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Cognitive Deficits Induced by Global Cerebral Ischaemia: Prospects for Transplant Therapy

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HODGES, H., A. NELSON, D. VIRLEY, T. R. KERSHAW AND J. D. SINDEN. *Cognitive deficits induced by global cerebral ischaemia: Prospects for transplant therapy.* PHARMACOL BIOCHEM BEHAV **56**(4) 763–780, 1997.—Global ischaemia induced by interruption of cerebral blood flow results in damage to vulnerable cells, notably in the CA1 and hilar hippocampal fields, and is frequently associated with memory deficits. This review examines cognitive deficits that occur in animal models of global ischaemia in rats and monkeys, the extent to which these deficits are associated with CA1 cell loss, and the evidence for functional recovery following transplants of foetal CA1 cells and grafts of conditionally immortalised precursor cells. In rats, impairments are seen most consistently in tasks of spatial learning and spatial working memory dependent on use of allocentric environmental cues. In monkeys, ischaemic deficits have been shown to a moderate extent in delayed object recognition tasks, but animals with a selective excitotoxic CA1 lesion show a profound impairment in conditional discrimination tasks, suggesting that these may be a more sensitive measure of ischaemic impairments. Several studies have reported correlational links between the extent of CA1 cell loss following two or four vessel occlusion (2 VO, 4 VO) in rats and behavioural impairments, but recent findings indicate that at intermediate levels of damage these relationships are weak and variable, and emerge clearly only when animals with maximal CA1 cell loss are included, suggesting that the deficits involve more than damage to the CA1 field. Nevertheless, ischaemic rats and CA1-lesioned marmosets with grafts of foetal CA1 cells show substantial improvements; in rats these are not found with grafts from other hippocampal fields. Conditionally immortalised cell lines and trophic grafts are currently being assessed for their functional potential in animal models, because clinical use of foetal cells will not be practicable. Recent findings suggest that an expanded population of neuroepithelial cells derived from the conditionally immortalised *H-2K*^{b}-tsA58 transgenic mouse improve spatial learning as effectively as CA1 foetal grafts in rats subjected to 4 VO, and clonal lines from the same source show similar promise. Lines derived from precursor cells have the potential to develop into different types of cell (neuronal or glial) depending on signals from the host brain. These cell lines may therefore have the capacity to repair damaged host circuits more precisely than is possible with foetal grafts, and offer a promising approach both to functional recovery and to elucidating graft–host interactions. © 1997 Elsevier Science Inc.

Global ischaemia Four vessel occlusion CA1 Spatial learning Conditional discriminations Intracerebral transplantation

ANIMAL MODELS OF GLOBAL ISCHAEMIA

Interruption of cerebral blood flow, as occurs in heart attack or coronary artery occlusion, is associated with moderate to severe memory deficits, which are chiefly anterograde (18, 53,104,117,142,151). These can be one of the most disabling consequences, and resemble deficits in temporal lobe amnesia (121). Postmortem examinations of patients showing post-ischaemic memory loss, such as R.B., have suggested that bilateral hippocampal damage is a key factor in this impairment (151), and a recent magnetic resonance imaging study (53) has confirmed restricted CA1–2 damage in an ischaemic patient suffering from both anterograde and retrograde amnesia. Animal models of global ischaemia (45,120) have sought to delineate the pattern of selective cell loss and cognitive deficits in a more controlled and reproducible way than is possible in clinical studies. In rodents, methods include two vessel occlusion (bilateral occlusion of the common carotid arteries, with hypotension induced pharmacologically or by exsanguination: 2 VO) or four vessel occlusion (cauterisation of the vertebral arteries under anaesthesia followed 24 h later

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by occlusion of the common carotids, with depth of ischaemia standardised by loss of righting reflex: 4 VO). In primates, restriction of blood flow has been attempted by use of a neck cuff combined with hypotension (119,153); by intracranial occlusion of the posterior cerebral artery, which partly supplies the hippocampus (5); or by eight vessel occlusion (133), which is comparable to 4 VO in the rat. These models have confirmed that hippocampal cells, notably hilar cell types and CA1 pyramidal cells (30,47,67,120), are highly vulnerable to effects of reduced blood supply. Increasing the duration of global ischaemia increases the extent of dorsal CA1 cell loss, to near maximum levels at durations of ca. 15 min in the rat 4 VO model (see Fig. 1), whilst longer durations lead also to damage in other hippocampal fields and in the cortex and dorsal striatum (106,120). Nunn et al. (89) reported a strong linear trend of increasing dorsal CA1 cell loss from 10% to 100% with increasing durations of 5, 10, 15, and 30 min of 4 VO, and a quadratic trend as damage approached asymptotic levels at 15 min. Limited durations of occlusion, therefore, appear to provide an opportunity: a) to examine cognitive deficits induced by graded intrahippocampal ischaemic damage, and to see if a relationship exists between the extent of ischaemic cell loss and behavioural deficit; b) to quantify the reduction in cell loss following cerebroprotective drug treatment against duration-related trends of damage in vehicle-treated controls; and c) to assess cognitive performance following drug treatment aimed at preventing cell loss or following transplant strategies aimed at promoting recovery from the effects of damage that has already occurred. This review will focus on the analysis of the cognitive impairments induced by global ischaemia, primarily in rats, and the extent to which transplant approaches show promise as a strategy for alleviating ischaemic deficits.

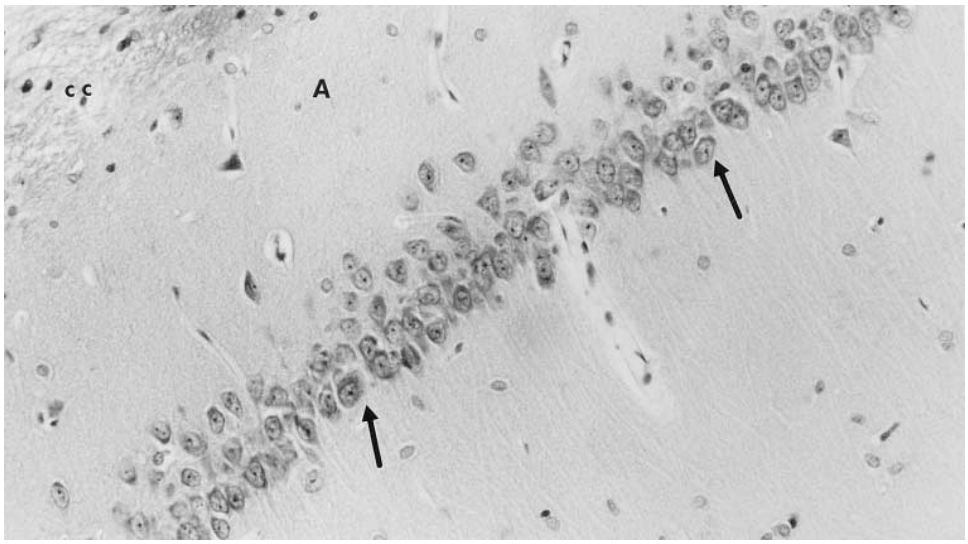
ISCHAEMIC PROFILE OF COGNITIVE DEFICITS: RELATIONSHIP TO HIPPOCAMPAL LESIONS

Deficits induced by hippocampal lesions have been manifest in three main types of task: those involving spatial learning, working memory, or conditional information processing. There is considerable variability in methods of lesioning (aspiration, fimbria fornix sectioning, electrolytic lesioning, or use of excitotoxins) and variability of results that have generated competing theories of hippocampal function [for reviews, see (25,50)]. However, since intrahippocampal damage is the most salient permanent result of global ischaemia, it is reasonable to assume that cognitive deficits will resemble the profile of impairment induced by hippocampal lesions of different origins. This assumption has been directly tested by Nunn et al. (92), who compared rats with an excitotoxic lesion of the CA1 field (designed to mimic effects of 15 min of 4 VO) with rats subjected to 4 VO, in a water maze task known to be sensitive to large hippocampal lesions (72). Lesioned animals were less impaired than ischaemic rats; indeed, they were not significantly worse than their control group in latency. However, lesioned and ischaemic animals both showed a less efficient search pattern than controls, so some parameters of learning were impaired to a comparable extent in both groups. Thus, while it is justifiable to look for ischaemic deficits in hippocampus-sensitive tasks, it cannot be assumed that effects of ischaemic lesions will closely resemble those induced by other means, any more than that effects of two different types of hippocampal lesion will be equivalent. There is also considerable variation between (and from time to time within) laboratories as to which tasks are significantly impaired by ischaemia, and variation in the extent of hippocampal damage induced by similar durations of occlusion, which has diminished hopes of seeing highly replicable results, despite use of fairly standardised methods across laboratories. Nevertheless, ischaemic deficits broadly conform to the pattern shown by animals with intrahippocampal lesions: dissociations between tasks that are and are not impaired point to a deficit that crucially involves spatial information processing and may extend to the processing of other types of "relational" (25) information, but the deficit does not appear to involve storage of information in intermediate (working) or long-term memory.

SPATIAL LEARNING: ISCHAEMIA DISRUPTS ALLOCENTRIC SPATIAL LEARNING BUT NOT LONG-TERM SPATIAL MEMORY

Since Tolman's (135) demonstrations that rats are able to make successful detours to reach a goal, views on spatial learning have come to emphasise the construction of "cognitive maps"—internal representations of the environment—rather than associative learning of specific routes or the chaining of motor responses. Visuospatial cues distributed around the environment play an important part in mapping processes, even in animals such as rats, which have poor visual acuity (7,24). This is shown by impaired performance when cues are clustered (95), removed (85), or moved around (6). O'Keefe's (94,96) identification of place neurons that fire when an animal is in a particular position provided evidence that the hippocampus plays a critical role in the construction of cognitive maps, so that ischaemic hippocampal damage might be expected to impair spatial learning. Global ischaemia has been found to disrupt spatial learning in several tasks that require the use of allocentric visuospatial cues. This has been demonstrated in the radial arm maze [(20,21,40,57,140,143); see (22) for a review], the T maze (43,141), the standard water maze [(43,44,46,85,89–92); see (87) for a review], and the water radial maze (44,83). However, the extent of impairment appears to vary as a function of experience and of opportunities for the use of alternative strategies for place learning. With respect to the influence of experience, Volpe's group has shown that with extensive postoperative and/or preoperative training, radial maze deficits become transient or disappear (21,22,140,143). Reference memory (long-term memory) errors are particularly amenable to training (21,22,140), suggesting that ischaemic rats can learn the location of arms that are consistently rewarded. Clearcut evidence that ischaemic rats do not show long-term deficits in the recall of locations has been provided by Netto et al. (85), who showed that with repeated phases of training, ischaemic rats learned to find the submerged platform in different positions in the water maze as rapidly as controls. However, when transferred to a new pool, with different extramaze cues, ischaemic deficits reemerged, indicating that the impairment involved spatial learning but not retention of spatial information.

