Neurotoxic dorsal CA1 lesions versus 4 VO ischaemic lesions: behavioural comparisons

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Anterograde amnesia, a common consequence of transient cerebral ischaemia, has been attributed to cell loss in the hippocampal CA1 subfield. However, variable, widespread damage outside hippocampal CA1 can also occur following ischaemia. We compared the functional consequences of ischaemia and ibotenate acid CA1 lesions on 2 spatial memory tasks (water maze 'place' and 'matching-to-position') to address the possibility that extra-CA1 loss contributes to ischaemia-induced memory deficits in the rat. During place task acquisition, ischaemic rats showed deficits on more measures than ibotenic rats, and during a 1 min probe trial, only ischaemic rats were impaired. On the matching-to-position task, ibotenic rats showed greater impairment than ischaemic rats in terms of one-trial learning, whereas ischaemic rats were more impaired after Trial 2. Ischaemia and ibotenic acid lesions resulted in equivalent CA1 loss, but silver impregnation revealed additional extra-CA1 cell loss in ischaemic rats. Together with the greater behavioural deficits of ischaemic rats, these data indicate a role for extra-CA1 cell loss in ischaemia-induced memory impairments in both animals and humans.

Keywords: Anterograde amnesia, global ischaemia, ibotenic acid, hippocampus, CA1, water maze, silver impregnation

1. Introduction

Cerebral ischaemia refers to a reduction in normal blood flow to the brain to a level that is insufficient to meet metabolic demands. Interruption of cerebral blood flow, as occurs in heart attack, bypass surgery, or coronary artery occlusion, results in a high incidence (up to 48%) of memory deficits, which are typically long-lasting. These memory impairments are characterised by a profound difficulty in learning new information but relatively preserved remote memory and general intelligence [5, 10, 29, 30]. The dramatic susceptibility of cell fields of the hippocampus, particularly the CA1 field, to ischaemia is well established in work with humans [27, 34, 43], and experimental animals including monkeys [2, 44] and rats [25, 28, 41]. Post-mortem and neuroimaging studies of patients with memory loss following ischaemic episodes have suggested that bilateral damage to the CA1 field may underlie impairments, which chiefly involve anterograde memory loss [14, 40, 43], and resemble deficits in temporal lobe amnesia [33]. However, an overemphasis on the CA1 region may exclude other mediators of flawed behavioural performance, and obscure a possibly complex relationship between ischaemia and the emergence of memory deficits that may be detrimental to effective clinical treatment.

If brief enough, ischaemia produces lesions that appear to be limited to the hippocampal CA1 field, but longer durations of ischaemia result in damage to other hippocampal subfields, and extra-hippocampal regions, including the dorsolateral striatum, thalamus, neocortex and various midbrain structures, in both animals and humans [5, 32, 34]. Despite the fact that widespread cerebral pathology following ischaemia is well-documented [16, 32], memory deficits have usually been directly attributed to hippocampal CA1 loss [1, 14, 35, 43], for three principal reasons. First, ischaemia-induced memory deficits are similar in some respects to those produced by hippocampal lesions [1, 18, 19, 41]. However, ischaemia-induced recognition impairments on delayed-matching-to-sample tasks are much more severe than impairments following complete surgical ablation of the hippocampus [2, 20], which strongly suggests that damage confined to the hippocampus cannot account for all aspects of ischaemia-induced memory deficits. Second, ischaemia-induced damage outside the CA1 can be hard to detect. A failure to detect specific brain damage after a heart attack when static neuroimaging techniques are used is a frequent occurrence [12]. Markowitsch and colleagues [16] for example, studied a patient who displayed severe and persistent anterograde and retrograde amnesia following a heart attack. Whilst structural magnetic resonance imaging (MRI) only indicated non-specific cortical atrophy, functional neuroimaging revealed severe

