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Observations on the relationship between verbal explicit and implicit memory and density of neurons in the hippocampus

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Abstract—The relationship between neuronal density and verbal memory in left and right hippocampal subfields was investigated in patients who underwent surgery for alleviation of temporal lobe epilepsy. The surgery consisted of unilateral partial removal of the hippocampus along with the anterior temporal lobe and amygdala. Study 1 looked at post-surgical explicit vs implicit verbal memory for lists of words while Study 2 looked at pre- and post-surgical explicit memory for word pairs. Left subfield CA1 appeared to be the most consistently involved in explicit and implicit memory. The results of the two studies confirm presence of hemispheric asymmetry in verbal memory. The notion that hippocampal control of memory is most apparent in post-surgical performance is discussed. © 1998 Elsevier Science Ltd. All rights reserved

Key Words: epilepsy; CA1; human; plasticity; mind-brain; hemispheric specialization.

Introduction

Sperry and Cajal

Around the turn of the 20th century, the eminent neuroanatomist Santiago Ramon y Cajal (Nobel Laureate, 1906) proposed the notion of regularity in neuronal connectivity in the brain. Axons connect with each other according to a predetermined specific plan and this through chemical signals emanating from the tips of the axons themselves. Much later, in the 1940's, Roger W. Sperry proposed the notion of chemoaffinity to explain how developing axons in one part of the nervous system "know" where their final target is located. He conducted a series of elegant, by now classic, critical animal experiments which illustrated the notion of specificity in neuronal connectivity. Sperry admired Cajal greatly; he remarked that "he was the greatest neuroscientist of this century". Despite his prodigious output, Cajal did not pay much attention to left–right differences in the brain nor to a huge bundle of fibers, the corpus callosum, that connected the two halves of the cerebrum. The left and right sides were the main focus of Sperry's work with cats, monkeys, and neurosurgical patients [47, 48]; his findings on functional brain asymmetries in commissurotomy patients ("split-brain") won him a Nobel Prize in 1981. A world-wide scientific interest in brain asymmetry was sparked by the work that he began and the observations on hippocampal asymmetries described here reflect one of the outcomes of this interest.

Ramon y Cajal conducted many anatomical investigations of the hippocampus and provided some of the most detailed descriptions of this structure to date [37]. His specimens were mostly (though not exclusively) from the mouse and rabbit which are now known to have somewhat different anatomical arrangements in the hippocampus than humans, especially with regards to commissural connection between the hippocampi in the two sides [3, 4, 13, 36]. The hippocampal commissure appears to have grown relatively smaller in evolution as one moves from rats, to cats, to monkeys, to humans [40, 58, 59]. From a phylogenetic perspective alone, as hemispheric specialization evolved the size of the corpus callosum grew; there may have also been a progression toward somewhat less direct communication between the left and right hippocampi but with increased ipsilateral structural/functional connections between hippocampus

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and neocortex [40]. Such an evolutionary trend would support the notion that human memory systems in the two sides are wired up differently to support separate but complementary functional specialization in the hemispheres [61].

Sperry

Anyone who has ever worked with Roger Sperry at Caltech, Pasadena (first author of this article, DWZ) sensed his love and commitment to science, especially to the biological basis of behavior. He was an original and creative thinker who had a deep understanding for current problems and issues in brain research, who always interpreted scientific findings within a global context and who always supported and encouraged innovative research. Setting an example, he worked continuously and involved himself in the nitty gritty aspects of research. His scientific approach was data driven; truths are to be found in observations themselves and in the data, not in elaborate theories or in artificial models, he said. Books or journal articles alone do not untangle the mysteries of the brain and mind. Ideas in themselves are not ''proofs''. If one persists in looking and exploring through direct experimentation and investigation, one could find the answer, or an important part of it. But, then, one must be committed and enjoy the scientific search. These were some of the lessons this important scientist of the 20th century imparted to those who worked closely with him.

Asymmetries in memory

The hippocampus has been considered a major anatomical structure for memory and learning functions, for both verbal and non-verbal material as well as for spatial orientation. The bulk of the evidence for selective unilateral hippocampal contribution to memory comes from cases with unilateral hippocampal damage. Left-sided anterior temporal lobectomy (LTL) due to epilepsy, which includes the hippocampus in resected tissue, may lead to worse memory for verbal material [31, 67], whereas right-sided resection (RTL) may lead to impairments in non-verbal memory such as faces, routes, or musical melodies [7, 23–26, 68, 69]. Support for right hippocampus activation in topographical knowledge has been shown in an fMRI study [28]. These findings on memory functions are consistent with left hemisphere specialization for language and right hemisphere specialization for non-verbal, visuo–spatial, or some musical abilities.

Hippocampus anatomy

There is a complete hippocampal formation in either side of the brain, each consisting of distinct cytoarchitectonic regions (the Amonic subfields) which are said to be linked to each other in a single, unidirectional synaptic circuit. Anatomical comparisons between the human left and right hippocampal formations have largely been neglected in contrast to the attention given to anatomical asymmetries in the human neocortex [17]. However, recently, we have reported asymmetries in regional interconnectivity in the presence of mostly symmetrical neuronal density [62, 65, 66]. Our findings imply that memory and learning have asymmetrical computational machinery. The present study examines the relationship between neurons in the hippocampus in the left and right sides and verbal memory.

Memory and hippocampal subfield specificity

Despite the fact that the hippocampal subfields are linked in a single synaptic circuitry, they have selective sensitivity to damage. For example, in humans, the most ''vulnerable'' subfield to hypoxia is CA1, and the least sensitive are CA2 and CA3 (see classifications in [14]). Given differential susceptibilities, it is reasonable to suppose that the hippocampal subfields play differential roles in memory and learning. A few recent studies correlated pre-surgical scores with neuronal density and found evidence for subfield specificity although not all focused on left–right differences [33, 44, 45]. Taken together, the left hippocampus was consistently implicated in cell loss and verbal memory, and the CA3 and hilar regions were significantly more involved than CA1 or CA2 in a study by Sass *et al.* [44]. However, these previous studies were somewhat limited in that they tested only explicit memory or measured only pre-surgical memory. Thus, they were narrowly focused.