FIG. 1. Nissl-stained (cresyl violet/luxol fast blue) coronal sections of the dorsal CA1 field at ca. 5.7 mm anterior to the interaural line, taken from a nonischaemic control rat (A) and rats subjected to global ischaemia (4 VO) for 10 min (B) and 20 min (C). Increasing durations of ischaemia resulted in progressively greater loss of CA1 cells (arrows), from 65% at 10 min to 90% at 20 min, in comparison with controls. cc, Corpus callosum. Magnification 200 ×.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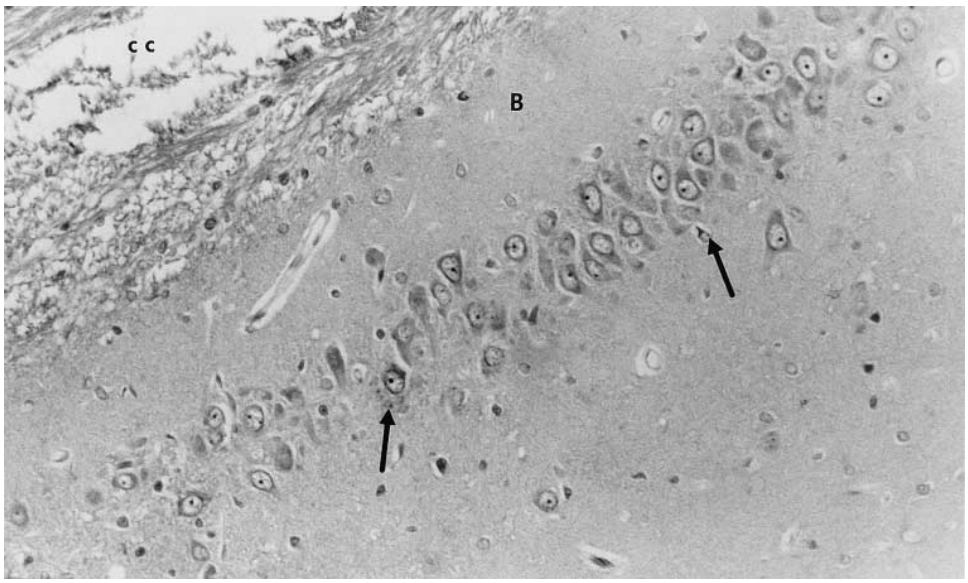
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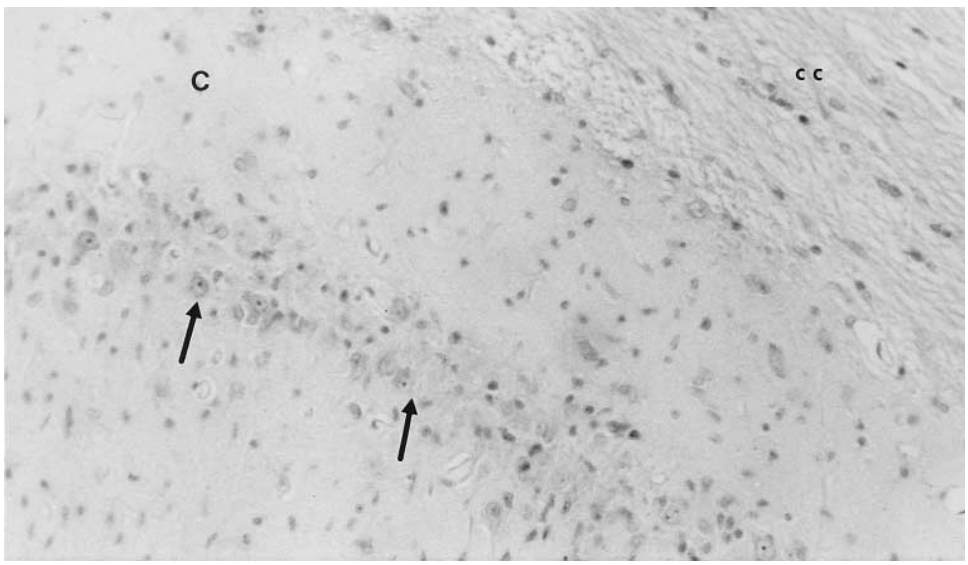
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Although the role of allocentric visuospatial cues has been emphasized in place learning, it is likely that several other types of information and information processing strategies contribute to place learning. These include use of odours, body movements, and learning of individual associations between particular cues and safe or rewarded locations, so animals with hippocampal damage can make use of alternative strategies that may not depend on hippocampal integrity (44). Eichenbaum et al. (26), for example, showed that rats with fimbria fornix (FF) lesions were impaired in finding the platform in a water maze when started from different positions in each trial, which required integration of visuospatial cues, but not when started from the same place, which permitted associative learning of a specific set of cues or a fixed route. Ischaemic rats may similarly be able to learn locations by alternative means, depending on the task, and this may account for discrepant accounts of their spatial abilities in the literature. Kiyota et al. (57), for example, found that rats subjected to 5 and 20 min of 4 VO were impaired in learning to find food in 5/8 arms in the radial maze, a task that measures both reference memory (choice of rewarded arms) and working memory (avoidance of arms already visited within a trial). The same animals were not impaired in place learning in the water maze, although some probe trial deficits were evident. In contrast, Nunn et al. (91) found that rats subjected to 4 VO for 15 and 30 min showed no impairment in radial arm maze learning (4/8 arms rewarded) using Jarrard's (49) "place" and "cue" tasks (see Fig. 2). The place task was the same as that used by Kiyota et al. (57), and the cue task involved learning which four distinctively textured arm inserts were associated with reward, even when these were moved to different arms in each trial. Despite normal acquisition in the radial maze, the same animals were impaired in learning the location of the platform in the water maze when assessed both before and after radial maze training, so lack of deficits in the radial maze cannot be attributable to time or previous training (see Fig. 2). The radial arm maze and water maze differ in many ways, including constrained versus free search, appetitive versus escape motivation, slow versus fast learning, and several versus one goal (44). It is important to know what critical differences contribute to the good performance of ischaemic rats in one task but not the other, as clues to the cognitive processes involved. Brown and coworkers (9,10) have suggested that associative links between local intramaze cues and rewarded arms, as well as allocentric extramaze spatial cues, could contribute to the formation of cognitive maps in the radial maze. Local cues are picked up by "microchoices" when rats investigate arm entrances without fully entering. Hence, one critical difference between the water maze and the radial maze may be that the latter permits associative learning of rewarded locations, which has been shown by Jarrard's cue task to be unimpaired in rats with hippocampal lesions (50). We (83) therefore investigated the performance of ischaemic rats in an eight-channel radial water maze (13) with a collapsible platform at the end of each arm for escape. Rats were trained in the Olton working memory task to find all the platforms without making reentries; when the platform was found it was collapsed, compelling the rat to search for another. This maze preserved the layout of the dry version but eliminated use of odour trails and visible intramaze markings. Although odours have been suggested not to guide working memory choices (99), they are impossible to remove in the dry maze and might contribute to microchoices. Local markings would change in the water maze, as it was hosed down each day. Rats in the water radial maze did not show microchoice

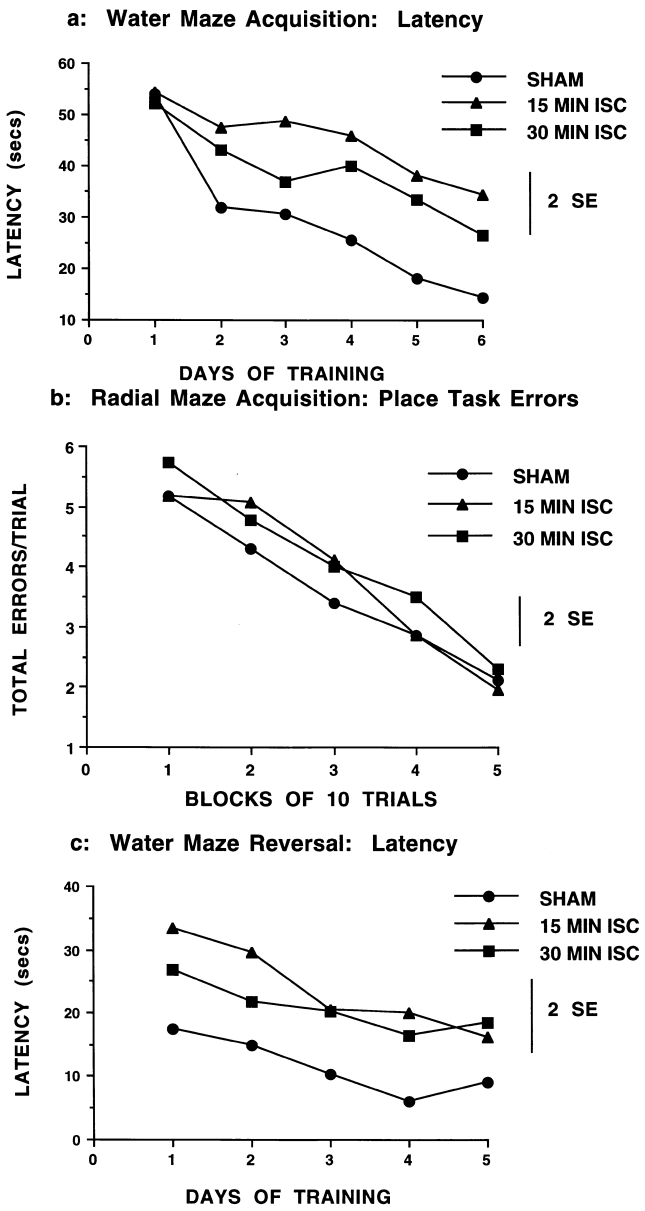

FIG. 2. Water maze and radial arm maze learning in rats subjected to 15 or 30 min of global cerebral ischaemia by 4 VO. Ischaemic rats were severely impaired in time taken to locate a submerged platform in the water maze, both initially (a) and 4 months later, after radial maze training, when the platform was in the opposite quadrant (c). However, ischaemic rats showed no impairment in radial maze reference (b) or working memory errors relative to sham-operated controls. Ischaemia resulted in 70–90% loss of dorsal CA1 cells, with some additional extrahippocampal cell loss in the 30-min group that did not exacerbate water maze impairment. Scale bars show 2 standard errors for the Groups × Days (a, c) or Groups × Blocks (b) interaction terms. (J. A. Nunn et al., in prep. Data reprinted from *Cognitive Brain Research* 3, H. Hodges, Maze procedures: The radial-arm and water maze compared, pp. 167–181. Copyright © 1966, with kind permission of Elsevier Science B.V.;, Amsterdam Publishing Division, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.)