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hypometabolism of the thalamus, medial and lateral temporal cortices. Additionally, post-mortem, the extent and distribution of extra-CA1 damage may well be underestimated by standard histological evaluations of ischaemic brains. Nunn and Jarrard [24] compared the cresyl violet stain with the Fink-Heimer method of silver impregnation [9], after 15 minutes of four-vessel occlusion ischaemia (4 VO; [28]) in the rat. Consistent with earlier results [32], the damage revealed by cresyl violet was almost exclusively confined to hippocampal CAl. The silver stain, however, revealed in the same animals, in adjacent slices, additional cell loss in hippocampal subfields CA2–4, dorsolateral striatum, somatosensory cortex, entorhinal and perirhinal cortex and cingulate cortex. Thus, extra-CA1 cell loss must be viewed as a potential co-mediator of ischaemia-induced memory impairments in humans. Third, some positive correlations have been observed between hippocampal CA1 loss and behavioural deficits in rats and primates. However, a general consensus on this matter has not yet been reached (see [23] for a review). Major discrepancies, such as the occurrence in rats of spatial memory deficits without any apparent CA1 cell loss [13], as opposed to lack of deficits with up to 50% CA1 cell loss [1], cast doubt upon the idea that CA1 damage alone is sufficient to explain ischaemia-induced memory impairments. Even in the presence of significant correlations between CA1 loss and behavioural indices, which have previously been seen as strong evidence of a direct role for hippocampal CA1 in ischaemia-induced deficits [35], CA1 damage may be a proxy for damage or dysfunction elsewhere.

Thus, contrary to the long-standing notion that hippocampal CA1 loss is responsible for ischaemia-induced memory deficits, the available evidence for this view is weak. A useful approach to this issue would be to determine whether the behavioural effects of CA1 lesions produced by other means lead to the same effects as seen following ischaemia, as suggested by Squire and colleagues [36]. The dearth of patients with selective, non-ischaemic lesions of hippocampal cell fields does not allow a direct comparison of ischaemia- versus lesion-induced memory deficits in humans. However, such a comparison is possible in experimental animals, although to our knowledge, it has been made in only one study in rats, in which such comparisons can be most readily carried out. Volpe et al. [41] found that rats subjected to 30 min 4 VO ischaemia, or high-dose ibotenic aid lesions of the entire dorsal hippocampus, were impaired to the same degree on a T-maze task. However, the high-dose IBO lesions were not selective for the CA1 region. A more explicit test of the hypothesis that IBO-induced CA1 cell loss yields the same impairment as that seen following ischaemia requires that the IBO lesion and ischaemia result in the same CA1 loss.

The present study mimicked the CA1 loss that is produced by 15 min 4 VO ischaemia, using an IBO lesion in rats, and directly compared the behavioural effects of the neurotoxic lesion with that of ischaemia. If damage outside hippocampal CA1 does not contribute to ischaemia- induced deficits, then the behavioural profile of the two groups should be the same. Both the water maze 'place' task, in which rats are trained to find a hidden platform located in a fixed place, and the water maze 'delayed-matching-to-position' task, in which rats are required to learn a new platform position each day, were used to assess the functional outcomes of ischaemic and neurotoxic lesions. Both tasks have been shown to be sensitive to the effects of 4 VO ischaemia and hippocampal lesions [11, 18, 19, 25].

2. Methods

2.1. Subjects

Male Wistar rats (initial weight 250–280 g, $N = 54$), were subjected to 15 min ischaemia (ISC), ischaemia sham-operation (ISC SHAM), ibotenic acid lesion of CA1 (CA1 IBO) or sham CA1 lesion (CA1 CON). Three animals from each group were randomly picked post-surgery for silver impregnation. Following Crain et al [8] a 5-day survival period was chosen to maximise the amount of damage that can be seen in brain areas sensitive to ischaemic insult. Because of this short survival time, these rats were not behaviourally tested ($N = 12$), but were representative of the 'behavioural' batch of animals.

2.2. Surgical procedures

2.2.1. Neurotoxic CA1 lesions

Rats were anaesthetised with Equithesin (3 ml/kg; i.p.), and the brain surface exposed. Using standard stereotaxic procedures, microinjections of IBO (0.033 ml) were given to 16 sites in the dorsal CA1 field at the following co-ordinates: bregma $= -3.0$ mm, midline $= \pm 0.8$ mm, depth $= -2.5$ mm; bregma $= -4.0$ mm, midline $= \pm 0.8$ mm, depth $= -1.8$ mm; bregma $= -4.0$ mm, midline $= \pm 2.0$ mm, depth $= -1.5$ mm; bregma $= -4.0$ mm, midline $= \pm 3.0$ mm,

depth = −2.0 mm; bregma = −5.0 mm, midline = ±1.5 mm, depth = −1.8 mm; bregma = −5.0 mm, midline = ±2.5 mm, depth = −1.8 mm; bregma = −5.0 mm, midline = ±3.5 mm, depth = −2.2 mm; bregma = −6.0 mm, midline = ±4.5 mm, depth = −2.4 mm. Sham-operated controls (CA1 CON) underwent anaesthesia and brain exposure, but no injections.

