden@mpib-berlin.mpg.de.

Recent evidence indicates experience-dependent brain volume changes in humans, but the functional and histological nature of such changes is unknown. Here, we report that adult men performing a cognitively demanding spatial navigation task every other day over 4 months display increases in hippocampal *N*-acetylaspartate (NAA) as measured with magnetic resonance spectroscopy. Unlike measures of brain volume, changes in NAA are sensitive to metabolic and functional aspects of neural and glia tissue and unlikely to reflect changes in microvasculature. Training-induced changes in NAA were, however, absent in carriers of the Met substitution in the brain-derived neurotrophic factor (BDNF) gene, which is known to reduce activity-dependent secretion of BDNF. Among BDNF Val homozygotes, increases in NAA were strongly related to the degree of practice-related improvement in navigation performance and normalized to pretraining levels 4 months after the last training session. We conclude that changes in demands on spatial navigation can alter hippocampal NAA concentrations, confirming epidemiological studies suggesting that mental experience may have direct effects on neural integrity and cognitive performance. BDNF genotype moderates these plastic changes, in line with the contention that gene–context interactions shape the ontogeny of complex phenotypes.

Keywords: brain-derived neurotrophic factor (BDNF), cognitive training, hippocampus, *N*-acetylaspartate (NAA), spatial navigation

Introduction

Experience-dependent changes in brain volume have been observed in the human hippocampus (Draganski et al. 2006), but the functional and histological nature of such changes is unknown. Unlike measures of brain volume, changes in hippocampal *N*-acetylaspartate (NAA) assayed in vivo with magnetic resonance spectroscopy (MRS) are specifically sensitive to functional and metabolic aspects of neural and glia tissue. Here, we investigated whether adults performing a spatial navigation task over a period of 4 months display performance-related increases in hippocampal NAA and whether genetic variations in the brain-derived neurotrophic factor (BDNF) gene moderate such effects.

Converging evidence from electrophysiological, neuroimaging, and lesion studies shows that both animal and human spatial navigation depend on the hippocampus (O'Keefe and Nadal 1978; Burgess et al. 2002). The hippocampus is also a structure known for its plasticity. For example, the relative volume of the hippocampus in nonhuman species varies as a function of demands on spatial memory (Healy and Krebs 1992; Barnea and Nottebohm 1994). In humans, Maguire et al. (Maguire et al. 2000, 2006) reported volume differences in the hippocampi of London taxi drivers as compared with controls. Draganski et al. (2006) found increases of gray matter in the posterior hippocampi of medical students studying intensively for a major exam. These findings indicate that variations in human hippocampal macroscale morphology can be driven by experience in adulthood. In line with this view, there is abundant evidence that the nonhuman adult brain, particularly the hippocampus, responds to experience with several types of changes at cellular, subcellular, molecular, and biochemical levels (Rosenzweig and Bennett 1996; van Praag et al. 2000; Jessberger and Gage 2008; Holtmaat and Svoboda 2009).

Neurotrophins play important roles in experience-dependent plasticity. One such protein, BDNF, is released in response to neural activity to modulate synaptic activity and hippocampal long-term potentiation. These actions are in addition to the typical roles of BDNF, which include involvement in cell proliferation, cell survival, axonal sprouting, and synaptic growth (Poo 2001; Lu 2003; Binder and Scharfman 2004). BDNF is distributed throughout the brain and is prevalent in the hippocampus (Murer et al. 2001). A common single nucleotide polymorphism of the human BDNF gene (Val66Met; rs6265; G196A) is linked to activity-dependent secretion of BDNF through alterations in the efficiency of the precursor peptide (Egan et al. 2003; Chen et al. 2004). The secretion of BDNF in neurons is lower in individuals carrying the Met allele (about 35% of the population) than in Val homozygotes (Egan et al. 2003). In line with the role of BDNF in plasticity, Met carriers show reduced cognitive performance (Egan et al. 2003; Li et al. 2010), smaller hippocampal volume (Egan et al. 2003; Pezewas et al. 2004), lower hippocampal activity during episodic encoding and retrieval (Hariri et al. 2003), and reduced reorganization of motor maps after short-term training (Kleim et al. 2006).

Carriers of the BDNF Met allele also evidence reduced concentrations of hippocampal NAA assayed in vivo with MRS (Egan et al. 2003; Stern et al. 2008). NAA is a putative marker of neuronal integrity, being located mainly within the body of neurons (where it is synthesized), but also in axons and dendrites. Lower levels of NAA are transported to glia cells

