Memory training alters hippocampal neurochemistry in healthy elderly

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Accumulating epidemiological evidence supports the notion of brain reserve, but there has been no investigation of neurobiological change associated with brief mental activation training in humans. Healthy older individuals were therefore investigated with magnetic resonance spectroscopy (MRS) in different brain regions before and after 5 weeks of focused memory training. Recall of a test-word list of $>$ 23 items was achieved accompanied by elevation of creatine and choline signals in the hippocampus. Those at risk for neural dysfunction, as indicated by lower neurometabolites at baseline, demonstrated the largest MRS increases after training. Biochemical changes related to cellular energy and cell-membrane turnover were found to increase after structured memory exercises and were limited to the medial temporal lobe. *NeuroReport* 14:1333–1337 © 2003 Lippincott Williams & Wilkins.

Key words: Ageing; Brain reserve; Creatine; Dementia; Magnetic resonance spectroscopy; Memory; Training

INTRODUCTION

How can lifetime patterns of mental activity modulate the pathogenesis or clinical manifestation of neurodegenerative disease? This concept, commonly referred to as brain reserve, has arisen from epidemiological studies showing that activities such as advanced education, occupational complexity, greater pre-morbid IQ and increased participation in post-retirement leisure activities independently relate to lower risk for cognitive decline and incident dementia [1]. Neuroimaging studies have shown that cognitive performance can be preserved in individuals with Alzheimer's disease (AD) perfusion deficits who also hold complex occupational histories or high educational levels [2]. Discovering the mechanisms by which protracted habits of mental activity should offer neuroprotection in late life is extremely challenging, with no satisfactory answers at present.

Studying the effects of mental stimulation over short-term periods, say a number of weeks, is one way of making this problem simpler. In rats, brief periods of enriched mental activity lead to a number of beneficial neurotrophic changes, including neurogenesis [3], enhanced synaptic budding and dendritic arborization complexity [4] and even protection from brain disease [5,6]. Induction of the ARC gene and increased brain-derived neurotrophic factor activity have been implicated in these brain changes [7]. Post-mortem human studies also confirm the close link between brain reserve indices like education and pre-morbid IQ and synaptic density [8]. The neurobiological effect of structured mental activity programs in adult humans, however, remains untested.

One cognitive memory program that is both brief and highly successful is the ancient method of loci (MOL; see Fig. 1a,b). First described in Cicero's *De Oratore* $\sim$40 BC, it asks the subject to link features of a familiar environment with items from a list requiring memorization. Sequential retrieval is aided by walking though one's mental landscape, each landmark acting as a cue for a list item via a self-generated mental association. MOL performance therefore depends on several cognitive functions including the generation of imagery, linguistic association, working memory, and in particular, mental map retrieval and navigation. Use of the MOL strategy can increase standard free recall from 7–10 word items to 30–40 items in sequence [9].

We used localized proton magnetic resonance spectroscopy (MRS) to measure biochemistry in three different brain regions, before and after five weeks of MOL exercises. The right hippocampus [10], midline parietal–occipital region [11] and left frontal lobes [12] were chosen for spectroscopic evaluation because of their implication with cognitive processes identified as critical for MOL. MRS allows measurement of several metabolic products central to neural energy pathways, cell membrane integrity and neural function [13] (Fig. 2a–c).

MATERIALS AND METHODS

Participants: Twenty healthy elderly lifelong residents of Sydney were recruited by community advertisement. Inclusion criteria were age > 60 years, the absence of neurological or psychiatric illness, English language proficiency and the absence of drug or alcohol dependency, psychotropic medication use or contraindications for MRI procedures. The average age of the sample was 70.1 years. Ten subjects were randomly allocated to the intervention and control groups. Institutional ethics approval was given for the study and written informed consent was obtained from all participants. Subjects provided a brief medical history and underwent MRI and MRS scans at baseline. The subsequent five weeks constituted the training phase. Control participants received no special instructions during this time. MRI and MRS scans were repeated 1–3 weeks after the end of the training phase.