2.2.2. Ischaemia

Global cerebral ischaemia was produced by the 4--vessel occlusion technique [28]. Briefly, rats were anaesthetised with halothane (2%) in a 3:1, $N_2O : O_2$ gas mixture and the vertebral arteries electrocauterised through the alar foramena. Silastic ligatures were also inserted around the common carotids and brought to the surface. 24 hrs later ischaemia was induced by clamping the carotid ligatures for 15 min. Loss of righting reflex reflex within 2 min of carotid clamping was used as a selection criterion. Animals which convulsed during the ischaemia or the reperfusion period were excluded. Body and head temperature were maintained at 37±0.5 °C and 36.5 °C respectively during ischaemia and reperfusion. Sham- operated controls (ISC SHAM) underwent the same surgical procedure as ischaemic rats except for carotid occlusion. For 2 weeks post-surgery, rats were tested daily for evidence of neurological deficits [4]. Only those animals which recovered normal neurological function within 10–13 days were included in subsequent behavioural testing; however no exclusions on neurological grounds were necessary.

2.3. Behavioural testing

Four weeks were allowed for recovery between surgery and the start of behavioural testing. Rats ($N = 42$) were tested first on the place task followed by the matching-to-position task. All rats were tested as one batch by the same experimenter. For both tasks, rats were trained to find the location of a platform (10 cm diameter) submerged 3 cm below the water surface of a circular pool filled with milky water. Temperature was maintained at approximately 22 °C [17]. The pool was conceptually divided into 4 quadrants of equal area, and 3 equal area annuli. The counter area was a circular area surrounding the platform, twice the platform's diameter. A trial began when the rat was placed in the water facing the pool wall and ended when the rat escaped from the water by climbing onto the submerged platform. Start locations were semi-randomly varied across trials. For the place task, the platform was placed approximately in the centre of a designated quadrant where it remained throughout the experiment. Four trials/day/rat were given for 5 days, with an inter-trial interval (ITI) of 5 min. On day 6 of place task training, retention of the platform's position was assessed in a 1 min probe trial, during which the platform was removed. For the *matching-to-position task*, the hidden platform was moved to a different, non-standard position (e.g., in the centre or near the edges of the pool) each day. Four trials/day were given for 4 days with a 30 s ITI. Swim path, latency to reach the platform, % time spent in each area of the pool and heading angle (a measure of divergence from the direct path to the platform) were recorded by an HVS image analysing system (VP112, HVS Image Ltd., UK).

2.4. Statistical analyses

Water maze measures were analysed by repeated measures analyses of variance (ANOVA) with Groups as the between-subjects (S) factor, and Trials and Days of testing as within-S factors, followed where appropriate by Student's t-tests, using the between-S pooled ANOVA error term and degrees of freedom. Following significant main effects or interactions, planned comparisons of behavioural performance were made between each lesion group and their respective control groups. The difference between CA1 cell loss following 15 min ischaemia and CA1 IBO lesions was assessed using the non-parametric test, Mann-Whitney U, since cell loss estimates took the form of grades.

2.5. Histology

Rats were terminally anaesthetised with pentobarbitone sodium (40 mg/kg, i.p.) and intracardially perfused with 0.9% saline followed by 10% neutralised formalin. Animals which had undergone behavioural testing ($N = 42$) were sacrificed 21 weeks post-surgery. For the *behavioural study*, non-embedded brains were cut into coronal sections 20 μm thick throughout the rostral-caudal extent of the hippocampus, prior to staining with cresyl fast violet. Cell loss was assessed bilaterally in the hippocampal subfields, striatum and neocortex by 2 independent observers unaware of the experimental condition. Measures were taken at 4 levels in cortex – 10.7, 9.7, 5.7 and 3.7 mm from the interaural line (IAL) – and at 2 levels in striatum and the CA1, CA2, CA3 and CA4 cell fields of the hippocampus (5.7 and 3.7 mm from the IAL). Quan-

titative histology of neuronal damage was based on a 6 point rank order scale [15], where 0 = 0–10% cell loss, 1 = 10–30% loss, 2 = 30–50% loss, 3 = 50–70% loss, 4 = 70–90% loss and 5 = 90–100% loss. Since silver staining techniques are extremely sensitive to neuronal degeneration, this method was used to check that the extent of CA1 cell loss in representative ischaemic and IBO rats was indeed equivalent, and to confirm the selectivity of the IBO lesion for the target area. Rats randomly selected for *silver staining* were sacrificed 5 days post-surgery, brains embedded in egg-yolk, and 40 μm coronal sections cut throughout the brain. Adjacent sections were chosen from each brain, and one of each randomly selected for silver or cresyl violet staining. Under light microscopic examination, damage was assessed in a semi-quantitative manner throughout the brain by two observers who were 'blind' as to lesion type.