The present article describes the results of two experiments in which verbal memory was tested in patients with unilateral hippocampal damage, that is, in LTL and RTL. In both experiments, memory scores were compared to hippocampal neuronal density. The first measured post-surgical memory for explicit vs implicit memory for lists of words, and the second measured pre- and post-surgical explicit immediate and delayed memory for unrelated paired-words. Together, the two studies provide convergent evidence for the importance of post-surgical testing in inferring hippocampal function and for the selective role of left hippocampal neurons in verbal memory.

Study 1: Explicit vs Implicit Memory and Neuronal Density

Introduction

It is important to distinguish between explicit and implicit memory in patients with suspected memory defi-

cits [8, 54, 55]. Studies which measure the relationship between hippocampal morphology and verbal memory rarely emphasize implicit memory. The distinction between explicit and implicit memory was first demonstrated in amnesic patients with bilateral cortical damage which includes the hippocampal formation due to alcoholism, to Alzheimer's disease, or to stroke. They had impaired explicit memory (recall or recognition) but unimpaired implicit memory (word-completion or skill learning) when performance was compared to control subjects [18–20]. A recent study [67] has investigated the status of verbal explicit vs implicit memory in unilateral anterior temporal lobectomy (TL) patients and found asymmetrical explicit verbal memory (worse in the left than in the right) in the face of symmetrical implicit memory (which was not different from normal). Taken together, these studies demonstrate that memory is not a unitary function but rather is made of multiple systems [49].

The memory impairments which follow unilateral TL typically involve explicit memory (measured in recall or recognition). However, explicit and implicit memory may not be equally dependent on the integrity of the hippocampus; it is critical for explicit memory but not to the same extent for implicit memory [49]. Neuronal density measurement could shed light on the morphological correlates of explicit and implicit memory in the left and right hippocampus and this was the purpose of the present study.

Neuronal cell death occurs normally in development (programmed cell death) but can also indicate a disease process. For example, hippocampal neuronal cell loss is common in temporal lobe epilepsy [5, 29, 39]. The degree of neuronal loss (determined by cell counts) predicts the pre-surgical verbal memory deficit in patients with a TL [44]; post-surgical memory deficits in TL patients are most pronounced, according to some investigators, when patients have good memory scores before surgery [10, 21, 22, 34, 38] and when there is least amount of cell loss [21, 34, 39]. That is, the healthier the hippocampus at time of surgery, the worse the memory after surgery. An MRI study of TL patients has reached similar conclusion regarding hippocampal volume [52]. This in turn suggests that the neuronal brain circuitry supporting memory is already established before hippocampal resection. Consequently, one may assume that in TL patients, post-surgical scores alone reflect partially the pre-surgical hippocampal status. That is, poor memory after surgery goes with relatively greater pre-surgical neuronal density while relatively good memory after surgery reflects the reverse situation for pre-surgical neuronal density. In this study, we only had post-surgical performance. So we correlated the scores for explicit and implicit memory with neuronal density in three hippocampal subfields and assumed that areas connected to the resected region contributed to the observed behavior after surgery.

Methods

Patients

Nineteen right-handed patients suffering from temporal lobe epilepsy were studied after they underwent *en bloc* unilateral anterior TL resection in the Radcliffe Infirmary, Oxford. TL consisted of resection of the anterior temporal lobe (approximately 5–5.5 cm on the left and 5.5–6 cm on the right), the amygdala and the anterior 3 cm of the hippocampus (approximately two-thirds) on either side [35]. In 11 cases, TL was on the on the left side (mean age at surgery = 19.2 years) and in eight cases, surgery was on the right side (mean age at surgery = 25.4 years). There was no significant difference between the two groups with respect to age at time of surgery ($P < 0.1$). The onset of epilepsy in the majority of cases dates back to early childhood (mean onset age in the left-side group = 7.5 years, and in the right-side = 10.0 years). There was no significant difference between the two groups with respect to age of onset of habitual epilepsy ($P < 0.3$). The mean age at which patients were tested (left-side age = 26 years, right-side age = 30.9 years) did not differ significantly ($P < 0.2$). All were left hemisphere dominant for language as determined by the intracarotid sodium amobarbital procedure performed prior to surgery (applied to most cases) and/or by clinical neuropsychological evaluation. All patients had a post-operative WAIS or WISC Full Scale IQ of 80 and above.

Neuropathology

Immediately upon surgical removal, specimens were placed in 10% neutral formalin for 5–7 days. They were then sliced coronally at 1 cm intervals, the slices were embedded in paraffin wax and 20 μm sections were stained with Luxol fast blue and cresyl violet.

We used an Olympus BH2 light microscope fitted with a 10×10 square eyepiece grid to count nucleolated neurons within the entire thickness of the slice (20 μm). Neurons which fell within the boundaries of the grid were counted by two observers (DWZ and MME) while being blind to patient's identity. The average of the counts of the two observers was entered into statistical analysis. Five different areas within a hippocampal subfield were sampled using the eyepiece grid. We counted pyramidal neurons in hippocampal subfields CA1 and CA4 and granule cells in the dentate gyrus (DG). Total magnification for counting neurons in CA1 and CA4 was $\times 250$ and for the granule cells in DG it was $\times 880$. Abercrombie's formula was used to correct for overestimation of counts which may arise from section thickness [1]. The hippocampal subfields were defined according to Duvernoy [14]. CA1 contained the pyramidal neurons extending from the border with neurons forming subfield CA2 to the subiculum. Subfield CA4 was defined as the area enclosed by the band of granule cell layer of the DG. DG was the distinctive C-shaped formation of close-packed pyramidal cells [14]. The hippocampal slices were from the body of the hippocampus.