Training: MOL subjects completed a group training session where they practised imaginary travel around 25 Sydney tourist sites (Fig. 1a), in fixed sequence, until all could do so forwards and backwards. Each was

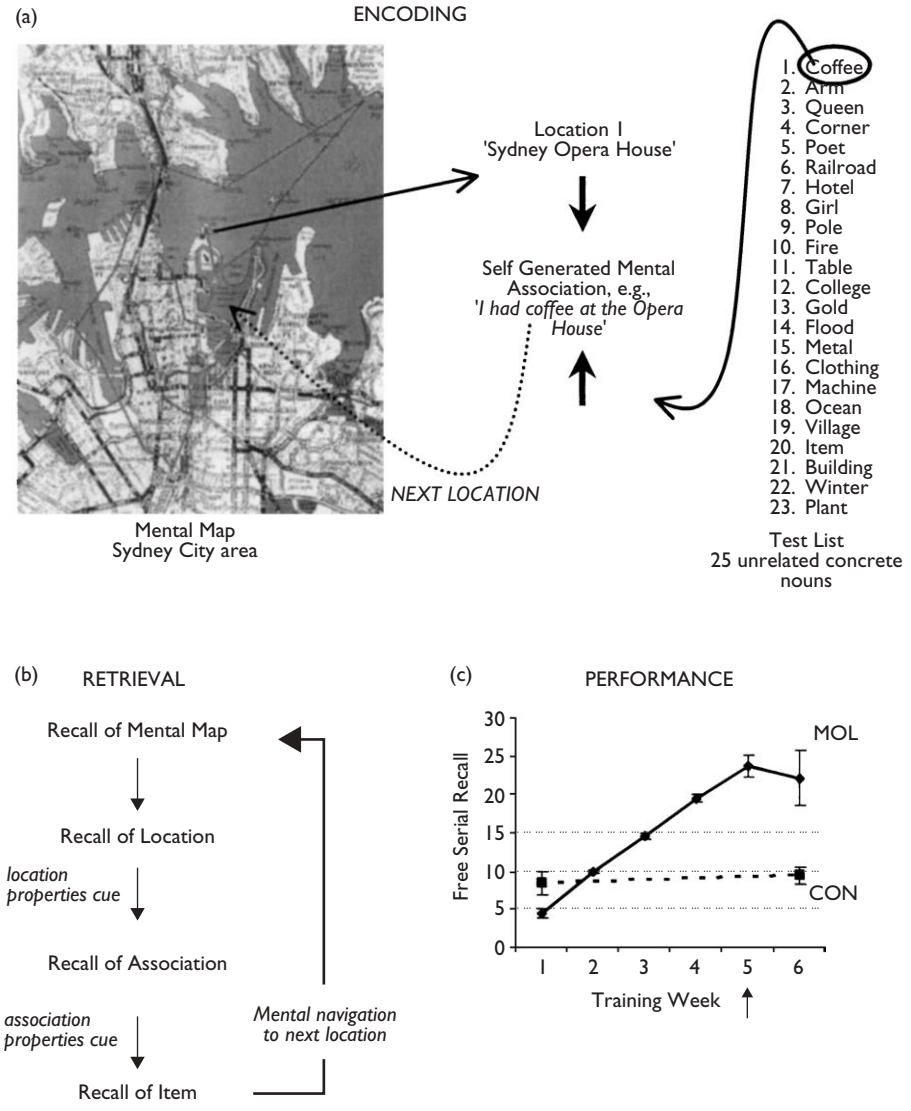

Fig. 1. (**a**) Overview of encoding processes in the method of loci (MOL). The schematic of the Sydney City area symbolizes the mental map that participants used in exercises. MOL involves retrieving a landmark from one's mental map representation and forming an imaginative association with an item from the test list. One then navigates to the next landmark and forms a new association with the next test list item and so on. (**b**) During retrieval, the mental map serves as the primary mnemonic aid. Retrieval of long lists is relatively easy because of reliance on an over-learnt spatial representation. Each landmark's properties cue recall of the self-generated mental association that cues recall of the test item. (**c**) Serial free recall performance in MOL subjects (solid line) and controls (CON, dashed line). Control performance was unchanged over the training period. MOL performance shows how subjects' performance improved with the increasing difficulty of weekly exercises ($p < 0.001$). Week 1 exercises involved using the first five landmarks of the mental map to recall the first five test items, with five more landmarks/items introduced each week. Training finished on week 5 (arrow) with 25 landmark/item exercises. s.d. shown as error bars.

then individually tested for recall of a five word-item list while using the following steps: (1) recalling the first landmark, (2) remembering a self-generated association with the landmark, (3) retrieving the word item by association, and finally (4) mentally navigating to the next landmark in the mental map and reiterating steps 2–4.

Subjects were then given an individualised homework book that coached participants in the MOL in pencil and paper format and served to check compliance. MOL subjects had to practice imagining travelling around the landmarks in order, then form associations between the test-list word items and each landmark, and then recall the test-list items by mentally navigating the circuit and