2.6. Staining procedures

2.6.1. Cresyl fast violet

Sections were immersed in Cresyl fast violet (Raymond A. Lamb, 10 g/l) for 1 min at 56 °C, rinsed in distilled water, and passed through ascending alcohol concentrations (95%, 100%) prior to clearing with Xylene.

2.6.2. Fink-Heimer silver impregnation [9]

Briefly, free-floating sections were thoroughly rinsed in distilled water, and the staining of normal grey matter suppressed by immersion in 0.05% potassium permanganate. Sections were decolourised in a bleaching solution (equal volumes of 1% oxalic acid and 1% hydroquinine), prior to staining in uranyl nitrate and silver nitrate, and immersion in an alcoholic formalin solution acidified by citric acid (the Nauta-Gygax reducer), until the sections turned brown. Following stabilisation in a sodium thiosulphate solution, sections were transferred to slides, passed through ascending alcohol concentrations (75%, 95%, 100%) and cleared in Xylene.

3. Results

3.1. Mortalities and exclusions

No deaths due to surgery occurred in any group. One rat developed seizures after IBO injections to CA1 and was excluded from the experiment thereafter. The number of animals that underwent behavioural testing in each group was as follows: ISC ($n = 8$), ISC SHAM ($n = 8$), CA1 IBO ($n = 17$), CA1 CON ($n = 8$). The CA1 IBO group was larger than that of the other groups to allow for possible elimination of animals after histological assessment due to inadequate lesions. Nine out of seventeen CA1 IBO rats were subsequently rejected (see below). Thus the analysis of behavioural performance below was conducted with 8 animals per group.

3.2. Water maze place task – acquisition

All groups of rats showed decreases in latencies to find the hidden platform over training ($F = 14.18$, df $= 1, 112$, $p < 0.001$ for the linear trend of Days), but only ISC rats were impaired relative to their control group (main effect of Group ($F = 3.38$, df $= 3, 28$, $p < 0.05$). Subsequent t-tests showed that this was due to ISC rats differing from controls ($t_{28} = 2.85$, $p < 0.05$) and from CA1 IBO rats ($t_{28} = 2.96$, $p < 0.05$); CA1 IBO rats did not differ from their controls ($t_{28} = 1.42$). The Days X Group interaction was non-significant ($F < 1$). In terms of percentage time spent in the training quadrant where the platform was located, all groups improved during training ($F = 25.11$, df $= 1, 112$, $p < 0.001$ for the linear trend of Days), but both ISC and CA1 IBO rats were impaired (main effect of Group ($F = 6.24$, df $= 3, 28$, $p < 0.01$), relative to their respective control group (ISC: $t_{28} = 2.37$, $p < 0.05$; CA1 IBO: $t_{28} = 2.68$, $p < 0.05$). The Days X Group interaction was non-significant ($F < 1$). The 4 groups of rats distributed their time amongst the three annuli of the pool in a similar fashion (all main effects of Group – $F < 1$), and there was no evidence for thigmotaxis. In terms of mean heading angle over the training period, lesioned rats were not distinguishable from controls (main effect of Group ($F < 1$).

3.3. Water maze place task – probe trial

Only ISC rats were impaired in terms of time spent in the former training quadrant (Quadrant 3; see Fig. 1), a measure of spatial bias towards the general area that contained the platform during training. Analysis of variance gave a significant main effect of Group ($F = 6.01$, df $= 3, 28$, $p < 0.01$), and subsequent t-tests showed that ISC rats spent significantly less time in this region of the pool than both ISC SHAM ($t_{28} = 2.98$, $p < 0.01$) and CA1 IBO rats ($t_{28} = 2.75$, $p < 0.05$). The measure of '% time spent in the