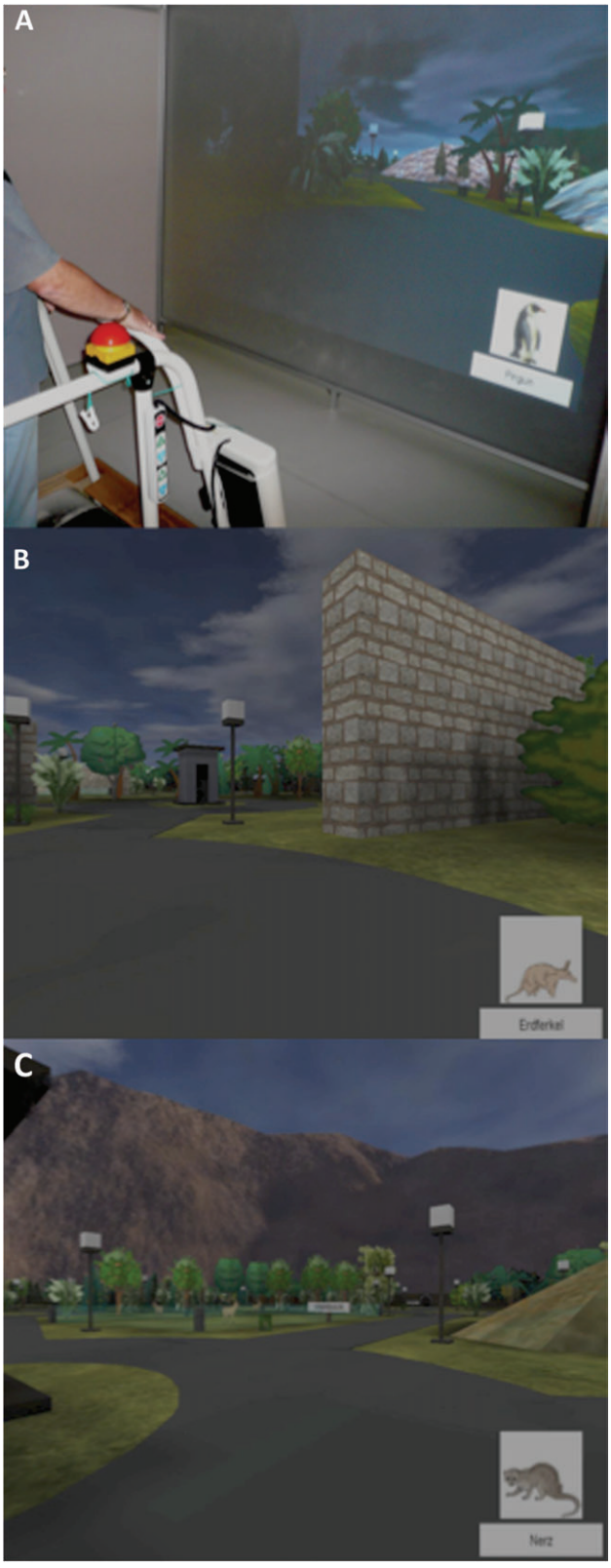

Figure 1. Participants in the navigation training group navigated in a VE while walking on a treadmill (*A*). The task was designed to demand functions known to depend on the hippocampus, such as spatial learning based on allocentric processes (e.g., navigation supported by triangulation of distal cues), associative memory (e.g., encoding and retrieval of associations between landmarks and navigational decisions), encoding of novel information (e.g., new navigation areas during training), and consolidation of information (e.g., retrieval of information encoded at previous training sessions was necessary for good performance). Participants started walking at the entrance to a virtual zoo and searched for the animal currently displayed at the lower right corner of the screen (*B* and *C*). When the animal had been found, a new animal was displayed and the participants searched for this animal. After all animals had been found, the participant completed the trial by finding the exit, received feedback, and started a new trial. After completing 4 trials, the participant received a new zoo. It was impossible to complete 4 trials within a 50-min training session.

where it is broken down to acetate and aspartate, and waste NAA is transported in the capillaries. Alterations in NAA concentrations may reflect multiple histological events in neural and glia tissue, such as changes in neural cell density, axonal and dendritric structure, and myelination, as well as metabolic alterations. Changes in NAA concentrations observed with MRS are also an important clinical diagnostic because almost all disorders involving neuronal loss or dysfunction (e.g., Canavan disease, brain cancer, multiple sclerosis, and Alzheimer disease) are associated with alterations in NAA (for review, see Moffett et al. 2007).

We assumed that if the BDNF gene produces variations in hippocampal NAA concentrations by modulating experience-dependent plasticity, then performing a demanding spatial -navigation task over a longer time period should: 1) increase hippocampal NAA levels and 2) alter hippocampal NAA levels to a greater extent in BDNF Val homozygotes than in Met carriers. To test these hypotheses, we used a spatial training task that demands several functions known to depend on the hippocampus (see Fig. 1). Training involved performing a navigation task in a virtual environment (VE), while walking on a regular exercise treadmill. The walking component was included in order to increase the immersion into the VE and to leverage the interacting role that self-motion may play in forming hippocampal spatial representations (McNaughton et al. 1996; Stackman et al. 2002) and in learning-related plasticity (Kempermann 2008). The walking was not physically demanding. Younger healthy men (n = 21) stratified on BDNF genotype underwent MRS of the right hippocampus before, immediately after, and 4 months after a 4-month long training period including a total of forty-two 50-min training sessions. The right hippocampus was selected for MRS based on its more prominent role in spatial location memory (Burgess et al. 2002). Participants in yoked control groups (n = 15) walked on the treadmill without the VE for an identical amount of time. We predicted larger increases in NAA for navigators than for walkers and that these changes would reflect adaptations to the experiential demands (Lövdén et al. 2010) so that NAA would return to pretraining levels 4 months after the last training session. We also predicted that the experience-dependent NAA increases would be larger for BDNF Val homozygotes than for Met carriers. In line with common spectroscopy procedures, we used creatine (Cr) as a control metabolite in the analyses.

Materials and Methods

Participants

One hundred and seven younger men (age range 20–30 years) were initially recruited through newspaper advertisements, word-of-mouth recommendation, and fliers circulated in Berlin, Germany. We screened these participants and obtained saliva samples for genotyping. In addition, we recruited 13 genotyped participants from a large database of participants taking part in a separate study including health assessments and cognitive tests (Nagel et al. 2008). From these pools

of participants, we recruited a total of 28 BDNF Val/Val, 25 Val/Met, and 3 Met/Met eligible participants. Due to the rarity of Met homozygotes and in line with common practice, participants were subdivided into 2 subgroups according to their BDNF Val66Met genotype status: Val/Val and Any Met participants. All were right-handed, had normal or corrected-to-normal vision, and reported no history of cardiovascular disease, neurological or psychiatric conditions, problems hindering gait or balance, or drug/alcohol abuse. They reported no use of antiseizure or antidepressant drugs. A clinical neurologist evaluated T_1- and T_2-weighted MR images collected at pretest and excluded one participant due to brain abnormality and 3 participants due to imaging artifacts (e.g., movement). Four participants dropped out during or immediately after the completion of pretest due to lack of motivation. Four participants dropped out during training due to health issues, starting a new job, and unspecified personal problems. Finally, 8 participants were excluded from the analyses due to nonacceptable quality of MRS spectra (see Data Analysis). Potential selectivity of the effective sample (n = 36) in relation to the initially recruited sample (n = 56) was computed on the criterion measure of navigation performance (see below) at pretest and expressed in an effect-size metric $[(M_{effective} - M_{total})/SD_{total}]$, separately for the BDNF Val/Val and Any Met groups. Selectivity, due to all sources of dropout, was negligible for both the BDNF Val/Val group (–0.14 standard deviation [SD]) and the Any Met group (0.13 SD).