Memory test

A test measuring verbal recall (explicit memory) and printing (implicit memory) for four different lists of words was administered post-surgically [19, 67]. There were ten different words in each list which were read to the subjects by the experimenter. For each of the four lists the following procedures applied: First, subjects heard the words on the list and indicated their preference for each word as it was read on a 5-point Likert

scale, where one was "liked very much" and five was "disliked very much". This rating procedure for the words promotes a memory association between the word and its meaning. Then, the list was read to the subject twice more, in a different order each time, and the subject was asked to remember the words. Immediately after the second time, the subject wrote down all the words recalled from the list. This was immediately followed by the priming task in which the subject was required to generate complete words from three letters with the first word that came to mind, leaving out proper names. There were 20 different 3-letter stems. Only 10 of the word stems matched the beginning three letters of words on the study list. A 2 min interval separated the beginning of each of the four lists.

In the context of this experiment, recall measured explicit memory and priming measured implicit memory. Completion of stems with words from lists never shown to the subjects (i.e. the remaining 10 words on the priming list) constituted chance level in generating words from 3-letter stems (baseline rate). In the past, these procedures have resulted in reliable explicit—implicit dissociation in both amnesic patients and normal subjects [67].

Results

Neuronal density

Figure 1 shows mean neuronal density in each hippocampal subfield. Three comparisons using *t*-tests (P level set to 0.01 to control for multiple comparisons) showed that the means did not differ significantly between the left and right hippocampi, except for subfield CA4 ($P < 0.03$).

Memory data

The LTL group recalled significantly fewer words (mean = 45%) than the right RTL group (mean = 62%) ($t = 2.74$, $P < 0.01$) but they did not differ significantly from each other on the priming task (mean LTL = 18.4%; mean RTL = 18.1%; mean normal = 12%). That is, they did not differ in the mean number of stem completed words from the word list that was not remembered in the recall stage. The priming scores were significantly different (t LTL = 7.7, $P < 0.0001$; t RTL = 5.7, $P < 0.0002$) from the baseline stem completion rate (mean LTL = 6.8%; mean RTL = 7.9%), as in normal subjects (mean = 8% [67]). Lower verbal recall in LTL compared to RTL is consistent with damage to the left hemisphere.

Correlation between neuronal density and memory scores

Explicit memory (recall) did not correlate significantly with neuronal density in any hippocampal subfield, in either side (Fig. 2). But implicit memory, which behavi-

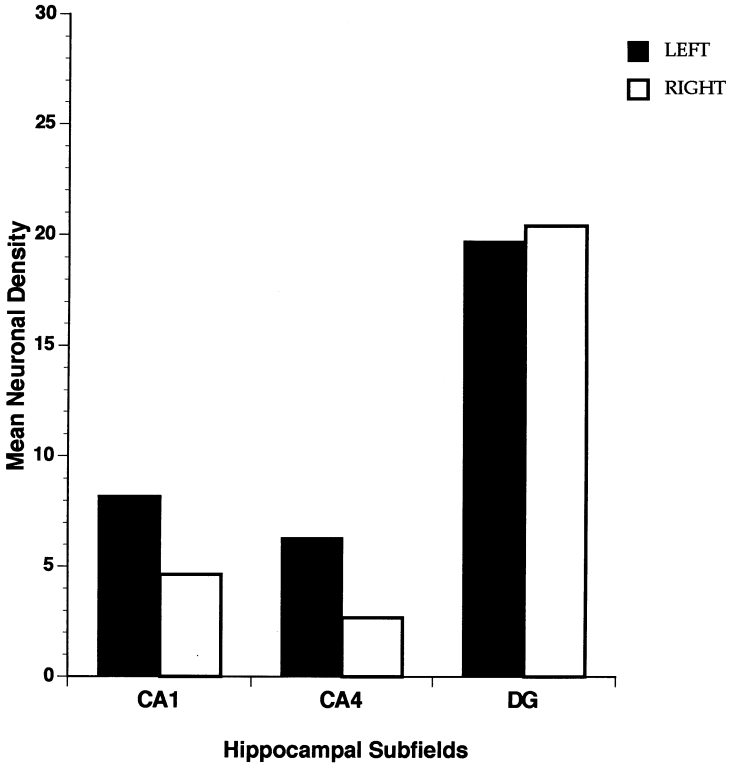

Fig. 1. Mean neuronal density in each of the three hippocampal subfields, in the left-side and right-side groups tested on the explicit vs implicit verbal memory test.

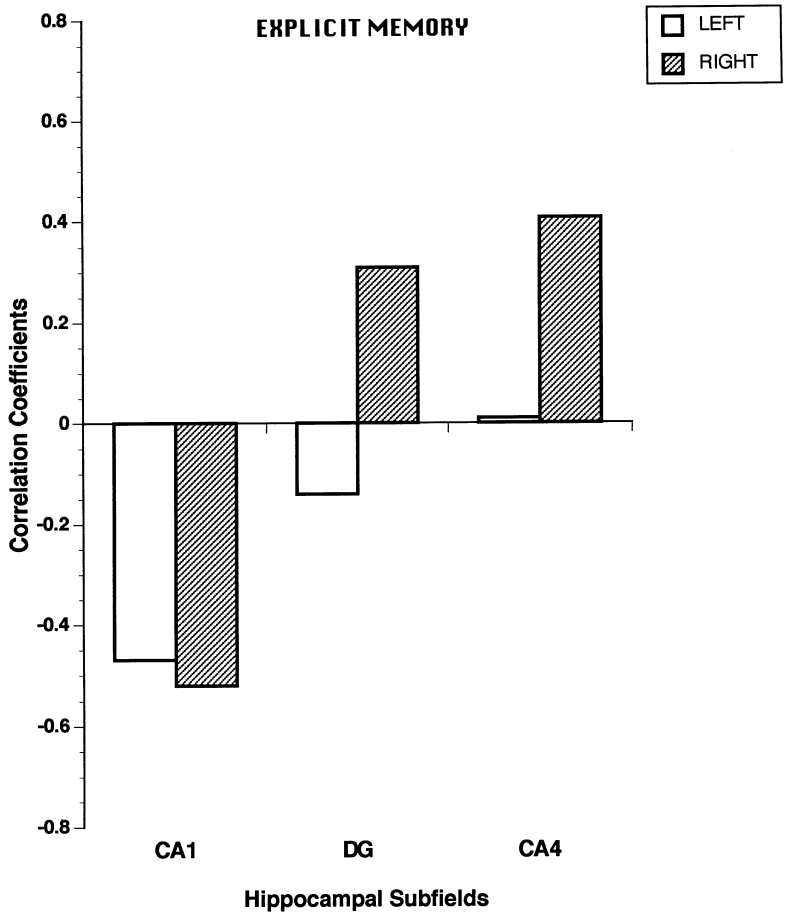

Fig. 2. This figure shows the correlation values (Spearman rank-order) between explicit recall of words from the target list by left and right unilateral anterior temporal lobectomy patients.