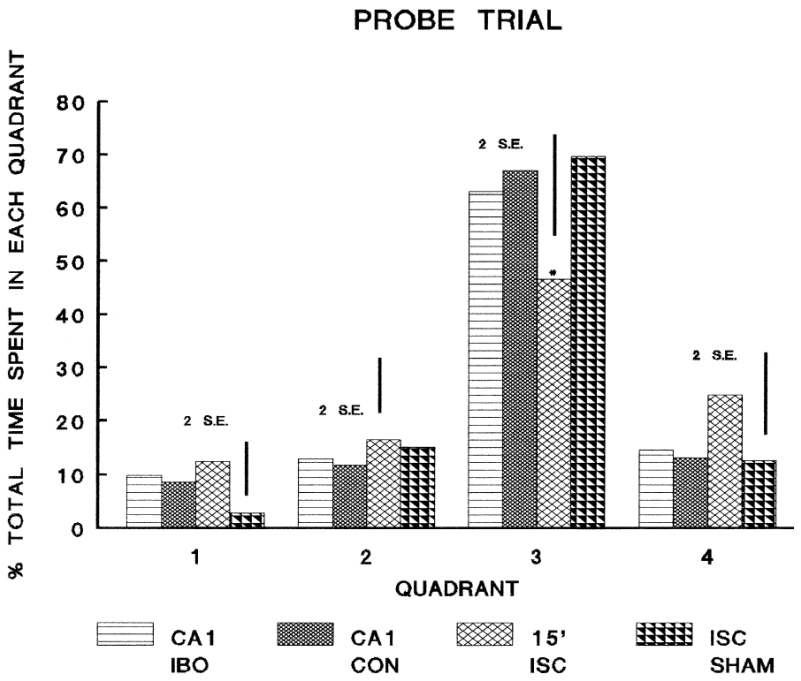

Fig. 1. Effects of 15 min 4 VO ischaemia and IBO CA1 lesions on percentage of time spent in each quadrant during the water maze probe trial. Quadrant three represents the previously correct quadrant. Scores are mean percentages. Bars show 2 $\times$ the standard error for main effects of Groups. Stars represent a significant difference between an experimental group and the sham-operated control group. $*p < 0.05$.

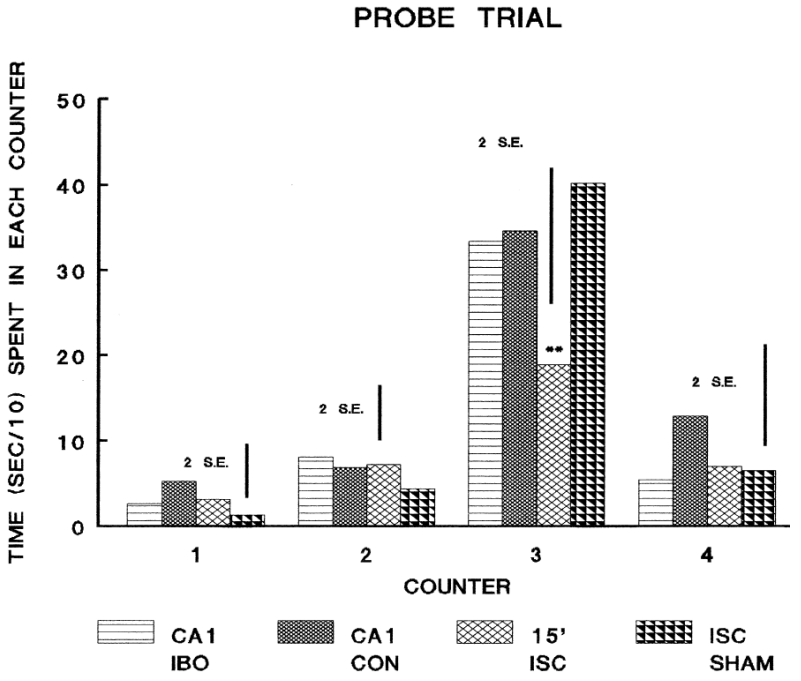

Fig. 2. Effects of 15 min 4 VO ischaemia and IBO CA1 lesions on time (sec/10) spent in each counter during the water maze probe trial. Counter 3 represents the immediate vicinity of the previously correct platform location; counters 1, 2 and 4 represent the immediate vicinity of other standard platform locations at the same radius. Bars show 2 $\times$ the standard error for the main effects of Groups. Stars represent a significant difference between an experimental group and the sham-operated control group. $**p < 0.01$.