recalling the test item via the self-generated mental association (Fig. 1b). Each week's assignment typically took participants 15–20 min to complete. In the first week the test list comprised five new test items, and five new words were added at the beginning of each subsequent week, so that the test list by week 5 comprised 25 items. A researcher rang the experimental subjects each week and obtained scores for that week's homework assignment. In addition, recall of the full test list was examined over the phone. The DASS (Depression Anxiety Stress Scale, 21 items) [14] was administered before and after the training phase to assess for possible motivational differences between the groups.

Imaging: All MR investigations were conducted on a 1.5 T Signa scanner (General Electric, echospeed with 8.3 level software) by an operator blind to the training status of the subjects. A sagittal scout image was acquired in the medial plane to replicate head position. This was followed by a 3D 1.5 mm thick coronal FSPGR T1-weighted anatomical scan of the whole brain (parameters: TR = 12.2, TE = 5.3). ^{1}H-MRS was performed in three brain regions. The right hippocampal region was defined by a 1.5 cm (superior–inferior) × 2.0 cm × 2.0 cm volume of interest (VOI). Localisation of the hippocampal VOI was completed from coronal images in the following manner. The right amygdala was identified and the operator then moved posteriorly slice by slice until the inferior horn of lateral ventricle was visible and the hippocampus was seen bordered by an approximately continuous rim of CSF both superiorly and medially (by the choroidal fissure). Lateral margins were defined by positioning the VOI in the middle of the structure and superior–inferior margins were defined by bisection of the CSF on top of the hippocampus and bisection of the entorhinal cortex below the hippocampus (Fig. 2b).

The left 2.0 × 2.0 × 2.0 cm frontal lobe area (FLA) was located anterior to the left lateral ventricular horn, comprising of frontal white matter and portions of orbital–frontal cortex and mid-frontal cortex as described previously [15]. The 2.0 × 2.0 × 2.7 cm (anterior–posterior) occipital–parietal (OPR) volume was located over the posterior longitudinal