orally did not differentiate between LTL and RTL, correlated significantly with neuronal density only in LTL, in subfield CA1 ($r_s = 0.65$, $P < 0.05$). This is shown in Fig. 3. That is, the higher the implicit memory scores in LTL, the higher the density in left CA1. Correlation values between explicit memory and CA4 or DG density were low in either side. By comparison, the ρ values between explicit memory and CA1 density were negative in both sides (left: $r_s = -0.4{,}6$, $P < 0.1$; right: $r_s = -0.52$, $P < 0.08$). The negative direction of the correlation indicates that the higher the memory scores, the lower the neuronal density.

The difference in sign in the correlation values (positive for implicit, negative for explicit) hints at a mutual relationship between explicit and implicit memory. Thus, for each patient, the total number of words remembered from the target list was calculated (explicit + implicit). Then, a relative difference score was calculated [(implicit − explicit) ÷ (explicit + implicit)]. The correlations between the relative difference score and subfield neuronal density were determined. Significant positive correlation was obtained only for left CA1 (left CA1, $r_s = 0.90$, $P < 0.0001$; right CA1, $r_s = 0.19$, $P < 0.5$) and indicates that the bigger the difference between explicit and implicit memory, the higher the density. That is, density in left CA1 may represent the morphological substrate of the strength of implicit relative to explicit memory, as well as the presence of a reciprocal relationship between explicit and implicit verbal memory.

Discussion

We obtained a significant positive correlation between implicit memory and neuronal density only in left CA1 (consistent with left hemisphere specialization in verbal processing) in the presence of negative correlations (though not significant) between explicit memory and either left or right CA1 neuronal density. This outcome is not consistent with previous conclusions regarding the role of the hippocampus in implicit memory. Those conclusions posited that the hippocampus is not critical in implicit memory. However, they were based on gross neuroanatomical studies while our study looked at detailed morphological features. Our behavioral results, on the other hand, showed a left–right difference in explicit memory, in the absence of a left–right difference in implicit memory.

Importantly, our behavioral findings revealed that implicit memory is processed in each side of the brain, a

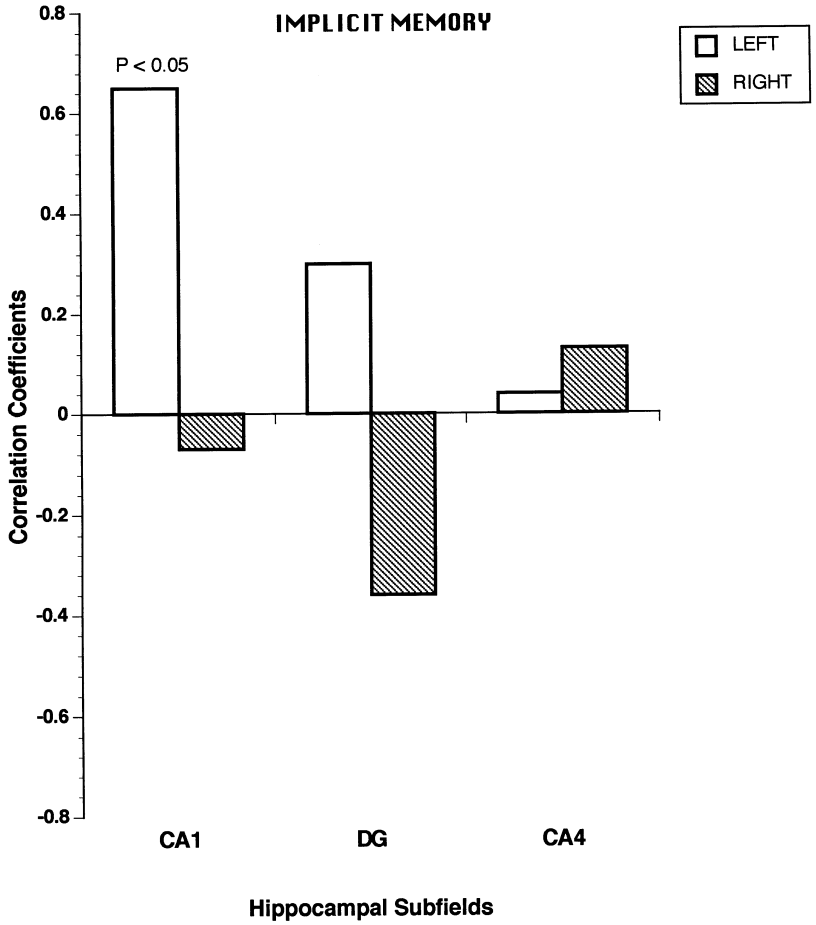

Fig. 3. This figure shows the correlation values (Spearman rank-order) between hippocampal neuronal density and implicit memory (words generated from 3-letter stems of words that were on the study list but were not recalled) and by left and right anterior temporal lobectomy patients.

finding that is consistent with the findings of Cronin–Golomb and associates in two split-brain patients from the Caltech series [12].

The present findings reveal a differential effect of hippocampal integrity on the two types of memory. Previously, Oxbury and Oxbury [34] and Hermann *et al.* [21] reported relatively pronounced memory (explicit) impairments in TL patients who had minimal hippocampal sclerosis; memory scores obtained prior to surgery went down appreciably in patients in whom presurgical scores were high [10, 22, 38]. Thus, a negative correlation between post-surgical explicit verbal memory and hippocampal integrity, as indexed by neuronal density, is expected in patients with minimal hippocampal sclerosis. Indeed, we found a trend in this direction for explicit memory, on either side, albeit the values obtained were not quite significant. This and the obtained positive correlation between density and priming (implicit memory) suggests a non-uniform effect of hippocampal neuronal loss on memory.