behaviour typical of behaviour in the dry radial maze. Moreover, duration-related impairments were clearly evident in the water radial maze in rats subjected to 10, 15, and 20 min of 4 VO. These differences in impairment in the dry and wet radial mazes suggest that an ischaemic deficit may only be manifested in spatial tasks that require the use of allocentric cues, and may not be detected if the task permits associative place leaning, in agreement with Eichenbaum's findings (26) with FF-lesioned rats.

WORKING MEMORY: ARE ISCHAEMIC DEFICITS APPARENT IN BOTH SPATIAL AND NONSPATIAL TASKS?

Working memory tasks require retention of trial-unique information for short periods ($\sim$ 30 s). Because animals with hippocampal damage reliably exhibit deficits in working memory tasks with a spatial component (radial maze, T-maze), and in some nonspatial tasks such as delayed object recognition, control of working memory has been proposed as one of the main functions of the hippocampus (98,100,108). However, although impairment in nonspatial working memory can occur after hippocampal damage (78,107), maximal deficits are seen in tasks that are subject to a high degree of interference between trials (109) or that contain concurrent or spatial components. Working memory deficits in both spatial and nonspatial tasks have been found to be more evident following rhinal cortex than hippocampal lesions in rodents and primates (36,77,80,146,147), although deficits after rhinal cortex damage are not restricted to working memory tasks—impairments in learning have also been reported (80,147). Studies that have specifically compared performance in spatial and nonspatial tasks report deficits in the former but not the latter (1,54,118) following damage to the hippocampus or its inputs. Primate studies of hippocampal damage have chiefly employed delayed object recognition paradigms (153), nonspatial working memory tasks that emphasise storage in working memory and that are also sensitive to amnesia in humans (151). Although hippocampal damage induces deficits in these tasks, impairment is more profound following damage to parahippocampal areas, particularly the perirhinal cortex (152). Thus, it seems that in both rodent and primate species, whilst damage to the hippocampus can lead to nonspatial working memory impairment, these tasks do not capture the essence of hippocampal function. In contrast, spatial working memory tasks appear to be highly sensitive to hippocampal damage in both species. In parallel studies, Murray et al. (79) and Markowska et al. (64) showed that primates and rodents were profoundly impaired in a T-maze runway working memory task but not in discrimination tasks that involved egocentric or associative place-reward learning. Gaffan (36) similarly demonstrated that monkeys with perirhinal cortex lesions were more impaired than those with fornix lesions in delayed nonmatching to sample (DNMTS) tasks, whereas the converse results occurred with spatial discrimination learning in the Wisconsin General Test Apparatus (WGTA). These types of dissociation have led Gaffan (36) and Eichenbaum et al. (25) to propose that the hippocampal formation and its limbic and cortical connections serve separate memory functions. Eichenbaum et al. (25) suggested that the hippocampus proper (pyramidal and granule cell fields) engages in relational information processing—extraction of relationships between stimuli, including their spatial layout, and links to recent or stored information—whereas the parahippocampal regions store information in an intermediate-term working memory buffer. This hypothesis would lead to the suggestion that animals with intrahippocampal cell loss following limited periods of global ischaemia would be likely to show impairment in spatial but not nonspatial working memory tasks.

Ischaemic deficits have, indeed, been shown in several spatial working memory tasks, including reentries in the radial arm maze (22,57), alternation in the T-maze (43,141), selection of correct opening doors in the three-door runway (46), and matching to platform position in the water maze (46,85). Discrepancies in performance suggest that the extent of spatial working memory deficit is related to the extent that tasks require processing of allocentric information; as discussed above, Nunn et al. (91) did not find working memory errors in the radial maze place task, which permits associative place learning, whereas reentries were much in evidence in the water radial maze. Further findings from our laboratory have confirmed that ischaemic deficits are not detected by less spatially demanding working memory tasks. Nelson et al. [(82,84); see (44) for a review] compared animals subjected to 5, 10, and 20 min of 4 VO and found a duration-related impairment in delayed matching to position in the water maze in a working memory task where rats were given four trials/day with a different platform position each day (see Fig. 3a). However, the same animals were not impaired in learning a delayed nonmatching to position (DNMTP) task in the Skinner box, in which the animals had to choose the lever that was not presented in the preceding information stage of the trial. When delays of 2–16 s were interpolated between the information and choice stages, all the animals showed a delay-dependent decrease in accuracy, but ischaemic rats were no worse than controls; indeed, they were were marginally more accurate at all delays (see Fig. 3b). These findings suggested that there is a marked difference between spatial information processing in exploratory tasks and those that require an animal to remember the position of objects in a confined area.

There have, however, been findings of impaired object recognition in both ischaemic monkeys and rats in the classic DNMTS task. Zola-Morgan et al. (153) found that animals with ischaemic cell loss largely limited to the CA1 field, induced by neck cuff occlusion, showed DNMTS impairments that were equivalent to those in monkeys with hippocampal lesions. In this model, cognitive deficits may be confounded by neurological impairments that occur after only 12 min of occlusion, a duration that produces no DNMTS deficits (119). Using a similar paradigm in rats, Wood et al. (148,149) found DNMTS deficits that were more severe than in animals with hippocampal/amygdalar lesions (78) but not as marked as in animals with rhinal cortex lesions (77). It is possible that these DNMTS tasks involve more than passive storage of information: for example, the learning of a nonmatching rule when faced with a large number of trial-unique objects. This ability to extract a relationship from multiple exemplars might require relational information processing capacity as well as storage in working memory, which, according Eichenbaum's (25) theory, would engage both parahippocampal and hippocampal-dependent processes. If so, current findings suggest that working memory tasks are relatively insensitive to global ischaemic damage unless they have a substantial spatial or relational component.

CONDITIONAL TASKS: NEED FOR ASSESSMENT IN ISCHAEMIC MODELS

Performance in tasks that require the learning of a conditional rule has been argued to be one of the most sensitive indicators of hippocampal damage in primates (112) and, by

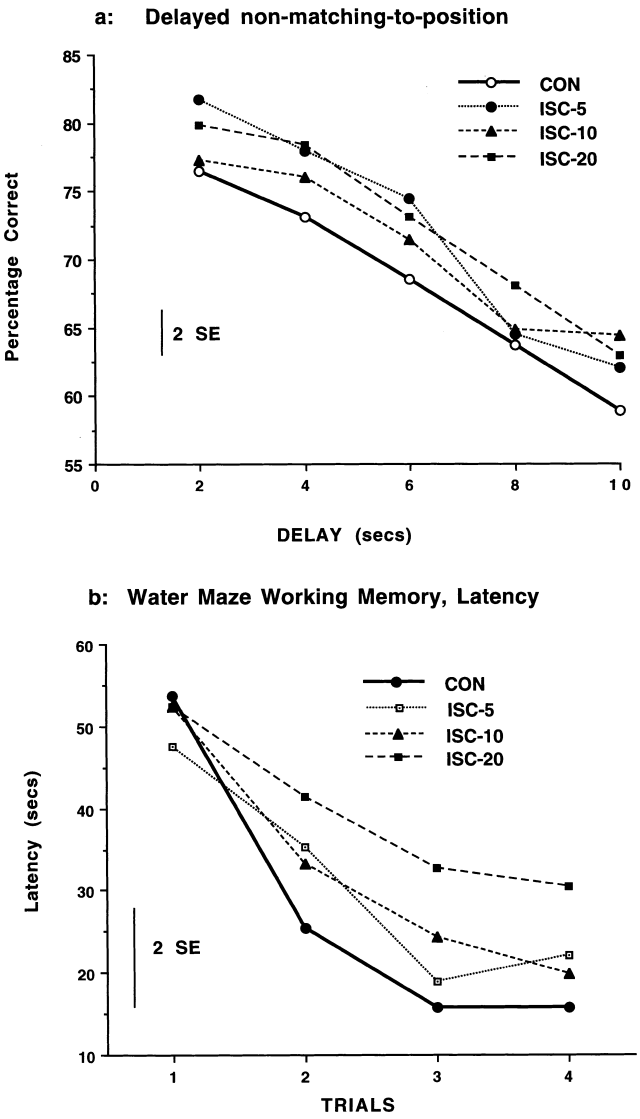

FIG. 3. Working memory performance in the Skinner box and water maze. Rats subjected to 5, 10, and 20 min of 4 VO showed no impairment in DNMTS in the Skinner box (a), but did show duration-related impairment in the more spatially demanding water maze working memory task (b). Bars show 2 × the standard error for the Groups × Delay (a) or Groups × Trials (b) interactions. (Data in panel a reprinted from *Behavioural Brain Research* 65, J. A. Nunn and H. Hodges, Cognitive deficits induced by global cerebral ischaemia, pp. 1–31. Copyright © 1994, with kind permission of Elsevier Science B.V., Amsterdam Publishing Division, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands. Data in panel b reprinted from *Cognitive Brain Research* 3, H. Hodges, Maze procedures: the radial-arm and water maze compared, pp. 167–181. Copyright © 1996, with kind permission of Elsevier Science B.V., Amsterdam Publishing Division, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.)