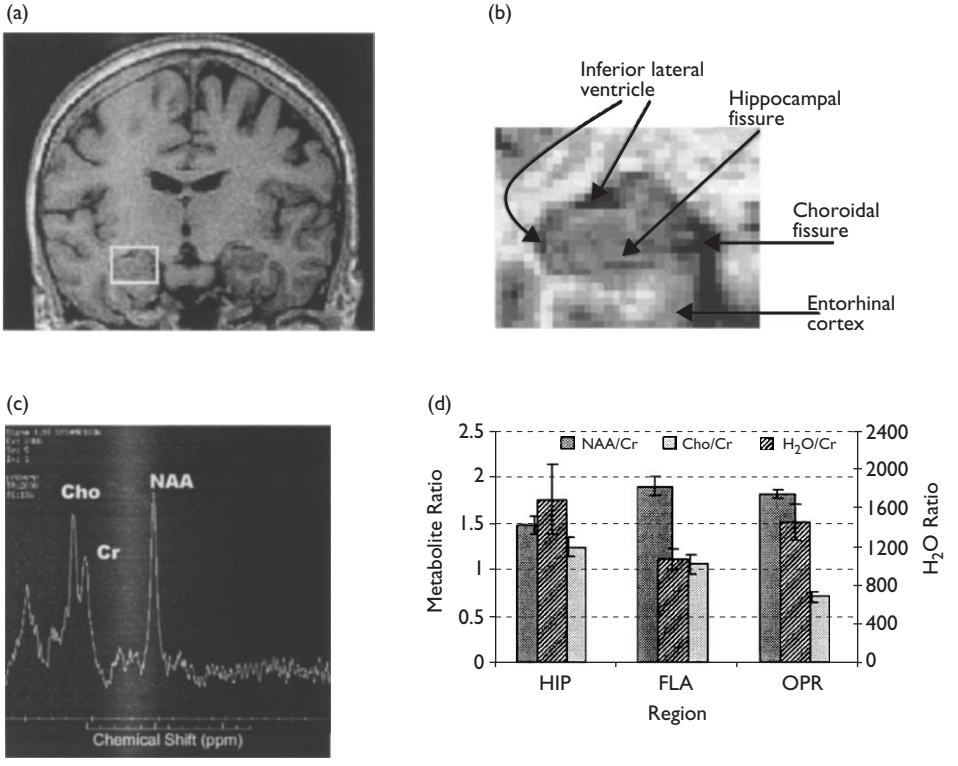

Fig. 2. (**a**) Example of the right hippocampal MRS volume of interest in a 72-year-old female. (**b**) Close-up of hippocampal MRS volume with anatomical localization landmarks. (**c**) Example of MR spectrum from hippocampal region. N-acetylaspartate (NAA), Creatine (Cr) and Choline (Cho) signals are indicated. (**d**) Average metabolite values at baseline for whole sample. NAA/Cr was significantly lower in the hippocampal (HIP) region than in the frontal lobe area (FLA) or occipital–parietal region (OPR; $p < 0.001$). Cho/Cr was greatest in the HIP region and lowest in the OPR, with FLA intermediate (all comparisons $p < 0.005$). H_2O/Cr was not significantly different between HIP and OPR, but the FLA demonstrated a lower signal ($p < 0.001$). s.d. shown as error bars.

fissure, including mainly posterior cingulate cortex, as described previously [15]. All regions were examined using the PRESS pulse sequence (parameters: TE 136, TR 2000, 2048 number of data acquisitions, 2500 Hz bandwidth). The hippocampal spectrum was averaged over 254 excitations, the FLA over 128 and the OPR over 64. An example of a hippocampal spectrum is given in Fig. 2c.

RESULTS

Behavioral data was examined to verify that the MOL had indeed worked as intended (Fig. 1c). Nine of 10 subjects in the MOL group could recall more than 23 unrelated concrete nouns, in order, by the end of training, significantly more than average free recall at the start of training (10 items, $p < 0.001$). There were no changes between groups on any of the DASS scales when comparing pre- and post-training scores ($F = 0.881$, $p = 0.362$).

MRS findings were also checked for reliability by successive hippocampal scans of one subject. N-acetyl-aspartate (NAA)/creatine (Cr) ratios in this case were 1.43, 1.37 and 1.47, a maximum error rate of $\pm 3.5\%$. Long-term reliability was assessed using the 5-week interval control hippocampal data, for which a non-significant difference of $\pm 2\%$ was found. Cerebrospinal fluid inclusion in the volumes of interest, as measured by a tissue segmentation algorithm, was equivalent over trials, also suggesting accurate retest localization. MRS signal to noise quality in the hippocampus was similar and identical during both sets of acquisitions (Cr SNR mean 12.20). Our data indicate a reliable and accurate MRS procedure.