In addition, the morphological data provide a clue regarding the nature of a relationship between explicit and implicit memory that could not have been revealed by the behavioral data alone. The imperfect explicit memory we all experience in everyday life or in laboratory experiments suggests some kind of interference in the explicit memory process [15]. The causes have been difficult to disentangle in purely behavioral experiments. We accounted for a relationship between implicit and explicit memory when the relative difference between explicit and implicit memory was correlated with neuronal density (see Results section). The correlation with density in the right side was low and non-significant. The high positive correlation value obtained in the left side implies stronger implicit than explicit memory in these subjects. This could reflect more neurons dedicated to implicit memory than to explicit memory, and thus would go against the notion that the hippocampus is not involved in implicit memory [49]. Generalizing to the normal brain, if the number of hippocampal neurons dedicated to implicit memory is greater than the number for explicit memory, interference with explicit memory may occur. Or, if implicit memory neurons are more functional than explicit memory neurons, the same behavioral outcome would be observed. Either interpretation may be useful for explaining why correct recall is rarely 100%. It has been suggested previously that explicit and implicit memory represent separable memory

systems [32, 49, 70] but what is proposed here is that they may instead be interdependent, perhaps interconnected systems.

The observations on implicit and explicit memory in this article are based on post-surgical behavior, but are likely to have relevance to pre-surgical deficits as well. First, previous studies [21, 34] showed no significant difference in pre- and post-surgical verbal memory in TL patients, including the present sample. Second, pre-surgical scores alone could reflect the combined effects of both the diseased and healthy tissues. That is, finding a positive correlation between pre-surgical scores and morphology may be misinterpreted as evidence that only the diseased tissue controlled the behavior. Third, to verify that hippocampal neurons indeed controlled the behavior, memory should be assessed in their total absence, that is, after surgical removal. A parsimonious assessment would be to account for both pre- and post-surgical performance. Unfortunately, this was not possible in this experiment. However, this is attempted in Study 2 below. Future studies in which explicit and implicit memory are studied, pre- and post-surgically, should help verify the conjectures advanced here.

Although our sample is relatively small, our findings reveal some statistically significant results. They are the first to provide clues regarding differential and asymmetrical hippocampal-morphological correlates for explicit and implicit memory and to suggest that in the normal brain there may be a neural substrate for a reciprocal relationship between them. Future combined morphological and behavioral studies of both hippocampi in neurologically-intact subjects should help determine if the observations made here can be generalized to the normal brain.

In the next study we describe pre- and post-surgical behavioral results, their relationship to hippocampal neuronal density, and show that post-surgical behavior can validly be compared to neuronal density.

Study 2: Verbal Memory for Unrelated Paired Words and Neuronal Density

Introduction

The previous study used a non-clinical test designed specifically to measure explicit vs implicit memory. We would have preferred to have had pre-surgical scores as well but this was not possible. Our main interest here is in comparing the status of hippocampal integrity to verbal memory. In Study 2 we report pre- and post-surgical findings on the relationship between neuronal density and memory for unrelated paired words test (part of Associate Learning subtest from the Wechsler Memory Scale [57]) often used in clinical assessment of memory (administered by one of us, EDB). This measures explicit memory. Previously, the total score on this subtest showed that young LTL patients obtained significantly worse scores than RTL patients [2]. However, direct comparisons between matched immediate vs delayed trials, comparisons between pre- and post-surgical performance, and the distinction between common vs novel pairs have not been reported. Here, both immediate and delayed recall were determined on a matched series of test trials.

Methods

Patients

The patients consisted of a consecutive series of 26 young English speaking cases (12 with left-sided, 14 with right-sided focus) suffering from temporal lobe epilepsy that could not be controlled with drugs alone. Average age of onset of habitual epilepsy was around five years in each group. They all underwent unilateral anterior temporal lobectomy (TL). The average age at surgery did not differ significantly between the two groups (mean left = 13.6 years; mean right = 12.5 years). Patients were included in the study if their pre- or post-surgical WISC IQ was 70 or above. The pre-surgical testing was three months before surgery and the post-surgical testing was six months after surgery. Surgical procedure was the same as in Study 1. All patients 12 years or older underwent the sodium Amytal procedure in order to determine the laterality of language dominance. Speech dominance was lateralized to the left hemisphere in all patients as determined by this procedure and/or by neuropsychological indications [35]. The cause of the epilepsy in the majority of cases was presumed to be hippocampal sclerosis (Ammon's Horn sclerosis). In a few cases, the cause was indolent glioma, present in the hippocampus or temporal lobe, presumably since birth. None suffered from traumatic head injury.

Neuropathology and neuronal counts

The identical procedures used in Study 1 were applied.

Materials and procedures

The verbal test consisted of a modified version (described below) of the Associate Learning subtest of the Wechsler Memory Scale [57]. Ten pairs of words made up the study-list. They were either related (easy) or unrelated (hard) associations. The related pairs consisted of highly-familiar associations in Western culture (e.g. North–South) while unrelated associations were novel (e.g. school-grocery). After the words on the study list were read to the subject, a cued-recall procedure began. It consisted of cueing the subject with one member of the pair and requesting the subject to produce the other member. This was done three consecutive times (always with the same ten pairs). However, the order in which the words were read from the study-list as well as cued for recall, was different each time. Immediate memory was measured immediately after the last pair was presented, while delayed memory was measured following a 40 min filled delay, beginning after the last cued-recall procedure (the third time immediate memory was tested). This is one of the earliest standardized tests of memory functions. The test has been widely used in clinical settings and some archaic features are retained. Only the unrelated associations are considered valid measures of memory functions (the easy pairs can be remembered through pure guessing) [39], and only items in the third presentation are meaningful for a comparison

between immediate and delayed memory, since the position of the pairs in the presentation order and in cued-recall is identical.

Results

Neuronal density

The results for neuronal density are shown in Fig. 4. There were no significant left–right differences.

Memory data

As predicted, only the unrelated word associations revealed meaningful relationships with hippocampal subfield neuronal density. Figure 5 shows the high scores for the related ("Easy") word associations. Since the correlations with neuronal density were consistently low and non-significant, the results are not shown.