Eichenbaum's theory (25), would be expected to reveal a dysfunction in all animals that possess a hippocampus. There are many variants of conditional tasks, including go/no-go discriminations (29) that involve response inhibition (if A, respond; if B, wait), visuospatial discriminations (112,113,115) (if objects are AA, go left; if objects are BB, go right), negative patterning (19,130) (A or B is rewarded, but not A+B), or visuovisual discrimination (114,115) (if objects are on black background, go right; if on white background, go left). Performance of animals in these tasks has been unexpectedly variable, so that, as with "spatial" tasks, there may be critical components that are variably taxed by the tasks or by lesion (35,37). For example, rats have been found to be impaired after electrolytic but not after aspiration lesions of the hippocampus in go/no-go delayed alternation tasks (29). Sutherland and Rudy (130) have argued that negative patterning tasks, which require "configural" information processing (i.e., construction of representations from combinations of elemental units; a concept similar to "relational" processing), are highly sensitive to hippocampal damage. However, Davidson et al. (19) showed that rats with hippocampal lesions caused by colchicine plus kainate, or more selective ibotenate, were not impaired in a negative patterning task, and transfer tests showed that lesioned rats were using configural processes to solve the task. Davidson et al. (19) therefore suggested that the hippocampus is not an important substrate for configural information processing.

Animals with ischaemic damage have not yet been assessed in conditional tasks. However, Ridley et al. (113–115) have examined effects of an *N*-methyl-D-aspartate (NMDA) lesion to the CA1 field designed to mimic effects of global ischaemia, which is difficult to achieve reliably in monkeys. Bearing in mind that ischaemic deficits may turn out to differ from those of the CA1 excitotoxic lesion, just as they differ to some extent from lesions to hippocampal inputs in monkeys (113), the selective pattern of impairments in conditional tasks identified by Ridley et al. provides valuable pointers to the probable effects of intrahippocampal ischaemic damage. Animals were impaired mainly in visuospatial and visuovisual discrimination tasks (see Fig. 4a), but not in simple visual discriminations (A+ vs. B−). An extensive series of tests (114) further showed that monkeys with CA1 lesions were *not* impaired in concurrent discriminations (up to eight simple discriminations), with or without distinctive backgrounds, which have been shown to improve performance in normal animals but not in those with fornix transection (36). Animals were not impaired in spatial tasks, including requirements to choose which of two identical stimuli was furthest away from a black object or to discriminate between two patterns that differed in orientation or that differed in the layout of lines on a black background. The tasks that the marmosets mastered all had a positive and a negative stimulus present, so the monkeys were simultaneously informed about what response to make and what *not* to make. Previous findings had shown that animals with hippocampal damage are not impaired in cue–reward evaluative learning (35,112). However, in the visuospatial and visuovisual discriminations, the animals were always presented with a rewarded pair of objects matched to two different equiprobable responses. If the stimuli were presented in consecutive trials until the animals were able to give three correct responses in a row (normal criterion was 27/30 correct), learning in the CA1-lesioned animals was not impaired. This suggests that split training enabled the animals to learn the task as separate discriminations, rather than learning the conditional rule. This impairment could involve response competition or disinhibition in the absence of a clear negative stimulus. Ridley et al. (114), however, suggested that it involves an impaired ability to process multiple aspects of a situation simultaneously, which is necessary for understanding the conditional relationships between the stimuli and the particular response

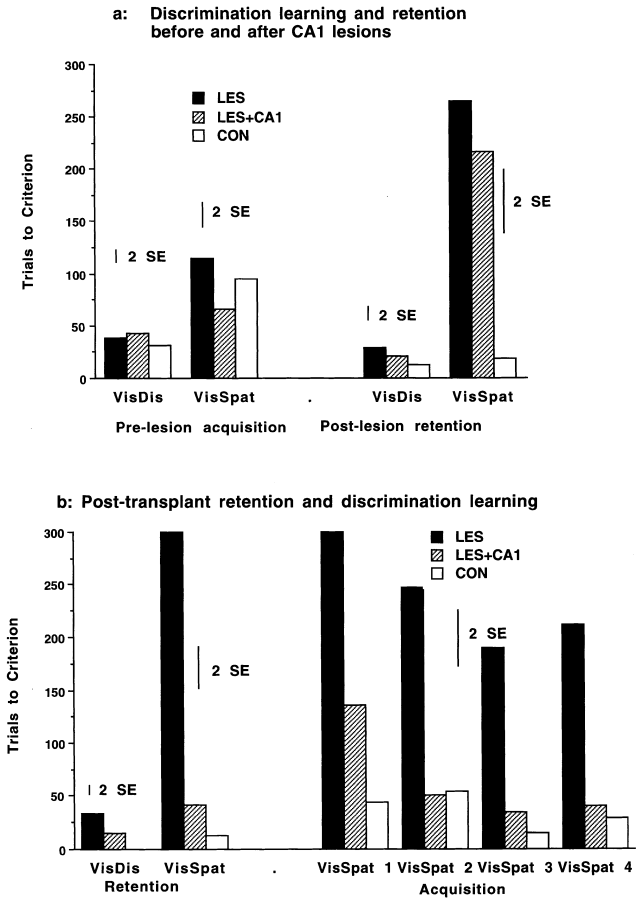

FIG. 4. CA1 lesion and transplant effects in marmosets. Marmosets were trained in simple visual discrimination (VisDis) and in visuospatial conditional discrimination (VisSpat), and then 9 of the 14 animals received NMDA lesions along a trajectory through the CA1 field. Before lesioning, trials to criterion were comparable in all groups, but after surgery lesioned animals (LES) showed profound impairment in retention of a visuospatial discrimination relative to nonlesioned controls (CON), without impairment in the ability to make simple visual discriminations (a). Lesioned animals later to receive transplants (LES + CA1) were similar to the lesion control group. Two to 3 weeks after lesioning, the LES + CA1 group received grafts of foetal E 94–96 CA1 cells implanted into the lesion sites. Grafted marmosets (b) showed improvement to the control level in retention of another visuospatial task learned before lesioning, and increasing superiority in learning new visuospatial discriminations, relative to lesioned controls, which remained substantially impaired throughout testing. Bars show 2 × the standard error for the main effects of Groups (Virley and Hodges, in prep.).

required. This failure to utilise stimulus attributes flexibly may be an example of impaired relational information processing postulated by Eichenbaum et al. (25). It is also reminiscent of Gaffan's (35) notion of "scene learning," where animals recall not only the identity of objects but also their spatial relationships and context, including their own responses, in a snapshot recollection analogous to human episodic memory. In agreement with rodent water maze studies, animals with CA1 lesions that eventually managed to learn the task were not subsequently impaired in recall (113), indicating impairment of acquisition, but not storage in long-term memory, of tasks learned in a lesioned state.

The highly selective impairment in visuospatial discrimination, but not in other types of spatial task, induced by an excitotoxic CA1 lesion in marmosets appears to be at variance with evidence for impairment in spatial learning and spatial working memory tasks induced by ischaemic and neurotoxic CA1 damage in the rat. This could relate to species-specific differences in information processing, or to lesion differences, but an obvious question to consider is whether "spatial" tasks were measuring the same abilities. Learning the positions of objects from a fixed position in front of the WGTA may not be as spatially demanding as place learning when moving around a large arena or maze. As we have previously noted, ischaemic rats were not impaired in DNMTP in the Skinner box (82,84), whereas both monkeys and rats with hippocampal damage show deficits in the T-maze (1,64,79). Thus, animals with hippocampal damage are sensitive to how spatial a task is, and whether it can be solved by processes (egocentric, associative) other than the use of allocentric spatial cues (44). A key feature of exploratory spatial tasks is that they require response flexibility that in some respects resembles the requirements of conditional visuospatial tasks: "if started from near the window, go towards the door; if started from near the poster go towards the experimenter." Eichenbaum et al.'s finding (26) that FF rats were impaired with random, but not fixed, start points (see above) illustrates the discrepancy between performance where single as opposed to variable and flexible responses are required; this is reminiscent of the ability of marmosets to learn visuospatial conditional discrimination as two separable responses with split training, but not as a conditional response with random or alternated training trials. Thus, the discrepancy between effects of CA1 damage in marmosets and rats may not be as great as it appears. In both cases, deficits are seen in tasks where several responses are appropriate and the animal must decide which to select in the given circumstances. A considerable amount of work needs to be done to increase our understanding of the nature of the cognitive deficits induced by global ischaemia and to see how far these are comparable to effects of selective lesions of the CA1 field. In particular, ischaemic rodents need to be assessed in conditional tasks to see if they are impaired in learning conditional rules with equiprobable response options. Conversely, the extent of impairment in tasks dependent on allocentric spatial information processing needs to be assessed in true primate models of global ischaemia. Nevertheless, robust and replicable deficits have been obtained in spatial learning and working memory tasks following global ischaemic damage in the rat (46), and in conditional discriminations following excitotoxic CA1 damage in the marmoset (115). These deficits provide baselines against which to assess functional effects of treatments designed to ameliorate the effects of ischaemic brain damage.

CAN DEFICITS INDUCED BY GLOBAL ISCHAEMIA BE RELATED TO CA1 CELL LOSS?