After pretests were completed, we randomly assigned participants to either the navigation ($n_{Val/Val}$ = 11; $n_{Any\ Met}$ = 10) or the walk-time yoked control group ($n_{Val/Val}$ = 8; $n_{Any\ Met}$ = 7). Unequal ns reflect unequal dropout. Table 1 shows background characteristics of these groups. The BDNF and training groups did not differ significantly in perceptual speed (digit symbol substitution; Wechsler 1981) or vocabulary (Lehrl et al. 1991) performance, Fs < 1, as assessed at pretest using standardized procedures.

Each participant was paid 1150 euro for completion of the whole study. The ethical review board of the Otto-von-Guericke University of Magdeburg approved the imaging part of the study, and the review board of the Max Planck for Human Development, Berlin, approved the behavioral part of the study. Written informed consent was obtained prior to the investigation.

Materials and Procedures

Training Protocol

Participants in the navigation groups performed a navigation task in a VE while walking on a treadmill. Participants in the walk-time yoked control group walked on a treadmill without the VE for an identical amount of time. Participants in the control groups were allowed to perform activities that they would normally do during walking (except explicit spatial navigation), such a listening to radio and music. A total of forty-two 50-min training sessions were administered. If a participant missed a scheduled session, the training period was prolonged so that all participants completed 42 sessions. The period between pretest and posttest 1, during which training took place, was on average 118 days (see Table 1), with no significant differences between training or BDNF groups (ts < 0.80). The period between posttest 1 and posttest 2, during which no training was administered, was on average 121 days, with no significant differences between groups (ts < 1.07).

Table 1
Subject characteristics across training group and BDNF genotype

Measures	Val/Val BDNF				Any Met BDNF			
	Navigators		Walkers		Navigators		Walkers	
	M	SD	M	SD	M	SD	M	SD
Age (years)	25.0	2.6	28.2	1.8	25.5	2.8	25.8	2.6
Digit symbol	57.8	8.4	58.6	14.5	53.5	11	58.7	9.5
Vocabulary	30.0	2.4	30.2	3.7	30.2	1.9	31.1	2.7
Walking speed (km/h)	3.6	0.7	3.4	0.6	3.7	0.6	3.8	0.6
Days between pre- and posttest 1	118	6	118	3	119	3	116	7
Days between posttest 1 and 2	119	8	122	5	122	4	123	3

All participants selected their preferred walking speed before pretest (see below) and before they were assigned to their respective group (walking/navigation) but after familiarization to the navigation task. Participants were instructed to select a nondemanding speed of walking (as if they took a Sunday walk in the park). This walking speed was kept constant throughout the study. Note that the visual flow of the VE was held constant for all participants, so that walking speed did not directly influence navigation performance. The training and BDNF groups did not differ significantly in walking speed (ts < 0.31; Table 1).

In the navigation laboratories, a VE was back-projected onto a screen situated approximately 150 cm in front of the walking area of a motorized treadmill that was positioned at the level of the floor (see Fig. 1*A*). The projection area was 176 × 236 cm, which allowed for an approximately 75 degree wide field of view. Operating 2 buttons attached to the handrail controlled navigation decisions (i.e., whether to turn left or right).

Maps of the VE were rendered in first-person view using the Quake 3 engine (http://www.idsoftware.com/games/quake/quake3-arena) and constructed in GtkRadiant (http://www.qeradiant.com/cgi-bin/trac.cgi). An interface for the experimenter was designed and programmed by us in Java to allow for control of the treadmill and the VE and for access to a MySQL database storing relevant data.

The task for participants in the navigation group was to start walking at the entrance to a virtual zoo and search for the animal currently displayed at the lower right corner of the screen (see Fig. 1*B,C*). Participants never had access to any survey map. When the currently displayed animal had been found, a new target animal was displayed in the lower right corner of the screen and participants started searching for this animal. After all animals had been found, the participant completed the trial by finding the exit of the zoo, received feedback on the number of minutes needed to complete the trial, and started a new trial in the same zoo. After completing 4 trials in a zoo, the participant received a new zoo (with different animals, topography, and layout) and proceeded with the task.

Twenty different training zoos were constructed. All zoos had identical skylines and distal landmarks (e.g., mountains, towers; see Fig. 1*C*) situated at identical places surrounding the area of the zoo (which was enclosed by walls). The length of the direct paths between the animals was kept constant across trials and zoos and generated such that it was impossible to complete 4 trials in a zoo within a 50-min session, and so that consecutive targets were not located next to each other. Subsequent training sessions began at the final position from previous session. The path structure of all training zoos was randomly generated under the constraint that they had 42 decision points that were connected by paths of identical total length across zoos. In all zoos, 14 animals were randomly distributed and an approximately equal amount of local landmarks (e.g., walls, trees, hills, cafeterias, and lampposts) was manually distributed over the zoos.

Decision points were always roundabouts (see Fig. 1*C*). Participants entered a roundabout clockwise (by clicking the left button) or counterclockwise (by clicking the right button). If no decision was made, then participants entered counterclockwise. Participants exited a roundabout with the appropriate button press (left or right depending on the direction of movement). They could not deviate from the paths in the VE (see Fig. 1*B,C*) and not stop walking (and moving in the VE) unless they requested the experimenter to stop the task (which was discouraged and a very rare event). All participants received the different training zoos in the same order but, depending on their performance, completed different amounts of zoos during the training period.