MRS variation in the three brain regions was tested for independence. Collapsing across groups at baseline, most metabolite measures relative to Cr were significantly different between regions (Fig. 2d). Furthermore, there were no significant correlations between equivalent metabolite measures in different brain regions, strongly suggesting that we observed region-specific biochemical variance.

One experimental subject declined a second MRI and one hippocampal spectra from each group was unusable due to poor linewidth and signal-to-noise, leading to follow-up data in eight MOL subjects and nine controls. Overall, metabolite values changed in the MOL group over the training period compared to controls. There was an $\sim 10\%$ reduction in the NAA/Cr measure in the hippocampus ($p < 0.01$). This finding was unexpected as lower NAA/Cr ratios are typically a feature of AD or other neurodegenerative conditions [13]. NAA is a neural amino acid derivative highly correlated with both neural density and neural phosphorylation potential and has been proposed as an *in vivo* marker of neurometabolic fitness [13]. Cr is often used as an internal reference in AD studies, but mixed results have been found when more rigorous quantitation methods are employed [13]. Phosphorous MRS studies, for example, point to a more specific phosphocreatine deficit in early AD [16].

Given these considerations, metabolite values were recalculated relative to tissue water (H_2O^*)[15] and re-analysed. Non-parametric analysis was carried out after categorizing all subjects' metabolite change scores as responders ($> 3.5\%$ change, based on reliability analysis) or non-responders, as data were not normally distributed (Shapiro-Wilks test of normality value of 0.612, $p = 0.01$). Once again the only significant difference between groups was found in the hippocampus: both creatine and choline were elevated more often after MOL training and unchanged in controls ($\chi^2 = 7.71$, $p = 0.005$ for both comparisons; Fig. 3).

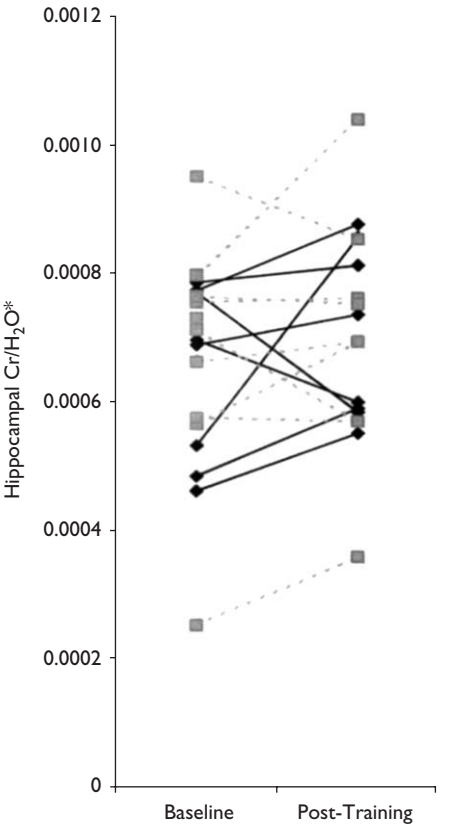

Fig. 3. Individuals' hippocampal creatine/H_2O^* values at baseline and after the 5-week interval period. Method of loci memory training participants are represented by the solid line, controls a dashed line. Overall, MOL subjects showed significantly more frequent creatine and choline elevation after the training phase than controls ($p = 0.005$ for both comparisons).

Finally, predictors of the induced creatine or choline response were examined and an inverse correlation found between baseline NAA/H_2O^* and Cr/H_2O^* percentage response (Spearman's rho $= -0.83$, $p = 0.01$). Choline and creatine response were highly correlated (rho $= 0.74$, $p < 0.04$).