Figure 6 summarizes mean scores for the unrelated ("Hard") word pairs. There were no significant differences between LTL and RTL (albeit, on the post-immediate, pre-delay, and post-delay conditions, the mean score for RTL was higher than in LTL). Within the LTL group, post-immediate memory was worse than pre-immediate memory (Wilcoxon $= 6.0$, $P < 0.04$).

Figure 7 summarizes the correlation coefficients between memory scores on these unrelated pairs and neuronal density. The Figure illustrates the following important outcomes: (1) The pre-surgical memory scores (immediate or delayed) did not correlate significantly with subfield density in either side. (2) Significant values emerged only for the LTL. (3) Accounting for both the pre-surgical and post-surgical performance was revealing and significant only for delayed memory. Indeed, pre-minus post-surgical scores revealed strong positive correlation with left side neuronal density, but only with the delayed unrelated word associations.

Discussion

A parsimonious measure of hippocampus involvement in memory is to take the difference between the pre- and post-surgical scores and determine the correlation between this difference and neuronal density. When this was done, strong positive correlations were obtained only between delayed memory for unrelated word pairs and density in the left side (Fig. 7). That is, the greater the difference between pre- and post-surgical scores, the higher the density. The side is consistent with left hemisphere specialization in language processing and the type of memory, delayed vs immediate, is consistent with previous findings on the critical role of medial temporal lobe regions in long-term memory. These results suggest that the sampled left hippocampal subfields play a role in verbal long-term memory, with CA1 being the most involved and DG the least.

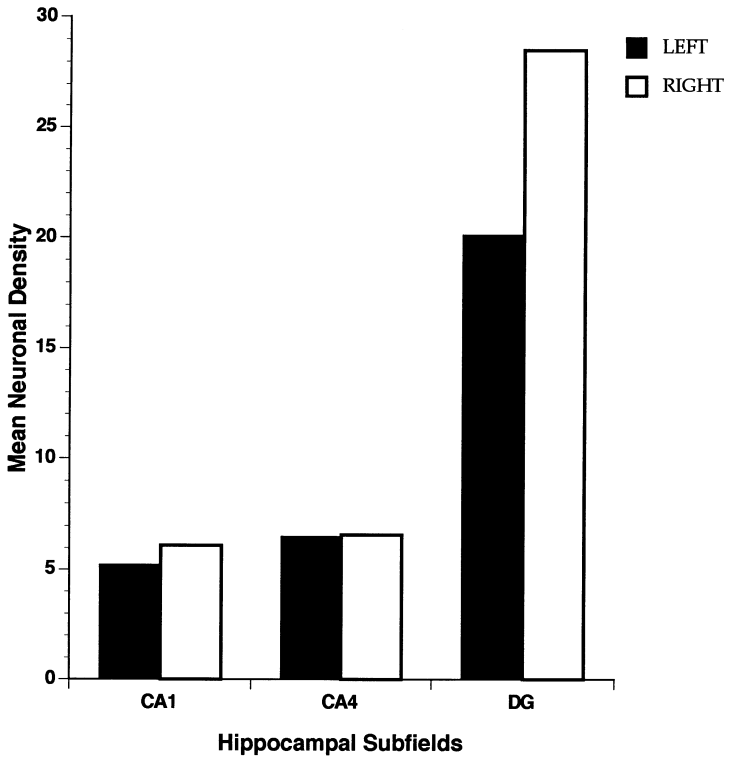

Fig. 4. Mean Neuronal density in the three hippocampal subfields, in each side, in the patients tested on explicit memory for paired-words.

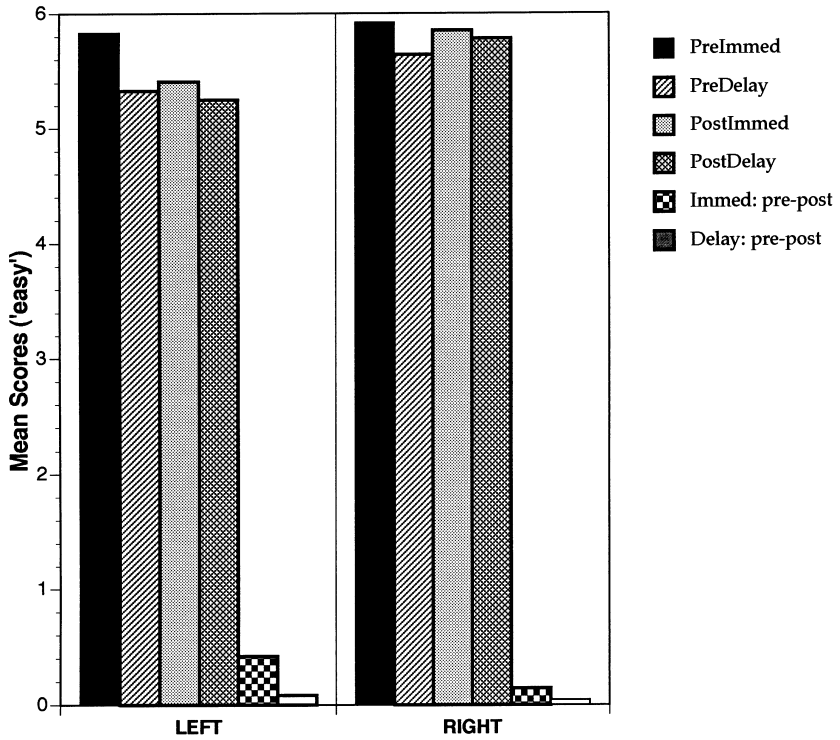

Fig. 5. Mean scores by the left and right unilateral anterior temporal lobectomy patients on the "easy" paired-word association, third trial. Maximum score is 6. PreImmed = preoperative, immediate memory; PreDelay = preoperative, delayed memory; PostImmed = postoperative, immediate memory; PostDelay = postoperative, delayed memory; Immed = preoperative minus postoperative scores on immediate memory; Delay = preoperative minus postoperative scores on delayed memory