Assessment of Navigation Performance at Pretest and Posttests

Pretest started with two 50-min sessions of familiarization to walking on the treadmill and navigating in the VE. After familiarization, participants were assessed on navigation performance with a task similar to the training task. Posttests included one familiarization session for the walk-time yoked control groups. Three different zoos were constructed, one for each assessment (pretest, posttest 1, and posttest 2). The 3 zoos had an identical path structure, and the animal cages were placed at identical locations. In addition, the correct path

between the animal cages was identical across zoos. However, the zoos contained different animals, different skylines, different distal landmarks (e.g., mountains, and towers), and a different layout of proximal landmarks (e.g., trees, hills, and buildings located in the area of the zoo). The dependent variable was the number of targets found in 50 min.

Assessment of Real-Life Navigational Strategies and Map-Drawing Performance

At posttest 2, we asked which of 8 strategies participants were using when navigating through unfamiliar environments in everyday life. Strategies were 1) using a map, 2) using an electronic navigation device, 3) writing down which route to take, 4) using street signs, 5) using close landmarks (remembering places and objects on the way), 6) using the sun and the shadow of objects to tell directions, 7) using distal landmarks (e.g., large buildings), and 8) asking other people for the way. For analyses, we summarized strategies 1, 2, 3, 4, and 8 as "relying on others and external aids." We classified using close landmarks (5) as a "cue-response" strategy and the reliance on the sun (6) and distal landmarks as an indication that participants were constructing a cognitive map.

After pretest assessment, participants were asked to place the animals into a map of the zoo, containing exit and entrance, distal landmarks, and 3 prominent close landmarks. The quality of the cognitive map was rated by 2 trained raters on a scale from 1 (excellent) to 6 (very poor), with very high interrater reliability (Cronbach's Alpha = 0.98). The correlation of map-drawing performance with navigation performance was high, $r = -0.75$ ($P < 0.01$), indicating that successful navigators obtained a high-quality mental representation of the zoo.

Magnetic Resonance Spectroscopy

MRS was performed on a 3 T MAGNETOM Trio scanner (Siemens), with an 8-channel phased-array head coil. The operator was blind to group status. Single voxel ^{1}H MR spectra (PRESS, echo time [TE] = 135 ms, repetition time [TR] = 2000 ms, 256 averages, bandwidth = 1200 Hz) of the right hippocampus were recorded subsequent to the high resolution T_1-weighted scans (MPRAGE, TE = 5.12 ms, TR = 2600 ms, inversion time = 1100 ms, flip angle = 7°, bandwidth = 140 Hz/pixel, matrix = 320 × 320 × 240, isometric voxel size = 0.8 mm^3). Voxels comprising mainly the medial part of the hippocampus (voxel size = 2 × 1 × 1 cm^3) were placed as indicated in Supplementary Figure 1. Manual shimming was performed to improve magnetic field homogeneity set by the automatic shim routine. Additionally, water reference data with radiofrequency pulses for water suppression switched off (TR = 10 s, 4 averages) were acquired for eddy current correction and scaling of the metabolite concentrations.

Genotyping

DNA was extracted from saliva using the commercial Oragene Kit (DNA Genotek). Genotyping of the BDNF Val66Met (rs6265; G196A) polymorphism was performed with polymerase chain reaction using melting curve detection analysis (Light Cycler System 1.5; Roche Diagnostics), according to standard procedures (e.g., Montag et al. 2008). The frequencies of the 2 BDNF genotypes in the initially recruited sample were 0.30 for Any Met and 0.70 for Val/Val. The allelic distributions did not deviate significantly from those expected according to the Hardy–Weinberg equilibrium, $\chi^2_1 = 0.01$.

Data Analysis

Behavioral Data

The inclusion of the walk-time yoked control groups enables differentiation of true navigation-related changes from confounding changes, such the effects of walking alone or other effects such as maturation, materialization, and time of year. With this design, the critical effect is revealed by an interaction between training group and time; that is, changes across the measurements for navigation groups that are significantly different from those for the walking groups. Similarly, BDNF-related differences in the effects of navigation training are revealed by an interaction between BDNF group, training group, and time of assessment. To investigate these effects, we used a mixed 2 (training group: navigators vs. walkers) × 2 (BDNF: Val/Val vs. Any Met) × 3 (time: pretest, posttest 1, posttest 2) analysis of variance (ANOVA). Polynomial contrasts were used to detect linear and quadratic effects involving time. In the presence of significant quadratic effects involving training by time, we followed up with ANOVAs and paired *t*-tests to trace the source of the effect. The alpha level was $P < 0.05$. Note that the 2 core research questions in this study come with the specific predictions of a training × time interaction and a training × BDNF × time interaction. For this reason, there is no need to correct the alpha level for multiple comparisons. Follow-up tests were applied only for the purpose of tracing the source of these predicted interactions and, therefore, there is no need for corrections for these tests either.

Magnetic Resonance Spectroscopy

Spectra were analyzed using LCModel (Provencher 1993; version 6.1.0, www.s-provencher.com/pages/lcmodel.shtml). The LCModel is a fully automatic quantitation method for in vivo ^{1}H MR spectra that fit each acquired spectrum as a linear combination of model spectra acquired from metabolite solutions. An example of hippocampal spectra and LCModel fits is shown in the Supplementary Figure 2. To determine metabolite concentrations, the metabolite signal is related to the water signal intensity of the same voxel. Participants were excluded if the analysis displayed Cramér–Rao lower bounds for NAA and Cr that were higher than 7% and 9%, respectively, at any time point.