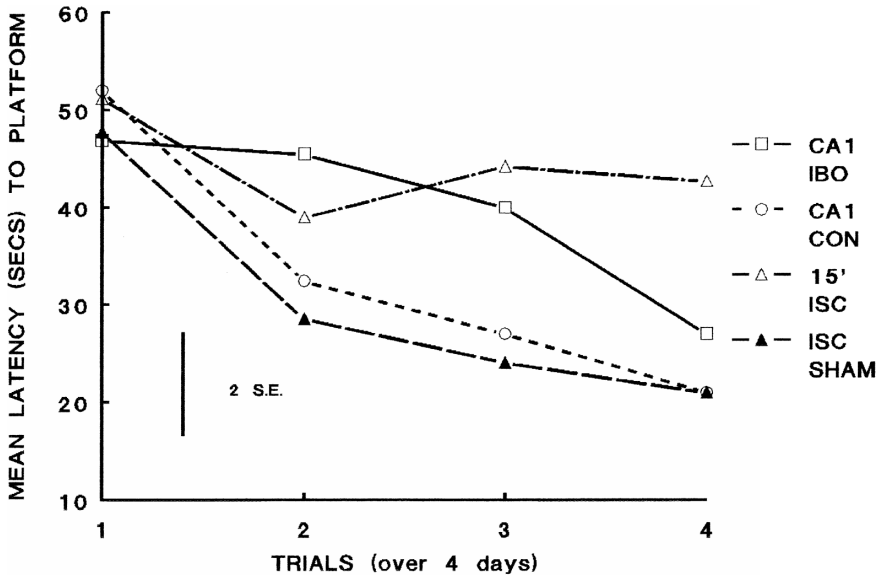

Fig. 3. Effects of 15 min 4 VO ischaemia and IBO CA1 lesions on latency to find the hidden platform in the water maze matching-to-position task. Scores are mean latencies for each trial summed over 4 days. Bar shows 2 × the standard error for the Groups × Trials interaction term.

counter area of the platform', provides a measure of recall of the precise former position of the platform. Again, only ISC rats were impaired on this measure ($F = 7.31$, df $= 3, 28$, $p < 0.01$), spending a reduced percentage of time searching in the immediate vicinity of the previously correct platform location (Counter 3; see Fig. 2), relative to both ISC SHAM ($t_{28} = 3.02$, $p < 0.01$) and CA1 IBO rats ($t_{28} = 2.56$, $p < 0.05$). Little time was spent in the counter areas of other potential platform locations by any group, showing that the high percentage of time spent in Counter 3 was not due to rats merely swimming a fixed distance from the side walls. Heading angle was worse in ISC rats than others, but the main effect of Group was non-significant ($F = 2.19$, df $= 3, 28$). All groups of rats distributed their time amongst the three annuli of the pool similarly to each other.

Swim speeds were derived from total swim path divided by time. Throughout the training period and probe trial mean swim speeds for all groups were nearly identical, and the main effect of Group for swim speed was non-significant ($F < 1$), indicating that differences in latencies were unlikely to be due to motor or motivational deficits.

3.4. Water maze matching-to-position task

This task was administered with the platform in a different position each day. Fig. 3 shows performance over the first to fourth trial averaged over four days of training. Latencies for all groups were similar on the first day; thereafter lesioned rats' latencies were greater than that of controls. Analysis of variance gave a significant main effect of Group ($F = 4.05$, df $= 3, 28$, $p < 0.05$), together with a significant decrease in latency over trials ($F = 34.83$, df $= 1, 336$, $p < 0.001$ for the linear trend of Trials), and a significant Trials X Group interaction ($F = 1.99$, df $= 9, 336$, $p < 0.05$). In separate analyses of each trial pooled across days, there was a significant effect of group on the critical second trial ($F = 2.98$, df $= 3, 28$, $p < 0.05$), due to reduced latencies in the ISC SHAM and CA1 CON groups relative to their controls (ISC: $t_{28} = 2.14$, $p < 0.05$; CA1: $t_{28} = 2.62$, $p < 0.05$). For all but the CA1 IBO group, most learning occurred between Trial 1 and 2. The CA1 IBO group did improve over trials 2 to 4, however. Group differences were maintained on Trial 3 ($F = 3.07$, df $= 3, 28$, $p < 0.05$) and were again attributable to a difference between each lesion group and its control group (ISC: $t_{28} = 3.34$, $p < 0.01$; CA1: $t_{28} = 2.55$, $p < 0.05$). On Trial 4 , group differences were still present ($F = 2.98$, df $= 3, 28$, $p < 0.05$), due to ISC and ISC SHAM groups differing significantly ($t_{28} = 2.58$, $p < 0.05$), and ISC rats showing significantly greater impairment than CA1 IBO rats ($t_{28} = 3.51$, $p < 0.05$), but on this last trial the difference between CA1 IBO and CA1 CON groups was not significant ($t_{28} = 1.92$). Thus, ISC and ISC SHAM groups differed from each other from Trial 2 onwards but CA1 IBO and CA1 CON groups

differed only on Trials 2 and 3. By Trial 4 the CA1 IBO deficit had subsided sufficiently for ISC and CA1 IBO group means to differ significantly. Distance measures followed an identical pattern to that of latency, and analysis of swim speeds showed that there were no differences between the groups (main effect of Group – F<1). Thus latency differences in this task, as in the place task, are not likely to have involved differences in motor performance.