The extent to which CA1 hippocampal cell loss is related to the degree of impairment in tasks of spatial learning and working memory has typically been investigated by calculating correlations between behavioural and cell loss indices. Although this cannot provide evidence for a causal relationship, it can indicate the strength of associations between discrete brain damage and particular behavioural deficits, which is attractive given the relatively selective nature of cell loss induced by global ischaemia. Positive correlations between the extent of ischaemic CA1 cell loss and behavioural deficits have

been reported in a variety of tasks, including the split stem T-maze (141), the radial maze (57), DNMTS (148), and the water maze standard (97) and learning set (116) tasks, providing evidence for an association between the extent of brain damage and cognitive impairment. However, a number of these studies [e.g., (97,116,148)] included nonischaemic controls in the correlations, which make the results difficult to interpret. Impairment in the absence of any histological evidence of CA1 damage has also been reported: for example, Jaspers et al. (51) found that water maze learning was impaired after both 4 VO when CA1 cell loss was evident and 2 VO when no loss was detected. Correlational findings from our laboratory have been mixed. Netto et al. (85) found no relationship between water maze acquisition latency and cell loss following 15 min of 4 VO. Nunn et al. (89) used periods of 5–30 min of 4 VO occlusion to obtain a spread of values in which cell loss was related to duration of occlusion by strong linear and quadratic functions. However, there was no significant relationship between the extent of CA1 cell loss across a variety of parameters in the water maze, though performance on these parameters was intercorrelated, indicating that the lack of correlation with CA1 cell loss was not likely to reflect random behavioural response.

In contrast to the results of Nunn et al. (89), Nelson et al., using comparable durations (5–20 min) and the same surgical and behavioural procedures in the same apparatus, have recently found positive correlations between the extent of CA1 cell loss and water maze measures (e.g., latency, percentage of time in the training quadrant) in both water maze acquisition and working memory tasks in two separate experiments (83,84). Correlations were also found between various parameters within the water maze tests (e.g., latency vs. heading angle) and between performance on water maze acquisition and working memory tasks. Interestingly, working memory errors in the radial water maze also correlated with hippocampal damage, but the association was far more reliable in rats with CA3 damage (which also sustained substantial CA1 loss) than in animals with CA1 damage alone (83). Moreover, water radial maze performance did not correlate with measures for either standard acquisition or working memory matching to position tasks in the standard water maze, suggesting some dissociation between these tasks (83). In a second experiment (84), there were no correlations between CA1 cell loss and trials to criterion in the Skinner box DNMTP, a task in which ischaemic rats were not impaired. However, in the same rats there were correlations between CA1 cell loss and water maze acquisition and working memory measures. Moreover, performance in the two water maze tasks was intercorrelated, whereas trials to criterion in DNMTP correlated with neither of these tasks. These results further support the suggestions that different processes are involved in Skinner box and water maze working memory tasks, and that ischaemia preferentially impairs performance in spatial tasks but does not disrupt storage in working memory. However, this lack of correlation between CA1 cell loss and trials to criterion in DNMTP is at variance with Wood et al.'s (149) findings of a significant relationship between trials to criterion and performance at a 30-s delay interval in a delayed object recognition (DNMTS) task in rats subjected to 2 VO, which were impaired in this procedure. Since Wood et al. included controls in the results, leaving only six ischaemic rats if controls are excluded, evidence for this relationship needs to be strengthened by replication with a larger group of ischaemic animals.

The discrepancy between the findings of Nunn et al. (89) and Nelson et al. (83,84) from the same laboratory may illustrate the critical importance of choice of animals for inclusion in the correlations. Both workers excluded nonischaemic controls, but Nunn et al. (89) also excluded animals with maximal CA1 cell loss (grade 5: 90–100% loss), arguing that if these animals were included, the variability of scores was reduced and the sample biased, whereas Nelson et al. (83,84) retained animals with grade 5 damage. If these are excluded, the relationships found by Nelson et al. diminish. This suggests that at intermediate levels of CA1 cell loss, relationships with behavioural impairment become weak or nonexistent and only emerge strongly if a number of animals with maximal dorsal CA1 cell loss, which is preferentially related to water maze impairment (73), are included in the correlations. It is typical to find animals with substantial but submaximal loss (e.g., $>$ 70%) performing almost at control level. Olsen et al. (97), for example, using an optical fractionation method to provide a reliable estimate of cell numbers throughout the hippocampus, found that rats with 4 VO-induced loss to 100,000 viable cells, as compared with 250,000 cells in controls (corresponding to grade 4 loss), showed little evidence of impaired learning in the water maze. Indeed, latency in 11/28 ischaemic rats was better than the mean plus standard deviation of controls However, there was a modest overall correlation ($p < 0.05$) between latency and the number of viable cells in this study of moderate ischaemic damage (6–12 min of 4 VO).

A further difficulty in quantitatively relating ischaemic brain damage and behaviour arises because conventional histology used for correlations may not detect a range of neurochemical and other changes induced by ischaemia. Although most studies that report behavioural impairment also find histological cell loss, indeed findings of Auer at al. (4) suggest that loss of 50% of dorsal CA1 cells may be necessary for cognitive deficits to be manifest, there have also been reports of impairments following ischaemic (51) or traumatic (61) brain injury in the absence of overt hippocampal damage. Global ischaemia induces a host of changes throughout the hippocampus, including changes in calcium channels and neurotransmitter levels, upregulation of heat shock protein (except in the CA1 field, where failure to upregulate may be a marker of vulnerability), expression of trophic factors, and changes in receptor binding and second messenger systems. These changes contribute to substantial remodelling of neuronal circuits and may continue long after the death of CA1 and hilar cells [(33,81,101,102); see (120) for a review]. These changes might also contribute to cognitive dysfunction or compensation in ways that would not be detected by conventional histological methods. This functional reorganisation may account for behavioural deficits after 2 VO without hypotension (51), where there is no CA1 cell loss. Furthermore, the functional changes induced by ischaemia—both in brain and in behaviour—may not be static. Fukuda et al. (33), for example, showed that GABAergic neurons in the CA1 sector are still declining some 6–8 weeks after global ischaemia in gerbils. Nelson et al. (84) found that rats subjected to 10 min of 4 VO were not impaired in water maze learning when tested 4 weeks after ischaemia but showed significant deficits when tested 43 weeks later, and that correlations with CA1 cell loss were seen for the late but not the early water maze scores. Thus, functional changes and reorganisation within the hippocampus may contribute to behavioural deficits, and these would not necessarily be related to the extent of CA1 cell loss. The progressive nature of ischaemic brain damage may also lead to the detection of relationships at some time intervals but not at others.

It must also be borne in mind that global ischaemia, particu-

larly at longer durations (>15 min), results in damage to brain regions beyond the hippocampus, notably to cortex and striatum (89,103), so that increased impairments with longer durations may reflect extrahippocampal cell loss. Degeneration of neurons in the cingulate cortex after 15 min of 4 VO has been detected by silver staining (88), and lesion studies have suggested that animals with cingulate damage show more marked impairment in water maze navigation than animals with intrahippocampal lesions, indicating that the cingulate cortex plays an important part in spatial learning (65). In principle, it would be possible to try to discriminate between the performance of animals with and without cortical ischaemic damage by use of tasks thought to be sensitive to cortical dysfunction, such as serial reaction time (76), but to our knowledge this has not yet been undertaken. Nevertheless, the possible contribution of extrahippocampal damage to ischaemic deficits would confound correlational evidence for an association between hippocampal cell loss and impairments at the longer durations of global ischaemia. In sum, although there is evidence for an association between CA1 cell loss and impairment across a range of tasks, at intermediate levels of cell loss this relationship is weak, and cognitive performance is also likely to reflect many other concomitant changes, including damage to other hippocampal fields or to other brain regions. Possibly only when dorsal CA1 cell loss is near total, so as to interrupt the major output from the hippocampus to the subiculum, are behavioural deficits significantly related to CA1 cell loss, but at this level of loss a contribution of extrahippocampal damage cannot be ruled out.

RATIONALE FOR THE DEVELOPMENT OF TRANSPLANTS AS A THERAPY FOR GLOBAL ISCHAEMIA

The cascade of changes induced by global ischaemia, including transient increases in glutamatergic transmission, changes in second messenger systems, ionic fluxes, changes in calcium homeostasis, energy depletion, and altered gene expression (33,79,101,102,120,124,125), has offered the promise of potential pharmacological interventions at many different points in the chain of events leading to the delayed death of vulnerable neurons. Thus, antagonists at both NMDA and non-NMDA receptors, free radical scavengers, calcium chelators, calcium and sodium channel blockers, and compounds with many other types of activity have been investigated for their capacity to reduce neuronal loss [see (71) for a review], and more rarely for their capacity to reduce functional deficits induced by global ischaemia (116,139,148). Apart from some notable exceptions with non-NMDA antagonists (121,150), sedative anticonvulsants (17,41), and sodium (127) and calcium (11) channel blockers, pharmacological reduction in CA1 cell loss has been difficult to achieve; there are relatively few examples of convincing hippocampal protection after significant durations of occlusion. Reduction in the extent of brain damage appears to be more common in "stroke" models of focal cerebral ischaemia: for example, following middle cerebral artery occlusion (MCAO), where the penumbral region with limited blood flow is capable of rescue (71). Models of reversible MCAO by intraluminal occlusion (58,132) and mixed models of middle cerebral artery plus carotid occlusion (12) have been developed to examine drug effects where reperfusion and delayed cell death occur, as in global ischaemia, which may be more realistic models of human ischaemic conditions and more sensitive to drug action. When cerebroprotection has been detected in animal models, it has generally not been followed by evidence of significant efficacy in clinical trials (48), although this may be because compounds have not been assessed within appropriate models or treatment regimes. Furthermore, protection in some animal drug studies may have arisen through secondary actions of treatment, such as reductions in temperature (14). For example, cerebroprotective effects of calcium channel blockers and AMPA antagonists have been attributed to their hypothermic effects (93). Pharmacological routes for reversal of ischaemic processes are clearly the simplest and most direct. However, drug treatments to date have not been found to be clinically efficacious (48) and may need to be administered during, or shortly after, an ischaemic episode (63), which is often not practicable. In these circumstances, the use of transplants to promote recovery from the effects of damage that has already occurred seems an option worth pursuing. The restricted location of cell loss in global ischaemia means that grafts can be targeted to the area of damage, and their siting and growth can be monitored by imaging techniques that are currently available (53,129). Because outgrowth from grafts appears to be limited (8,126), transplants sited in a restricted area of hippocampal damage would not be required to extend long axons but would be in receipt of a rich and highly organised innervation from the host (28). Moreover, since ischaemic incidents can occur in otherwise healthy brains, grafts would not be placed in a degenerating environment, as would occur with grafts designed to alleviate neurodegenerative diseases (126).