We targeted NAA in the analyses, using Cr as a control metabolite, with the same statistical approach and logic as for the behavioral data. We applied a mixed 2 (training group: navigators vs. walkers) × 2 (BDNF group: Val/Val vs. Any Met) × 2 (metabolite: NAA vs. Cr) × 3 (time: pretest, posttest 1, posttest 2) ANOVA to analyze the NAA and Cr concentrations, predicting a 4-way interaction among time, training group, BDNF, and metabolite, reflecting selective increases in NAA over time that are larger for navigators than for walkers and for the BDNF Val/Val group than for the Any Met group. In the presence of significant effects involving training and time, we followed up with ANOVAs and paired *t*-tests to determine the source of the effect. Finally, we correlated, separately for the 4 groups, changes from pretest to posttest 1 in the NAA and Cr metabolites with changes in navigation performance. We opted for not including any NAA/Cr ratio score in these correlations because of interpretational ambiguities reflecting the spurious correlation between the ratio and its denominator (Pearson 1897; Atchley et al. 1976; Van Petten 2004). The alpha level was again $P < 0.05$.

Results

Navigation Performance

No reliable effects involving training group or BDNF were detected in analyses of pretest data, $Fs_{1,32} < 1.09$, indicating that the groups were comparable in baseline performance on the criterion task (see Fig. 2). In addition, we observed no significant effects involving BDNF or training status on self-reported real-life navigational strategies or map-drawing performance, $Fs_{1,32} < 2.27$.

Analyses addressing training-related changes in navigation performance revealed significant linear, $F_{1,32} = 12.05$, $P = 0.002$, and quadratic, $F_{1,32} = 14.78$, $P = 0.001$, training group × time interactions. While the walking group exhibited modest increases in spatial navigation performance that likely reflect the mere effect of retest, the navigation group showed performance increases beyond those of the walking group (see Fig. 2). As indicated by a significant training group × time interaction in analyses comparing pretest and posttest 2, navigation-related improvements were partly maintained 4 months after termination of training, $F_{1,32} = 35.76$, $P < 0.001$. However, as indicated by the significant quadratic training × time interaction, the navigation-related training effect declined in magnitude during the maintenance period.

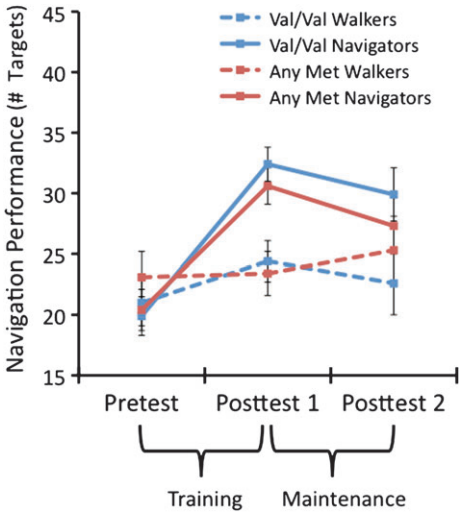

Figure 2. Mean (±standard error) navigation performance (number of targets found within 50 min of navigation) as a function of training group (navigation/walk-time yoked control), BDNF genotype (Val/Val vs. Any Met), and time of assessment (pretest/posttest 1/posttest 2).

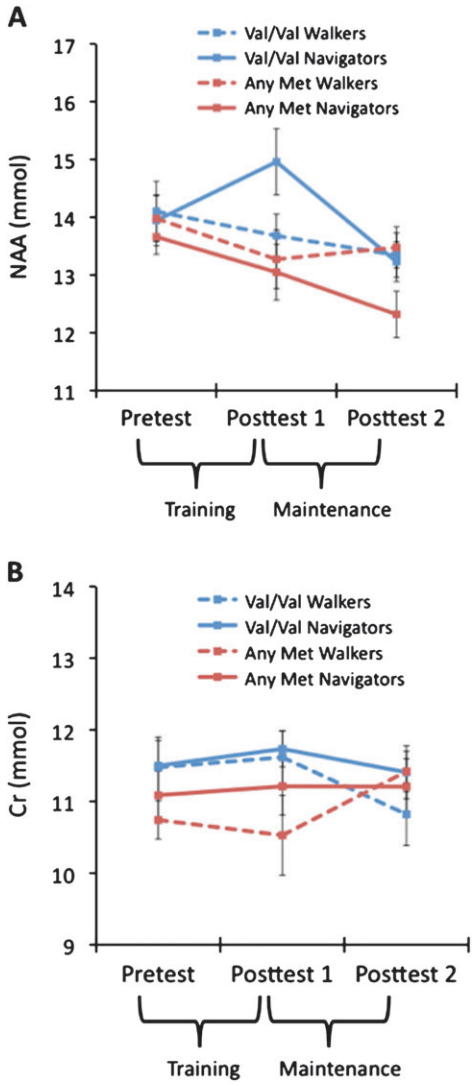

Figure 3. (*A*) Mean (±standard error) NAA as a function of training group (navigation/walk-time yoked control), BDNF genotype (Val/Val vs. Any Met), and time of assessment (pretest/posttest 1/posttest 2) and (*B*) a corresponding plot for Cr.

MRS Results

No significant effects involving training group or BDNF group were obtained in analyses of pretest NAA and Cr, $Fs_{1,32} < 2.40$, indicating that the groups were comparable in these measures at baseline (see Fig. 3).

A 4-way ANOVA testing training-related changes in the metabolite concentrations (NAA and Cr) revealed a significant quadratic training × BDNF × time metabolite interaction, $F_{1,32} = 4.34$, $P = 0.045$. An inspection of Figure 3*A* indicates that NAA displayed transient increases from training for Val/Val navigators only and that no changes in Cr (Fig. 3*B*) occurred for any of the groups. A 3-way ANOVA on Cr, including training, BDNF, and time as factors, confirmed this interpretation through the absence of reliable effects involving training or time, $Fs_{1,32} < 2.35$. In addition, a 3-way ANOVA on NAA revealed a significant quadratic training × time, $F_{1,32} = 6.03$, $P = 0.020$ and a significant BDNF × time effect, $F_{1,32} = 4.82$, $P = 0.035$. However, the training × BDNF × time interaction failed to reach significance, which led us to follow up the original 4-way interaction with training × time ANOVAs on NAA for the 2 BDNF groups separately. Critically, the quadratic training × time interaction was significant for BDNF Val/Val participants, $F_{1,32} = 7.04$, $P = 0.017$, but not for Any Met participants, $F_{1,32} < 1$. Finally, we compared the Val/Val navigators with the Any Met navigators in a BDNF × time ANOVA on NAA, which revealed a significant quadratic BDNF × time interaction, $F_{1,32} = 8.03$, $P = 0.011$. Paired *t*-tests showed that Val/Val navigators increased significantly in NAA between pretest and posttest 1, $t_{10} = 4.34$, $P = 0.001$, and decreased significantly in NAA over the maintenance phase, $t_{10} = 3.65$, $P = 0.004$. We conclude that the transient navigation-related increases were selective for NAA and BDNF Val/Val navigators.