3.5. Histological analysis

3.5.1. Behavioural groups: cresyl violet staining at 21 weeks post-surgery

Neither sham-operation (ischaemia or IBO) resulted in any cell loss. Of the original 17 CA1 IBO rats, 9 were rejected from the behavioural analysis described above, due to either patchy or asymmetrical lesions of CA1. Decisions regarding these exclusions were made without reference to the behavioural data. The remaining 8 showed a very similar magnitude of CA1 loss to that seen following 15 min ischaemia (ISC group), which in turn was similar to that observed in previous experiments carried out in this laboratory [11, 21, 22, 25]. CA1 loss was greatest in anterior CA1; ventral CA1 was completely spared by both treatments.

Five out of eight ISC rats showed 90-100% cell loss in anterior CA1; in the remaining three ISC rats cell loss ranged from 50-90%. Four out of eight CA1 IBO rats exhibited maximal cell loss in anterior CA1; the other four showed cell loss of between 70-90%. In posterior CA1 both lesion groups showed an average of 50% loss, with little variability. In both ISC and IBO groups, CA1 loss began and ended at similar rostral-caudal levels (approx. 6.5 mm and 3.2 mm from the IAL, respectively). Additional intra- and extra-CA1 loss was present in only one ISC rat, which demonstrated approximately 25% loss of CA4 (hilar) neurons. The CA1 IBO lesion resulted in some very slight damage to the overlying neocortex in $2/8$ rats, but otherwise did not cause cell loss in any area other than hippocampal CA1. There was very little left/right hemisphere variability in CA1 loss in either group. There were no significant differences between CA1 cell loss grades in CA1 IBO and ISC rats in either anterior ($U = 22$, df $= 14$) or posterior ($U = 19$, df $= 14$) CA1.

3.5.2. Silver impregnation study: Fink-Heimer versus cresyl violet stains at 5 days post-surgery

No degeneration was seen in CA1 CON ($n = 3$) or ISC SHAM ($n = 3$) rats. Cell loss was observed primarily in hippocampal dorsal CA1 irrespective of staining technique in both ISC ($n = 3$) and CA1 IBO ($n = 3$) rats. Cresyl violet stained sections did not reveal any damage in ISC or IBO rats beyond the CA1 region. The pattern of cell loss observed in silver impregnated sections from ISC rats has been reported previously (see [24]), and will be described only briefly here. All ISC brains impregnated with silver showed variable loss in hippocampal subfields CA2–4, the dorsolateral striatum, somatosensory cortex (mainly layer III), dorsal perirhinal (up to 10%) and cingulate cortex (up to 30%), that was not observed in adjacent, cresyl violet stained sections. One ISC rat showed evidence of slight, additional damage to ventral CA1, the basal lateral nucleus of the amygdala, ventral posterior thalamic region, and dorsolateral septal nucleus. Silver impregnated sections from CA1 IBO rats did not reveal any extra-CA1 loss apart from some very slight degeneration in overlying neocortex.

4. Discussion

The behavioural effects of a neurosurgical lesion were directly compared with those of an ischaemic lesion, to determine whether hippocampal CA1 loss alone is responsible for ischaemia-induced memory deficits. Silver impregnation confirmed that the amount of CA1 damage in IBO and ISC groups was equivalent, and revealed extra-CA1 loss in ischaemic rats. The two treatments did not, however, produce equivalent performance in either place or matching-to-position water maze tasks. IBO-lesioned animals were less impaired than ISC rats overall, although IBO rats were impaired on some measures in both tasks. These data indicate that, in humans, memory impairments observed following ischaemia are only partially due to hippocampal CA1 loss, and that damage in other brain regions also contributes.