DISCUSSION

The MOL intervention was extremely successful, with older subjects able to recall > 23 unrelated words in order by the end of training. This was accomplished not by repetitive rote learning, but by activation of a mental map stored in long-term memory and facilitating cued recall via self-generated mental associations. Tissue water referencing revealed significant increments in the Cr and Cho signals in the hippocampal region and not in the frontal or occipital–

parietal areas. These results were not related to any group differences in mood or motivation.

The proton spectroscopy Cr signal reflects resting levels of the high-energy phosphate buffer system, that is, concentration of both phosphocreatine (PCr) and creatine [17], which act via creatine kinase to regulate adenosine triphosphate. The increased processing demands of MOL exercises, which focus on an integration of spatial and episodic memory, may have upregulated resting oxidative metabolism in the hippocampus of participants. Topographic selectivity of memory-activity-induced change corresponds with other cognitive neuroscience findings in the medial temporal lobe [18].

A beneficial activity-dependent neurochemical effect is suggested in a brain region particularly susceptible to degenerative damage. Higher resting Cr levels have been associated with better neuropsychological performance in various cognitive domains [19], perhaps because PCr provides the most immediate energy source for cellular repolarisation [20]. Exogenous PCr has also proved an effective neuroprotective agent in rodent models of degenerative brain disease [21] and human Cr supplementation trials indicate a benefit on time-pressured psychometric tests [22]. Focused mental activity may therefore reactivate dormant neural populations in process-dependent areas by increasing resting endogenous levels of PCr; the net effect would be towards counteracting phosphocreatine deficits found in early AD [16] and increasing cellular energy available for synaptic transmission. Whether the induced Cr signal elevation we witnessed relates more specifically to increased PCr stores could potentially be distinguished by phosphorous spectroscopy.

NAA was unchanged and so no evidence was found for neurotrophic change predicted to accompany short-term mental activity [3]. Clearly, the possibility that neurogenesis may have occurred cannot be overlooked but it is unlikely to be to an extent detectable by MRS. However, low NAA has been associated with neural dysfunction [13] and those individuals with low NAA at baseline experienced the greatest Cr increments after our training program. It may turn out that those who benefit most from memory work, at a neurobiological level, are those at most risk, as has been the experience in some cognitive intervention programs [23]. Longitudinal research is needed to determine if memory exercises like the MOL are specifically protective against degenerative change or whether there was a general effect of increased mental activity.

Choline moieties increased in the hippocampus in a similar fashion to Cr augmentation, but a clear understanding for this change is not available. While many of the synapses in the hippocampus are cholinergic, acetylcholine is thought to make only a minor contribution to the Cho signal, with the majority determined by levels of cell membrane phosphatidylcholine precursors and breakdown products [17]. Thus increased membrane turnover due to increased mental work is one explanation. Another relies on the observation that the Cho signal correlates with dendritic density [24], which is in turn responsive to neural activity levels [25]. Choline changes due to age or inflammatory gliosis are unlikely to be involved due to the design of the study. A possible artefactual reason may stem from the technical challenges imposed by proton spectroscopy in the medial temporal lobe. The area is susceptible to both bone and air artefacts, tending to reduce signal quality and increase linewidth. While the overall Cr linewidth in our study was adequate, it was in the low range, and overlap with the Cho resonance may not have been fully adjusted for by post-processing. Further advances in MRS technology should allow more anatomically and biochemically refined assays to be implemented.

CONCLUSION

This study showed that focused memory exercises in the elderly can induce measurable and persisting biochemical changes in the hippocampus. Increased creatine and phosphocreatine signals indicate that the MOL memory program may have augmented resting oxidative phosphorylation in this region, an effect of possible neuroprotective value. Combining brief cognitive activation programs and modern neuroimaging tools may provide further insights into the mechanisms behind the brain reserve effect.

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