Next, we correlated changes in navigation performance between pretest and posttest 1 with changes in NAA and Cr separately for the 4 training × BDNF groups. Results showed a significant positive association between changes in navigation performance and NAA for the Val/Val navigators, $r = 0.78$, $P = 0.004$. No other significant correlations were observed, $rs = -0.57$ to 0.36. Figure 4 shows the scatterplots of the associations between changes in navigation performance and changes in NAA for the Val/Val and Any Met navigators, as well as the corresponding plots for Cr.

Discussion

The present results show that changes in demands on spatial navigation induce increases of NAA in the right hippocampus of male BDNF Val homozygotes but not of Met carriers. These changes returned to baseline 4 months after termination of training. Importantly, increases in navigation performance correlated with NAA changes in BDNF Val/Val navigators.

The increments in hippocampal NAA may reflect increases in neuronal cell density, altered metabolism, gliogenesis, or growth of cells, axons, and dendrites (Moffett et al. 2007), in response to the training-induced increases in demands on hippocampal spatial processing. Unlike previous observations of experience-dependent changes in hippocampal volume (Draganski et al. 2006), plastic alterations in NAA are

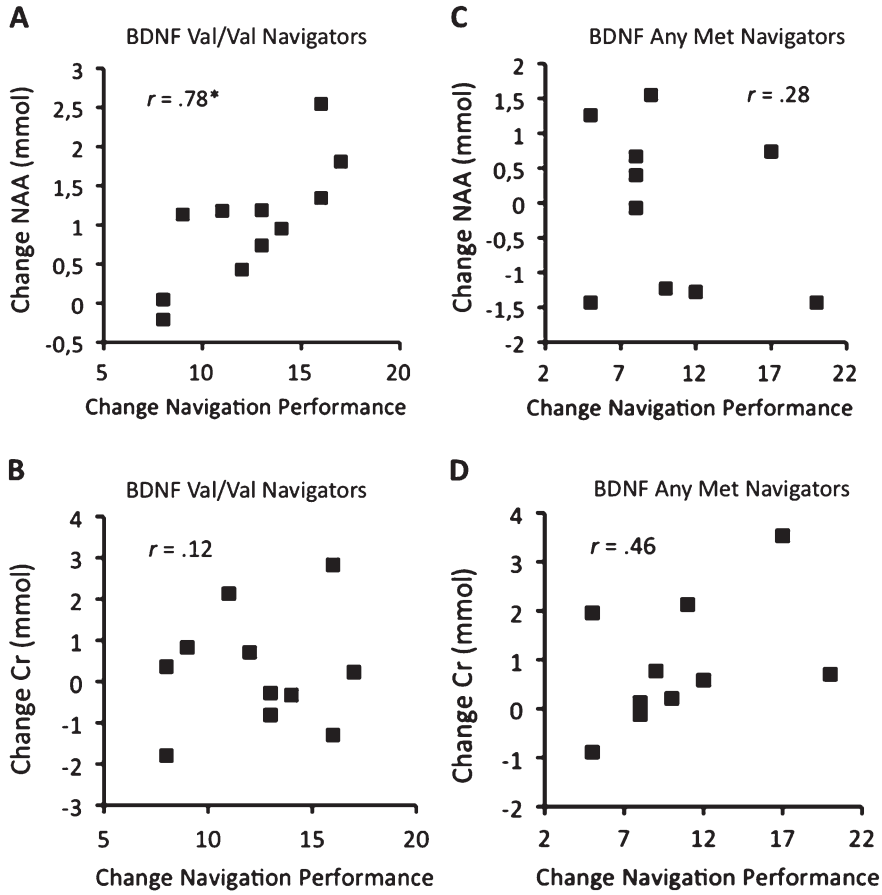

Figure 4. (*A*) Scatterplots of the associations for BDNF Val/Val navigators between changes from pretest to posttest 1 in navigation performance and changes in NAA and (*B*) a corresponding plot for Cr. (*C*) Scatterplots of the associations for Any Met navigators between changes in navigation performance and changes in NAA and (*D*) a corresponding plot for Cr.

specifically sensitive to functional properties and metabolic aspects of neural and glia tissue and unlikely to reflect alterations in microvasculature. In line with our findings, such experience-dependent changes have been observed in the nonhuman hippocampus (Rosenzweig and Bennett 1996; van Praag et al. 2000; Jessberger and Gage 2008; Holtmaat and Svoboda 2009). The effects of BDNF genotype on training-related increases in NAA concentrations may reflect the role of activity-dependent secretion of BDNF in such forms of hippocampal plasticity (Poo 2001; Lu 2003; Binder and Scharfman 2004). With lower BDNF secretion, these changes may not occur or require larger or more persistent changes in experiential demand to materialize (Lövdén et al. 2010). This interpretation is consistent with findings of reduced reorganization of motor maps from short-term training in BDNF Met carriers (Kleim et al. 2006).