The performance of ISC rats on the place task was similar to that reported previously in our laboratory [11, 21, 25] and in other laboratories [13, 26]. ISC rats showed deficits in both acquisition and retention of the platform's position, whereas IBO rats, although impaired on one measure during acquisition (latency to find the platform), showed good retention of the

platform's position once its location had been learned. This pattern of results is consistent with a transient learning deficit in IBO rats, and a more severe learning deficit in ISC rats.

On the matching-to-position task, both ISC and IBO rats were impaired relative to their respective control groups, but in somewhat different ways. IBO rats showed a greater deficit in terms of one-trial learning, whereas ischaemic rats were more impaired in terms of matching-to- position trials from Trial 2 onwards. This dissociation between one-trial learning and slower learning may simply reflect a difference in rate of acquisition or the impairment of different processes induced to varying degrees by the two treatments. Specifically, a reduction in savings from Trial 1 to Trial 2 is consistent with a working memory impairment, but this kind of impairment would also be expected to contribute to a rate of learning deficit.

Other research in this laboratory has shown that transplants of foetal CA1 neurons into ischaemic rats alleviate aspects of water maze place learning [11, 21] demonstrating that a lack of viable CA1 cells is crucially involved in water maze impairments. However, ischaemic rats implanted with foetal CA1 grafts still show problems with fine localisation – as shown by reduced time spent in the counter area relative to controls during water maze probe trials [11]. Thus graft-induced recovery was only partial. These data again indicate that factors other than CA1 loss were additional mediators of ischaemic water maze deficits.

The most obvious cause of the greater memory impairment associated with ischaemia is the extra-CA1 loss revealed by silver staining. However, establishing the exact extent of this damage is difficult. It should be noted that these neuropathological changes were observed following one short (5 days) survival time, in order to maximise the amount of damage seen in brain areas sensitive to ischaemia [8]. However, neuronal death outside the hippocampus can occur up to 3 weeks following the ischaemic episode [31], and therefore further cell loss may have been missed by the use of a short survival period. On the other hand, whatever the technique employed, histological evaluations performed months after the insult, when the degeneration process is complete, likely fail to detect widely distributed, slight-to-moderate cell loss that may have functional relevance. Thus, post-mortem studies of ischaemic patients who survive the ischaemic episode by months or years may have suffered cell loss that is undetectable at the time of the neuropathological examination.

Considering the extra-CA1 loss that was observed at 5 days post-ischaemia in the present study, it is possible that many of the brain areas affected could have contributed to the water maze impairments observed, and, by inference, to the anterograde amnesia observed in ischaemic patients. Small, discrete lesions of hippocampal dentate gyrus and CA3 subregions [7, 37] disrupt water maze performance. Lesions of both anterior and posterior (retrosplenial) cingulate cortex can result in water maze place learning deficits ([38, 39], and in humans, a transient topographical amnesia has been reported following an angioma in the cingulate cortex [6]. Perirhinal cortex lesions in rats can result in mild impairments in the water maze place task [42], and in humans the importance of this region for visual recognition memory performance is undisputed. In fact, two patients with complete damage to the perirhinal cortex have been reported to perform worse than other amnesics (with damage limited to the hippocampal formation or diencephalic structures) at recognition memory for complex visual scenes stimuli at delays of 25s or longer [3].

The long-lasting functional changes in *surviving* neurons that accompany ischaemia includes changes in calcium channels and neurotransmitter levels, up-regulation of heat shock protein, 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 hippocampal CA1 cells (see [32] for a review). Attenuation of brain function may also be mediated by post-ischaemic oedema, which has been reported in rats [32] and monkeys [2], and presumably may also occur in humans.

In summary, the present findings do not support the long-held view [43] that hippocampal CA1 loss is the sole cause of ischaemia-induced memory impairments. Rather, they add to the growing body of evidence, described above in the Introduction, indicating that both intra- and extra-hippocampal damage contribute to the pattern of memory impairments observed following ischaemia. Consequently, although animal models of global ischaemia represent valuable models for neuropathological and therapeutic studies, they may not be appropriate as a clean example of selective CA1 loss that will in turn point unequivocally to the functional effects of this particular break in the hippocampal circuit. The clinical implications are that patients suffering cardiac arrest may have widespread brain damage which goes beyond the frequently assumed medial temporal lobe pathology. The standard

histological techniques employed in postmortem examinations may not be sufficient sensitive to detect this widespread pathology. Accordingly, the effectiveness of cerebroprotective agents should be demonstrated at the behavioural, as well as the histological level, since deficits in brain function, not brain histology, represent the ultimate costs of ischaemic brain damage.

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