USE OF FOETAL TRANSPLANTS IN MODELS OF GLOBAL ISCHAEMIA

Studies of the effects of transplants following global ischaemia in rats have taken advantage of the foetal dissection methods developed by Field et al. (28), who showed that the hippocampal fields can be separated at embryonic day (E) 18–19, when the areas are clearly discriminable, to obtain different cell types (CA1, CA3, and dentate granule) for grafting. Field et al. showed that grafts of CA3 cells, but not CA1 cells, implanted in the lesioned CA3 field, attracted normal laminar innervation from host mossy fibres, restoring 20% of normal synaptic density. Contacts between the misplaced CA1 cells and the host were sparse and abnormal. These findings, supported by convergent electrophysiological (23,75), autoradiographic (2), anatomical (74), and tract tracing (136,137) studies, suggested that in animals with discrete intrahippocampal lesions, homotypic foetal grafts placed into the appropriate regions of damage develop normally and become well integrated into the host hippocampal circuit, raising the possibility that they might have selective and beneficial functional effects. Accordingly, rodent functional studies have focussed on the issue of whether grafts from the CA1 field, but not from other hippocampal areas or other brain regions, promote recovery from the effects of long-term CA1 cell loss induced by global ischaemia, using a limited range of tasks that are reliably impaired by ischaemia.

A series of studies from our laboratory suggests that functional effects of foetal grafts are highly selective. Netto et al. (85) compared the performance of nongrafted ischaemic and nonischaemic controls with that of animals receiving E 18–19 CA1 and dentate granule grafts, and with that of rats receiving cholinergic-rich E 15 grafts from the basal forebrain (previously shown to be effective in animals with lesion-, age-, or alcohol-induced cholinergic depletion). Animals were assigned to graft groups on the basis of performance in the water maze, where a very clearcut ischaemic deficit was evident, and were retested in several phases of retention, reversal, new

position learning, and working memory from 4 to 20 weeks after grafting. All of the animals improved with experience, so that although rats with CA1 grafts performed better than the other ischaemic groups, the improvements were only marginal. When animals were tested in a new pool, ischemic deficits reemerged, and improvement to the control level was seen in animals with CA1 grafts but not in those with dentate granule or basal forebrain grafts, which remained as impaired as the ischaemic controls. These findings of a selective CA1 graft effect were followed up by a series of replications (46) that compared effects of CA1 grafts with those of the structurally and pharmacologically similar CA3 cells. A group of rats with dentate granule grafts was also included, with tissue taken at postnatal day 1, rather than E 18–19, to try to increase survival in these later-developing cells. However, these grafts largely failed to thrive, illustrating the crucial importance of donor age in graft survival (126). Graft effects were assessed in animals that had no previous experience in the water maze, so that ischaemic deficits were maximal. Against this baseline, improvement in animals with CA1 grafts was substantial; in most parameters they performed as well as controls. However, rats with CA3 grafts showed no improvements, and in several respects, such as the degree of thigmotaxis, were significantly worse than ischaemic controls. Working memory performance in the water maze and three-door runway afforded an interesting contrast. Rats with CA1 grafts were highly superior to ischaemic and CA3 graft groups, exhibiting one-trial learning in the water maze, in a manner comparable to controls. However, in the three-door runway, the CA1 grafted group, although superior to ischaemic groups, was also impaired relative to controls, so that functional recovery was only partial (see Fig. 5). This task (34) required rats to pass through four barriers, each with three doors, only one of which could open, to find food at the end of the runway. On the first trial each day, the opening doors were cued white, but on the remaining trials (2–6) all doors were black, and the rats had to remember the position of the white doors. A different pattern of opening door was used on each day, and pushes against nonopening doors were counted as errors. Normal rats take several weeks to learn this task and to run the maze within 20 s (average speeds of trained rats were ca. 5 s). Unlike water maze acquisition and working memory tasks, where improvement is evident over days of training, effects of ischaemia are relatively constant ("steady state"), so the three-door runway may be useful for repeated assessments of treatment effects.

There have been no assessments of effects of grafts in primate arterial occlusion models of ischaemia. However, Ridley et al. (114) have examined effects of late foetal tissue grafts (E 95), dissected from the CA1 field, in marmosets that showed deficits in visuospatial conditional discrimination following NMDA lesions of the CA1 field. Animals were assessed from 3 months after grafting in a range of tasks (conditional, concurrent, and spatial discriminations) used to determine the lesion deficit (see above). Lesioned and grafted animals performed similarly across the range of tasks, except for the two types of task that were impaired by the lesion—visuospatial and visuovisual discriminations. In these tasks, grafted marmosets were significantly better than their lesion-only counterparts, but they remained impaired relative to intact controls. These results are similar to those for three-door runway performance in grafted rats, a task in which steady-state deficits were also apparent. The lack of effect of grafts in the lesion-insensitive tasks suggests that motivation and ability to learn complex tasks were equivalent in control, lesioned, and grafted marmosets, so the grafts did not induce further damage as space-occupying lesions. Following up these findings, Virley and Hodges (in prep.; see Fig. 4b) have examined effects of E 94–96 foetal grafts after CA1 lesions in marmosets on retention followed by repeated assessment of new visuospatial conditional discriminations. All of the grafted animals showed good

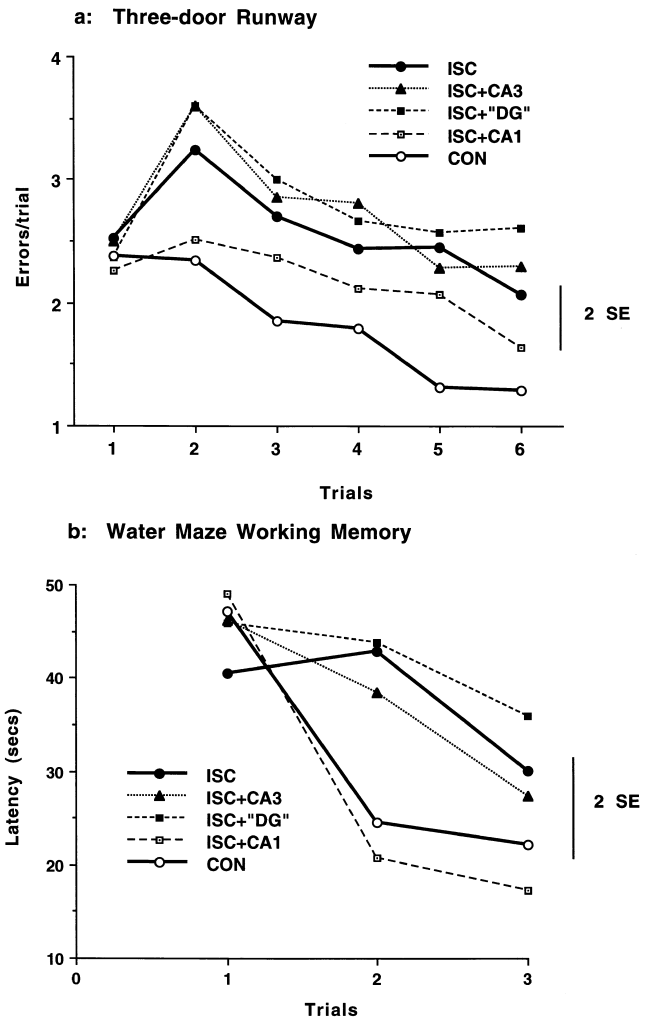

FIG. 5. Effects of ischaemia (15 min of 4 VO) and transplants on spatial working memory assessed in the three-door runway (a) and water maze (b). Ischaemic controls (ISC) and ischaemic groups with foetal grafts from the CA3 (ISC + CA3) and dentate gyrus (ISC + "DG"; these cells showed poor survival) hippocampal fields showed marked impairment in pressing at opening doors in the runway in trials 2–6 when all doors were black, relative to controls and rats with CA1 grafts (ISC + CA1). In Trial 1 in the runway, all groups made a similar number of errors in learning to discriminate black (closed) from white (opening) doors. The ISC + CA1 group was also significantly better than ischaemic controls and the other grafted groups in the water maze working memory task (b). However, graft effects differed in the three-door runway and water maze. In the water maze, rats with CA1 grafts improved to the control (CON) level, whereas in the runway, the ISC + CA1 group still showed substantial impairment relative to controls. (Data reproduced from *Neuroscience* 72, H. Hodges et al., Contrasting effects of fetal CA1 and CA3 hippocampal grafts on spatial learning and working memory induced by global cerebral ischaemia in rats, pp. 959–988. Copyright © 1996, with kind permission of Elsevier Science Ltd., The Boulevard, Langford Lane, Kidlington, OX5 1GB, UK.)

recollection of a visuospatial discrimination learned before lesioning, whereas lesioned animals took substantially more trials to reach the criterion of 27/30 correct choices. Grafted animals showed intermediate recovery in the first new visuospatial discrimination, in agreement with the findings of Ridley et al. (114). That is, they were significantly better than lesioned animals but significantly worse than controls. However, in three subsequent new discriminations, rate of learning improved to the control level in grafted marmosets, suggesting that time and/or experience is required for the grafts to modify behaviour maximally. Lesioned animals failed to show significant improvement over time.

Taken together, these findings suggest that CA1 grafts promote functional recovery in both primate and rodent species when placed close to the region of CA1 damage. Effects appear to be dramatic in tasks in which animals show a profound though transient deficit (46), but this may only amount to a more rapid normalization of performance in tasks that would eventually be learned. Intermediate levels of improvement in tasks such as the three-door runway (46) or visuospatial conditional discrimination (114) may demonstrate recovery that is more relevant to long-lasting cognitive impairments, and there is evidence that grafted animals may continue to show improvement, possibly involving increasing functional integration with use (Virley and Hodges, in prep.). These findings argue for a high degree of specificity of graft action, because CA3 cells, which are structurally similar to CA1 cells and also glutamatergic, are functionally ineffective in rats (46). The findings imply that homotypic cell replacement may serve to reconstruct the host hippocampal circuit, and there is some support from electrophysiological studies for this conjecture (23). However, the detailed anatomical studies that are needed to characterise graft–host connectivity have not yet been carried out.