In addition, BDNF Val homozygotes and Met carriers may use spatial strategies that differ in the extent to which they require hippocampal involvement, and therefore in the extent to which they cause experience-dependent hippocampal changes. Specifically, BDNF Val homozygotes may have used hippocampal-dependent cognitive-map strategies to a greater extent, whereas BDNF Met carriers may have relied more on cue-response strategies, which draw on, for example, the caudate nucleus (Hartley et al. 2003; Iaria et al. 2003). Such differences in spatial navigation strategies are correlated with hippocampal and caudate volumes (Moffat et al. 2006; Bohbot et al. 2007) and discriminate between younger and older adults (Lövdén et al. 2005) and between men and women (Lövdén et al. 2007).

The strategy account is supported by the transient nature of the training-dependent NAA changes. Specifically, these findings indicate that higher NAA concentrations may only be maintained if they are accompanied by more permanent changes in behavior. The strategy explanation is also in line with the absence of significant effects of BDNF genotype on changes in navigation performance, suggesting that the regional neural correlates producing changes in navigation performance may be different for any Met participants (e.g., the caudate) than for Val/Val participants (i.e., the hippocampus). Note, however, that navigation performance displays a numerical trend for larger improvements in BDNF Val navigators than in Met navigators (see Fig. 2), motivating cautious interpretations. Likewise, we are hesitant to draw strong conclusions from the absence of differences in NAA between BDNF genotypes at baseline. Specifically, though the effective longitudinal sample was representative of the initially recruited sample, this null finding may reflect sampling error at recruitment. In addition, previous spectroscopy work on the effects of BDNF genotype has detected NAA differences only in the left hippocampus (Egan et al. 2003; Stern et al. 2008), whereas we, based on lateralization of spatial memory for

location (Burgess et al. 2002), measured NAA in the right hippocampus. Finally, we observed no BDNF differences in self-reported real-life navigation strategies, some of which correspond to the use of cue-response–based strategies and some to strategies based on spatial processing. In a similar vein, we observed no BDNF differences in map-drawing performance at pretest, which may be taken as evidence of equivalent allocentric spatial representations of the VE across genotypes. Though we are reluctant to rule out the presence of strategy differences based on these latter null effects, both accounts of the BDNF effect (i.e., direct effects of BDNF on NAA and effects of BDNF on hippocampal NAA through different strategies) are consistent with the important take-home message that cognitive demands can alter hippocampal NAA concentrations and that BDNF genotype moderates these effects.

An increase of levels of BDNF messenger RNA in response to physical exercise (Neeper et al. 1995) has been suggested as a mechanism underlying effects of cardiovascular-fitness training on cognitive functioning (Colcombe and Kramer 2003). In addition, self-motion may play an important role in learning-related plasticity (Kempermann 2008). Based on our comparison with the walk-time yoked control group, we can rule out that the effects reflect influences of the walking component per se. However, we cannot rule out that the walking component is a contributing factor in interaction with the navigation. That said, the low demands of the slow walking speed selected by our healthy and fit participants are unlikely to alter physical fitness. In addition, in real life, some type of self-motion typically accompanies navigation behavior. In this sense, our paradigm possesses high ecological validity. Finally, generalization from the study is limited due to the exclusive focus on men, which was implemented to reduce interindividual differences in navigation strategies. On average, men tend to rely more on cognitive-map strategies (Lövdén et al. 2007) and to activate the hippocampus (Gron et al. 2000) to a greater extent during navigation tasks than women.

We conclude that changes in demands on spatial navigation can alter hippocampal NAA concentrations, experimentally confirming epidemiological evidence (Lövdén et al. 2005; Hertzog et al. 2009) suggesting that mental experience may have direct effects on neural integrity and cognitive performance. BDNF genotype moderates these plastic changes, in line with the contention that gene–context interactions shape the ontogeny of complex phenotypes (McLearn 2006).

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

Funding

Max Planck Society, including a grant from the innovation fund the Sofja Kovalevskaja Award to M.L. administered by the Alexander von Humboldt foundation and donated by the German Federal Ministry for Education and Research (BMBF); the German Research Council; the Swedish Research Council; and by an Alexander von Humboldt Research Award to L.B.

Notes

The authors thank Colin Bauer, Daniel Bittner, Marcel Derks, Isabel Dziobek, Gabriele Faust, Kristen Kennedy, Shireen Kwiatkowska-Naqvi, Naftali Raz, Karen Rodrigue, Michael Schellenbach, Elisabeth Wenger, and all student assistants. *Conflict of Interest* : None declared.

References

Atchley WR, Gaskins CT, Anderson D. 1976. Statistical properties of ratios. I. Empirical results. Syst Zool. 25:137–148.

Barnea A, Nottebohm F. 1994. Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. Proc Natl Acad Sci U S A. 91:11217–11221.

Binder DK, Scharfman HE. 2004. Brain-derived neurotrophic factors. Growth Factors. 22:123–131.

Bohbot VD, Lerch J, Thorndycraft B, Iaria G, Zijdenbos AP. 2007. Gray matter differences correlate with spontaneous strategies in a human virtual navigation task. J Neurosci. 27:10078–10083.

Burgess N, Maguire E, O'Keefe J. 2002. The human hippocampus and spatial and episodic memory. Neuron. 35:625–641.

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS. 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wildtype BDNF in neurosecretory cells and cortical neurons. J Neurosci. 24:4401–4411.

Colcombe S, Kramer AF. 2003. Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol Sci. 14:125–130.

Draganski B, Gaser C, Kempermann G, Kuhn HG, Winkler J, Büchel C, May A. 2006. Temporal and spatial dynamics of brain structure changes during extensive learning. J Neurosci. 26:6314–6317.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Betolino A, Zaitsev E, Gold B, Goldman D, Dean M, et al. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 112:257–269.

Gron G, Wunderlich AP, Spitzer M, Tomczak R, Riepe MW. 2000. Brain activation during human navigation: gender-different neural networks as substrate of performance. Nat Neurosci. 3:404–408.

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egena MF, Weinberger DR. 2003. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci. 23:6690–6694.

Hartley T, Maguire EA, Spiers HJ, Burgess N. 2003. The well-worn route and the path less traveled; distinct neural bases of route following and wayfinding in humans. Neuron. 37:877–888.