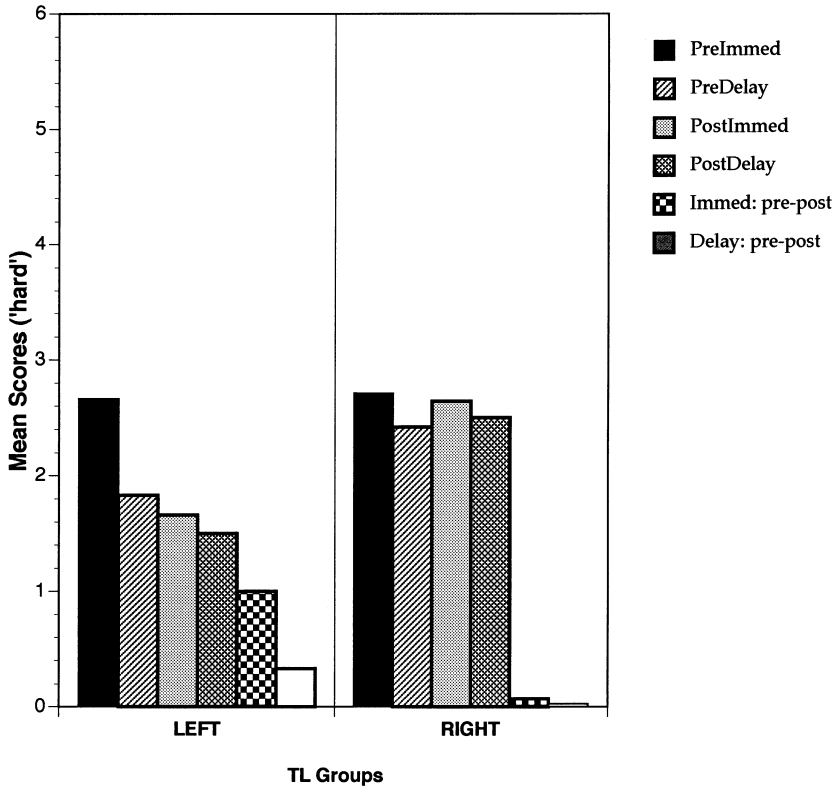

Fig. 6. Mean scores by the left and right unilateral anterior temporal lobectomy patients on the "hard" paired-word association, third trial. Maximum score is 4. PreImmed = preoperative, immediate memory; PreDelay = preoperative, delayed memory; PostImmed = postoperative, immediate memory; PostDelay = postoperative, delayed memory; Immed = preoperative minus postoperative scores on immediate memory; Delay = preoperative minus postoperative scores on delayed memory

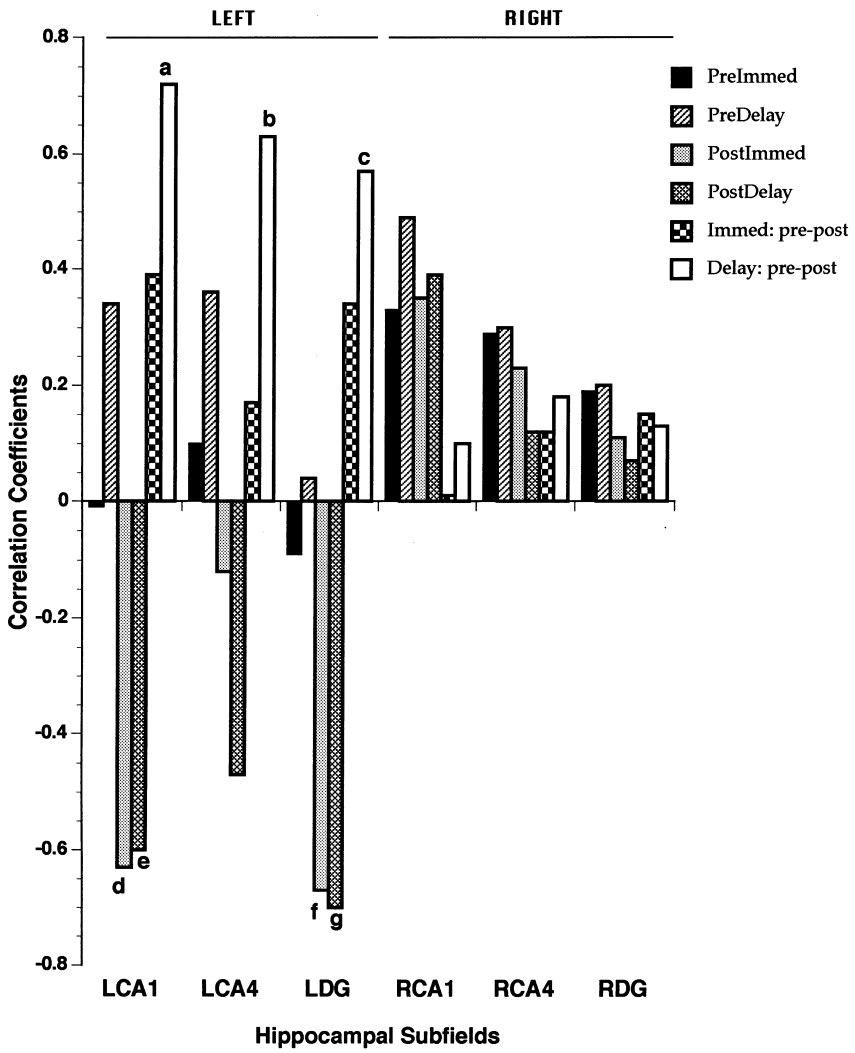

Fig. 7. Correlations (Spearman rank-order) between neuronal density and performance on the "hard" paired-word associations, third trial. The scores on all conditions were correlated with density separately: PreImmed = preoperative, immediate memory; PreDelay = preoperative, delayed memory; PostImmed = postoperative, immediate memory; PostDelay = postoperative, delayed memory; Immed = preoperative minus postoperative scores on immediate memory; Delay = preoperative minus postoperative scores on delayed memory. a = $P < 0.004$; b = $P < 0.01$; c = $P < 0.03$; d = $P < 0.01$; e = $P < 0.02$; f = $P < 0.01$; g = $P < 0.001$

Not surprisingly, the related word pairs did not correlate with neuronal density in either side (results not shown). The difference between the two kinds of word associations can most likely be attributed to what was really being measured: These related pairs were highly familiar associations which could be "remembered" purely through guessing. Indeed, it is plausible to consider that they tap cultural "semantic memory". The unrelated pairs were novel and likely tapped true memory capacity. It should not be surprising that hippocampal integrity is more critical for the latter than for the former.