USE OF NONFOETAL TISSUE IN MODELS OF GLOBAL ISCHAEMIA: TROPHIC AND CELL LINE GRAFTS

Although findings to date provide encouraging evidence that foetal CA1 grafts can promote functional recovery following global ischaemia, implications for therapy are limited, since clinical use of foetal tissue will be practically and ethically problematic, particularly given the requirement for a late donor age. Arnold and Trojanowski (3) suggested that at least 15 weeks is required for the development of human hippocampal cytoarchitecture. Much research effort is therefore being devoted to the generation of nonfoetal sources of donor tissue. There are several developments that are of potential relevance to ischaemic brain damage, notably the use of trophic grafts (31,32) and the production for grafting of "progenitor," "precursor," or "stem" cells (15,38,39,111,128,131) that have flexible developmental capacity.

Global ischaemia induces region-specific increases in the synthesis of neurotrophic factors including brain-derived growth factor (BDNF) (16,60,134,138), nerve growth factor (NGF) (60,134), and neurotrophin-3 (NT-3) (60,134) and growth factors such as basic fibroblast growth factor (bFGF) (144). The time course and distribution of these changes suggest that they play an important part in damage limitation and remodelling of circuits. Delivery of appropriate factors might therefore increase cell survival, particularly in the CA1 region, where failure to upregulate NGF may contribute to vulnerability to ischaemia (60). A range of factors have been shown to be cerebroprotective in ischaemic models. Intraventricular injections of insulin growth factor-1 (IGF-1), transforming growth factor-B_1 (TGF-B1), and NGF have been shown to reduce infarction after ischaemic-hypoxic or cortical devascularising lesions (42,59,69,70, 105,144). Delivery of large molecules to appropriate sites is a major problem to be overcome. Multiple intracranial infusions may lead to traumatic or inflammatory reactions (68). Therefore, implantation of carrier cells transfected to release appropriate factors at target sites is a promising alternative approach. Fibroblasts genetically modified to express NGF have been shown to attenuate CA1–2 hippocampal cell loss when implanted 7 days before global ischaemia (103). However, a fibroblast carrier is subject to overgrowth, and we have found that NGF-releasing fibroblasts

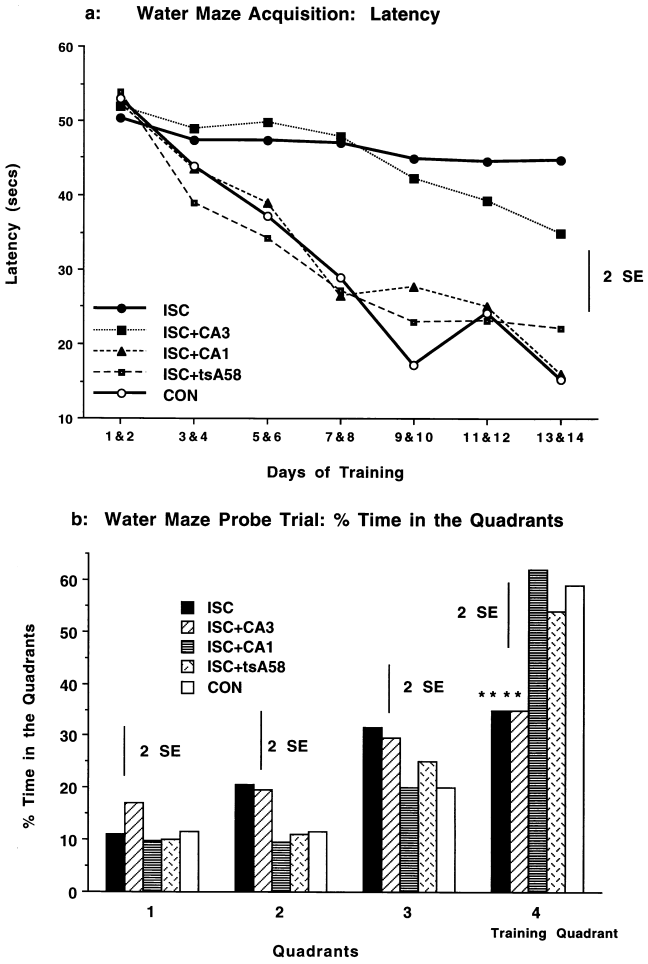

FIG. 6. Comparison of the effects of foetal hippocampal grafts and cells from an expanded hippocampal neuroepithelial (NE) population derived from the *H-2K*^{b}tsA58 transgenic mouse. Ischaemic rats (ISC; 15 min of 4 VO) took substantially longer to find the submerged platform in the water maze (a) than did sham-operated controls (CON). Grafts of CA3 cells (ISC + CA3) failed to improve performance. However, ischaemic rats that received grafts of NE cells (ISC + tsA58) learned as rapidly as rats grafted with foetal cells from the CA1 hippocampal field (ISC + CA1). In the probe trial with the platform removed, the control, ISC + CA1, and ISC + tsA58 groups spent significantly longer ($p < 0.01$) searching in the training quadrant than did the ISC and ISC + CA3 groups. (Data from T. R. Kershaw, The development and transplantation of neural cell lines from the *H-2K*^{b}tsA58 transgenic mouse. Ph.D. Thesis, University of London, 1996.)

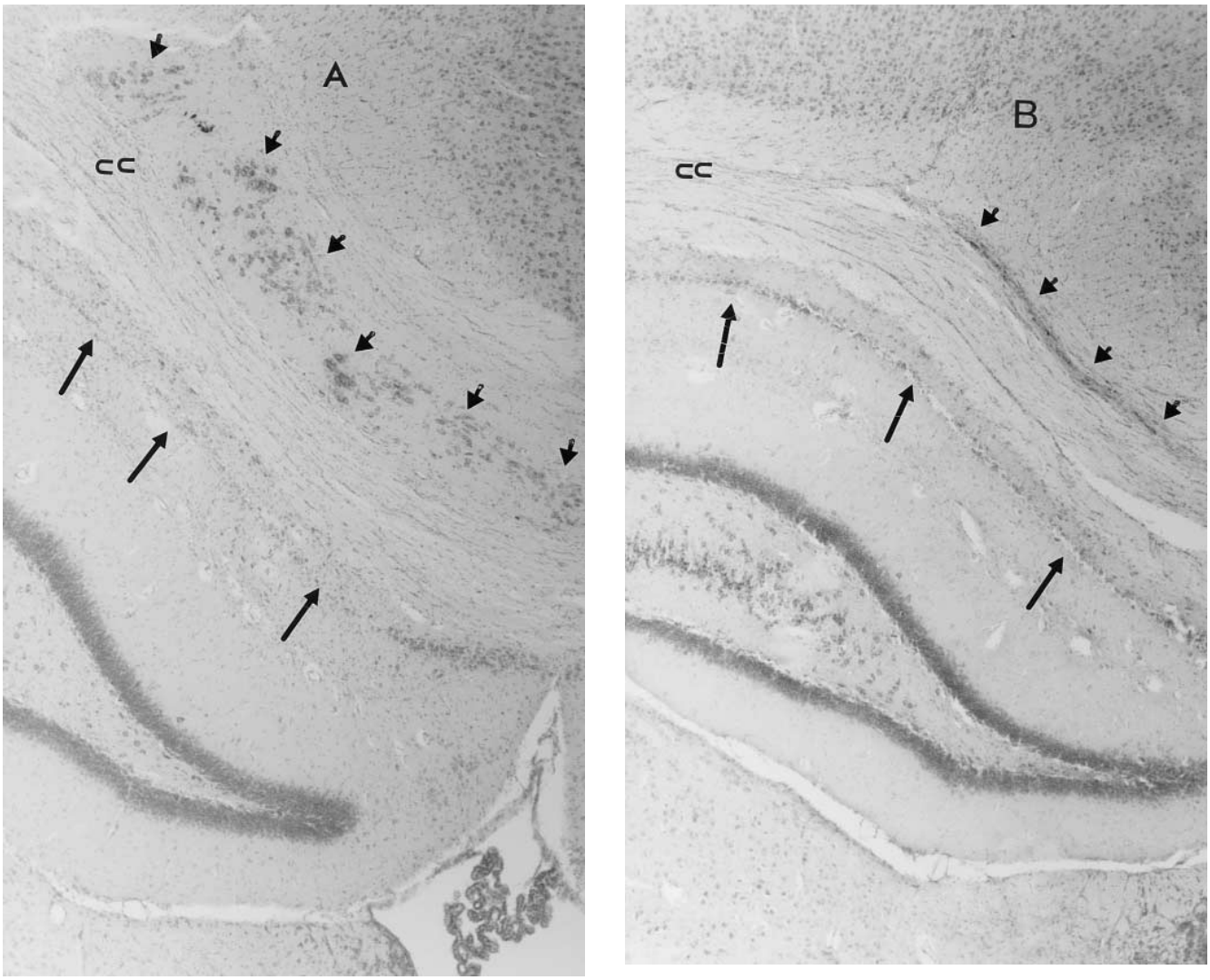

FIG. 7. Coronal Nissl-stained sections of the hippocampus showing a foetal (E 18) CA1 cell suspension graft (A) and a graft of neuroepithelial cells from the *H-2K*^{b}tsA58 transgenic mouse (B) in rats subjected to 15 min of 4 VO. (A) Grafted foetal cells (arrowheads) lie in thick clumps along the corpus callosum (cc) above the region of ischaemic cell loss in the host CA1 field (arrows). (B) Grafted NE cells (arrowheads) have formed a narrow strand within the corpus callosum (cc) above the damaged host CA1 field (arrows). Both rats showed improvement to the control level in water maze acquisition. Grafting took place 2–3 weeks after ischaemia, and animals were taken for histology ca. 9 months after grafting. Magnification 40 ×.

do not attenuate ischaemic deficits or cell loss when implanted 2 weeks after 4 VO ischaemia (H. Watts, unpubl. data), so the cerebroprotective mechanism may only be proactive, and hence of doubtful clinical relevance. Polymer encapsulation of cells that release NGF (or other protective factors) may provide a biologically inert and stable carrier for grafting. Maysinger et al. (69) have shown that encapsulated grafts attenuate the effects of cortical devascularising lesions. Cell lines such as the HiB5 line developed by McKay and colleagues (110), when transfected with the NGF gene, may provide another safe route for delivery of neurotrophins (27,66). These grafts have been shown to increase basal forebrain cholinergic cell bodies in size and number in aged or lesioned rats, and to produce hypertrophy in young nonlesioned animals. The trophic approach to reduction of ischaemic brain damage holds great promise, although it is in early stages of development.