Healy SD, Krebs JR. 1992. Food-storing and the hippocampus in corvids: amount and volume are correlated. Proc R Soc Lond. 248:241–245.

Hertzog C, Kramer AF, Wilson RS, Lindenberger U. 2009. Enrichment effects on adult cognitive development: can functional capacity of older adults be preserved and enhanced. Psychol Sci Public Interest. 9:1–65.

Holtmaat A, Svoboda K. 2009. Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci. 10:647–658.

Iaria G, Petrides M, Dagher A, Pike B, Bohbot VD. 2003. Cognitive strategies dependent on the hippocampus and caudate nucleus in human navigation: variability and change with practice. J Neurosci. 23:5945–5952.

Jessberger S, Gage FH. 2008. Stem-cell-associated structural and functional plasticity in the aging hippocampus. Psychol Aging. 23:684–691.

Kempermann G. 2008. The neurogenic reserve hypothesis: what is adult hippocampal neurogenesis goof for? TINS. 31:163–169.

Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC. 2006. BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat Neurosci. 9:735–737.

Lehrl S, Merz J, Burkard G, Fischer B. 1991. Manual zum MWT-a [Manual for MWT-a]. Erlangen (Germany): Perimed.

Li S-C, Chicherio C, Nyberg L, von Oertzen T, Nagel IE, Papenberg G, Sander T, Heekeren HR, Lindenberger U, Bäckman L. 2010.

Ebbinghaus revisited: influences of the BDNF Val66Met polymorphism on backward serial recall are modulated by human aging. J Cogn Neurosci. 22:2164–2173.
Lövdén M, Bäckman L, Lindenberger U, Schaefer S, Schmiedek F. 2010. A theoretical framework for the study of adult cognitive plasticity. Psychol Bull. 136:659–676.
Lövdén M, Ghisletta P, Lindenberger U. 2005. Social participation attenuates decline in perceptual speed in old and very old age. Psychol Aging. 20:423–434.
Lövdén M, Herlitz A, Schellenbach M, Grossman-Hutter B, Krüger A, Lindenberger U. 2007. Quantitative and qualitative sex differences in spatial navigation. Scand J Psychol. 48:352–358.
Lövdén M, Schellenbach M, Grossman-Hutter B, Krüger A, Lindenberger U. 2005. Environmental topography and postural control demands shape aging-associated decrements in spatial navigation performance. Psychol Aging. 20:683–694.
Lu B. 2003. BDNF and activity-dependent synaptic modulation. Learn Mem. 10:86–98.
Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak RSJ, Frith CD. 2000. Navigation-related structural change in the hippocampi of taxi drivers. Proc Natl Acad Sci U S A. 97:4398–4403.
Maguire EA, Woollett K, Spiers HJ. 2006. London taxi drivers and bus drivers: a structural MRI and neuropsychological analysis. Hippocampus. 16:1091–1101.
McLearn GE. 2006. Contextual genetics. Trends Genet. 22:314–319.
McNaughton BL, Barnes CA, Gerrard JL, Gothard K, Jung MW, Knierim JJ, Kudrimoti H, Qin Y, Skaggs WE, Suster M, et al. 1996. Deciphering the hippocampal polyglot: the hippocampus as a path integration system. J Exp Biol. 199:173–185.
Moffat SD, Kennedy KM, Rodrigue KM, Raz N. 2006. Extrahippocampal contributions to age differences in human spatial navigation. Cereb Cortex. 17:1274–1282.
Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AMA. 2007. N-acetylaspartate in the CNS: from neurodiagnostic to neurobiology. Prog Neurobiol. 81:89–131.
Montag C, Reuter M, Newport B, Elger C, Weber B. 2008. The BDNF Val66Met polymorphism affects amygdala activity in response to emotional stimuli: evidence from a genetic imaging study. NeuroImage. 42:1554–1559.
Murer MG, Yan Q, Raisman-Vozari R. 2001. Brain-derived neurotrophic factors in the control human brain and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol. 63:71–124.
Nagel IE, Chicherio C, Li S-C, von Oertzen T, Sander T, Villringer A, Hekkeren HR, Bäckman L, Lindenberger U. 2008. Human aging magnifies genetic effects on executive functioning and working memory. Front Hum Neurosci. 2:1–8.
Neeper SA, Gomez PF, Choi J, Cotman C. 1995. Exercise and brain neurotrophins. Nature. 373:109–111.
O'Keefe J, Nadal L. 1978. The hippocampus as a cognitive map. Oxford: Oxford University Press.
Pearson K. 1897. On a form of spurious correlation which may arise when indices are used in the measurement of organs. Proc R Soc Lond. 60:489–502.
Pezewas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR. 2004. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci. 24:10099–10101.
Poo M. 2001. Neurotrophins as synaptic modulators. Nat Rev Neurosci. 2:24–32.
Provencher SW. 1993. Estimation of metabolite concentrations from localised in vivo proton NMR spectra. Magn Reson Med. 30:672–679.
Rosenzweig MR, Bennett EL. 1996. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res. 78:57–65.
Stackman RW, Clark AS, Taube JS. 2002. Hippocampal spatial representations require vestibular input. Hippocampus. 12:291–303.
Stern AJ, Savostyanova AA, Goldman A, Barnett AS, van der Veen JWC, Caillicott JH, Mattay VS, Weinberger DR, Marenco S. 2008. Impact of the brain-derived neurotrophic factor vall66met polymorphism on levels of hippocampal N-Acetyl-Aspartate assessed by magnetic resonance spectroscopic imaging at 3 Tesla. Biol Psychiatry. 64:856–862.
Van Petten C. 2004. Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. Neuropsychologia. 42:1394–1413.
van Praag H, Kempermann G, Gage FH. 2000. Neural consequences of environmental enrichment. Nat Rev Neurosci. 1:191–198.
Wechsler D. 1981. Manual for the Wechsler Adult Intelligence Scale—Revised. New York: Psychological Corporation.