Given the behavioral results alone, one would expect pre-surgical performance to be positively correlated with neuronal density. The fact that this did not happen here supports the notion described above (see also Introduction to Study 2), namely that pre-surgical performance represents the combined effects of diseased and healthy tissue. Specifically, not only the neurons in the resected hippocampus controlled pre-surgical memory, other regions, anatomically and functionally connected to the hippocampus, must have been involved in the memory process as well. Hence the low non-significant correlation values with density.

The negative correlations between post-surgical scores and density indicate a functional relationship between high density with low scores. This pattern is expected in cases where resected hippocampus is relatively intact (the toxic effects of the seizures did not cause much neuronal destruction) and may be interpreted in terms of plasticity and functional reorganization. Thus, the low scores may reflect an incomplete state of compensatory adjustment after surgery. In cases with substantial neuronal loss, on the other hand, compensatory adjustment could have begun well before the surgery, and was in place following surgery. In such cases, post-surgical memory should be relatively better than the cases with relatively little neu-

ronal loss. This may appear to be a flat contradiction of the ideas in the previous paragraph, but it is not. A parsimonious explanation is that the post-surgical correlations reflect the combined effects of removal of the tissue which controlled the behavior prior to surgery, as well as the status of hippocampal integrity before surgery.

General discussion

We set out to determine the relationship between hippocampal laterality and hippocampal subfield specificity in verbal memory. We found consistent left hemisphere correlation between verbal behavior and neuronal density, in accord with left hemisphere specialization for language functions. Subfield specificity appears consistent for CA1 and slightly less so for subfields, CA4 and DG. What the unique neuronal and synaptic properties of CA1 are, as compared to the other two subfields, remain to be determined. All that can be said now is that the data suggest that this subfield, in the left side, plays an important role in verbal memory.

Unfortunately, there is remarkable paucity of reports on left–right differences in neuronal density and memory performance. Typically, density calculations are collapsed together for the sides in correlation analyses with memory [33, 39, 45]. Sass *et al*. [44] did report correlations between density and long-term verbal memory for each side separately; positive significant correlations were obtained for left subfields CA3 and CA4 (hilus). No post-surgical analysis was reported. Our study did not look at CA3. We did find significant positive correlations between memory for unrelated paired words (Study 2) and left CA4, as well as for left CA1 and DG. In the explicit vs implicit memory experiment (Study 1), only left CA1 was maximally involved. Thus, across studies, there seems to be a consistent involvement of CA1 in the left side in verbal memory. However, more studies of the type reported in the present paper are needed to underscore the selective roles of hippocampal subfields in memory.

The results of Study 2 highlight an important issue raised by the results of Study 1, namely inferring function from pre-surgical performance alone. The common approach used by most investigators is to determine the correlation value between pre-surgical memory and morphology. The results are typically interpreted as evidence that the removed tissue controlled the behavior, and in most studies has been so interpreted [33, 39, 44, 45]. There is an inherent weakness in this approach. First, it is possible that pre-surgical behavior reflects absence of effective functions in the diseased tissue. Second, before surgery seizure rate and medication levels are high, and on logical grounds alone it is unlikely that they do not have some negative effects on memory. Third, after successful surgery, both the seizures and medications are reduced. To continue this argument and discussion, consider the eight possible logical outcomes of correlations between pre- and post-surgical memory and morphology (Table 1). Taken together, the two studies reported here allow a convergent analysis of the relationship between verbal memory and the integrity of the hippocampus.

With early brain damage it has generally been assumed that the earlier the damage, the greater the likelihood of functional reorganization, and alleviation of behavioral symptoms. The reorganization notion was based on the assumption that parts of the brain that were not yet committed to particular functions, would "take over" those functions that became impaired [6, 51]. Or, even if committed, important functions such as language would "squeeze out" less important functions. There has always been very little evidence to support this assumption [16, 41]. Patterns of hemispheric functional specialization, for instance, remain the same despite early brain damage, as long as the damage is not massive [30, 42, 60]. That is not to say that early brain damage cannot lead to greater improvement in performance with time than late damage. However, not all issues concerning plasticity and functional reorganization in the human brain have been explored [9, 27, 56]. They remain to be differentiated for specific types of brain damage such as epilepsy, head injury, tumor, brain surgery, or stroke.

The selective contribution of neuronal density in the hippocampal subfields to memory functions can at present be best studied through microscopy [64]; the current state-of-the-art for MRI, PET, or SPECT technology does not allow visualization of neurons. The published

Table 1. Possible outcomes and logical interpretations of pre- and post-operative correlations between behavior and morphology (+ = correlation value significantly different from zero; − = non-significant correlation value)

	Possible outcomes			
	A	B	C	D
Pre-operative	+	−	−	+
Post-operative	−	+	−	+

A: The removed region controlled the behavior before surgery but not after surgery; significant correlation is obtained pre-operatively only.
B: The removed region did not control the behavior but the surgery resulted in functional reorganization so that an area connected to the removed region contributed to the function after but not before the surgery.
C: Most likely *a-priori* outcome not related to the damaged area, that is, no significant correlation between neuronal density and behavior, either before or after surgery.
D: An area connected to the removed region contributed to the behavior before and after surgery.
Summary: The usual interpretation of a significant pre-operative correlation value in the absence of post-operative data is A rather than D. Instead, we believe the most likely interpretation of our data is D. But the logical possibility that C is true cannot be ruled out.

reports that certain drugs used in anesthetics (e.g. oxazepan) can selectively impair implicit but not explicit memory, or vice versa [11, 46, 50] may shed further light on neuronal function in memory processes. The present results and similar reports elsewhere in the literature may go a long way to explain hippocampal mechanisms unique to human memory [63]; future studies of this type could prove even more illuminating.

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