Development of stem or progenitor neural cell lines suitable for grafting is also being vigorously pursued. For maximal utility, these lines need to be conditionally immortalised: for example, by transfection with a temperature-sensitive oncogene such as the SV 40 large T antigen, which permits proliferation at a low permissive temperature (33°C) for rapid expansion and manipulation in vitro, but on transplantation to a nonpermissive brain temperature (38°C), the cells stop dividing and differentiate into mature neurons or glia. Secondly, the cells need to be derived from a progenitor source, such as neuroepithelial (NE) stem cells, with pluripotent developmental capacity, so that they can differentiate into various types of cell according to signals from the region of the host brain into which they have been grafted. Grafts derived from progenitor cells have been shown to develop into neurons or glia according to placement in the cortex, hippocampus, or

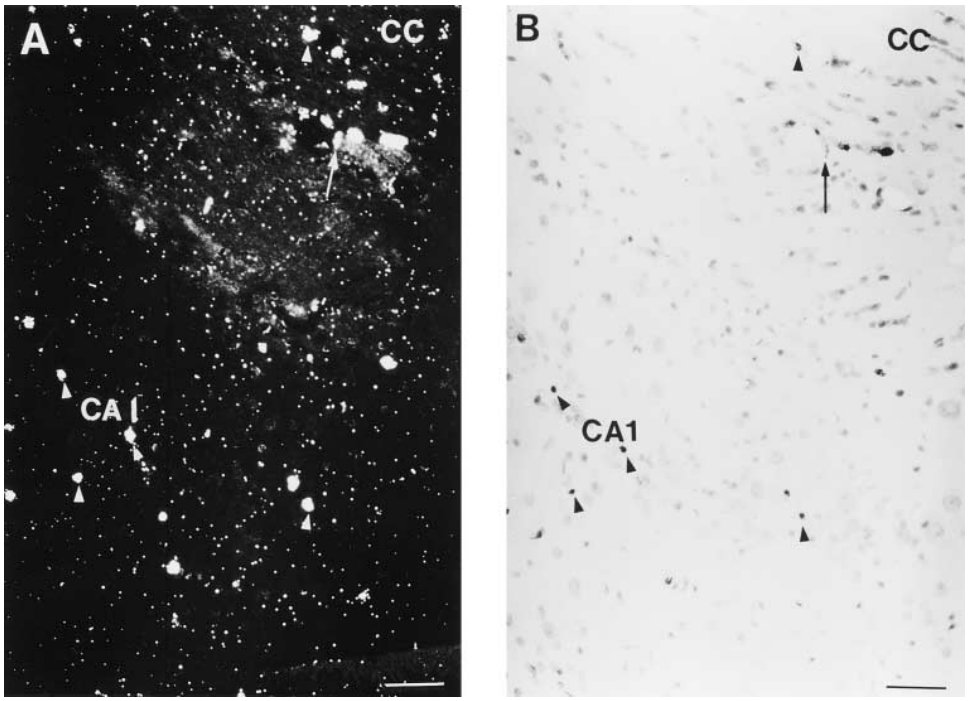

FIG. 8. Coronal sections of the hippocampus showing grafts from an expanded population of neuroepithelial cells from the *H-2K*^{b}tsA58 transgenic mouse labelled with ^{3}H-thymidine (arrowheads). Grafted cells were scattered within the ischaemic CA1 cell layer of the hippocampus and in the corpus callosum (cc), in some cases surrounding blood vessels (arrows). A is a darkfield image of B. Scale bar = 50 μm. (From T. R. Kershaw, The development and transplantation of neural cell lines from the *H-2K*^{b}-tsA58 transgenic mouse. Ph.D. Thesis, University of London, 1996.)

cerebellum of neonatal or adult rats (15,39,62,110,111,128, 131,145).

Kershaw et al. (55,56) have characterised an expanded population of conditionally immortalised cells derived from *H-2K*^{b}-tsA58 transgenic mouse (52) neuroepithelium that expresses a mutant allele of the SV 40 large T antigen under the control of the interferon-inducible *H-2K*^{b} promoter (55,56). At a permissive temperature (33°C), these cells expanded over long periods in vitro with the addition of bFGF to the media, expressed nestin (a marker of precursor cells), and incorporated bromodeoxyuridine (BrdU; a marker for dividing cells). However, when switched to a nonpermissive temperature (37°C) with bFGF withdrawn, the proportion of BrdU-labelled cells fell rapidly. Administration of dibutyryl cAMP triggered differentiation into several types of cell; some expressed neuronal and neurofilament markers (neuron-specific enolase, RT97), some showed transmitter-specific reactivity (GABA, glutamate), whilst others expressed the glial marker GFAP. This population therefore contained precursor cells with varied differentiation capabilities. These cells would thus be likely to respond to an ischaemic environment in which growth factors, cytokines, neurotrophins, and their high-affinity receptors (60,134,144) are available to foster graft survival, differentiation, and synapse formation. We therefore assessed the functional effects of an expanded population of NE cells from the transgenic mouse in ischaemic rats (15 min of 4 VO), in comparison with animals grafted with foetal CA1 and CA3 cells as positive and negative controls, and sham-grafted ischaemic and nonischaemic controls, in tests of water maze acquisition and working memory. Both the CA1- and NE-grafted groups showed substantial recovery relative to ischaemic controls and rats with CA3 grafts, learning as rapidly as nonischaemic controls (see Fig. 6). Histological examination of cresyl violet-stained sections showed that the extent of CA1 cell loss was equivalent in all groups. Foetal CA1 and CA3 grafts formed typical discrete masses above the damaged CA1 field, or in the corpus callosum, whereas NE cells, although widely distributed, also formed thin strands along the corpus callosum (see Fig. 7). Prior incubation in ^{3}H-thymidine enabled labelled NE cells to be identified in scattered locations throughout the hippocampal formation, the corpus callosum, and overlying neocortex, although some clusters occupied the CA1 field (see Fig. 8). Improvements in spatial learning following grafts of an expanded immortalised NE cell population are among the first demonstrations of cognitive recovery following grafts of precursor cells. These findings also confirmed that recovery from effects of global ischaemia requires homotypic replacement of foetal CA1 cells and not cells from the adjacent CA3 field. The success of NE grafts, and encouraging preliminary results with clonal cell lines from the same transgenic mouse, suggest that grafts of precursor cells are able to adopt a neuronlike phenotype that is capable of restoring hippocampal function as effectively as homotypic foetal grafts. It will be necessary to compare patterns of connectivity of foetal, trophic, and cell line grafts to understand their mechanisms of action. It will also be important to investigate the inductive triggers that send pluripotent grafts along different developmental routes, to see how the cells respond to different types of lesion (123) and to see how far they can be encouraged to integrate into different regions of damage. In particular, it will

be of interest to see whether precursor cell lines develop differentially in different regions of infarction following focal ischaemia, and whether, like foetal grafts (86), they also promote functional recovery in stroke models.

In conclusion, studies of the effects of global ischaemia in our laboratory suggest that deficits in general resemble the pattern of impairments induced by discrete hippocampal lesions, although a direct comparison of animals with CA1 cell loss induced by 4 VO and excitotoxic lesions indicated a lesser degree of spatial impairment with lesioning. Our findings suggest that ischaemia results in marked impairment of spatial learning in exploratory tasks that require the use of allocentric spatial cues, but not of place learning that can be accomplished using associative or egocentric strategies, or of two-choice position learning from a static viewpoint. However, once ischaemic animals have learned a location, they remember the environment, so that with repeated testing they can find new locations as rapidly as controls; thus, storage and retrieval of spatial information do not seem to be impaired. Working memory also appears to be spared, although performance in working memory tasks with an allocentric spatial component is severely disrupted. On the basis of hippocampal lesion studies, impairments in learning and retention of conditional visuospatial tasks would be expected following global ischaemia. This has been shown in the marmoset following a selective lesion to the CA1 field but has not been investigated in ischaemic primates or rodents. These profiles of deficit shown by ischaemic animals conform to the proposal of Eichenbaum et al. (25) that damage to the CA fields and dentate gyrus impairs "relational" (including spatial) information processing but not storage in working memory. The extent to which behavioural deficits can be correlated with CA1 cell loss induced by 4 VO is controversial. Our recent findings suggest that strong positive correlations emerge if animals with maximum cell loss are included, but at intermediate levels of damage the relationship between the extent of cell loss and behavioural impairment is weak. Nevertheless, participation of the CA1 field in the deficits induced by global ischaemia is supported by our findings that implantation of foetal CA1 cells, but not of cells from other hippocampal fields or other brain regions, improves the performance of ischaemic rats to control level in water maze acquisition and working memory tasks, and significantly reduces deficits in the three-door runway. Of greater importance for the eventual development of transplants as a therapy for deficits induced by ischaemic brain damage, a conditionally immortalised expanded population of neuroepithelial cells has also been shown to reduce spatial learning deficits in ischaemic rats as potently as foetal CA1 grafts, and neurotrophic grafts have been shown to exert cerebroprotective effects. Because these types of graft can be cloned, standardised, and produced in unlimited numbers, they avoid many of the ethical and practical problems associated with the use of foetal tissue. Development of clonal and neurotrophic cell lines suitable for grafting represents a promising approach to the use of transplants as a therapy for global ischaemia and is likely to advance rapidly in the next decade.

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