

Research report

Chronic cerebral hypoperfusion: loss of pupillary reflex, visual impairment and retinal neurodegeneration

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Abstract

Adult rats underwent permanent bilateral occlusion of the common carotid arteries (2VO) to determine the effect of chronic cerebral ischemia on vision and retina. They were monitored post-surgically for the presence of the pupillary reflex to light. Some rats were tested for 6 months post-surgically on a radial arm maze task and then tested in another water-escape task which explicitly tested visual function. Another group of rats were tested post-surgically for 3 months on a task which simultaneously assessed visual and tactile discrimination ability. The thicknesses of the retinal sub-layers were then measured for some rats. Fourteen of the 25 rats that underwent 2VO lost the pupillary reflex. This seemed to occur within 5 days. Rats that lost the pupillary reflex but not rats whose reflex was intact, were impaired on all visually guided mazes. Tactile discrimination ability was unaffected. Only rats that lost the pupillary reflex showed reduced thickness of the retinal outer nuclear and plexiform layers, reduced cell density in the retinal ganglion cell layer and astrocytosis and degeneration of the optic tract. We conclude that 2VO can eliminate the pupillary reflex. Photoreceptors and retinal ganglion cells degenerate, but it is unclear if these are the cause(s) or result(s) of the loss of the pupillary reflex. These effects are accompanied by impairment of visually guided behavior. The possibility that visual system damage may also occur in acute ischemia merits further investigation. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chronic bilateral occlusion of the common carotid arteries (2VO) of the rat reduces cortical/hippocampal blood flow to ~25–50% of normal levels at 2.5 h post-occlusion. Seven days later, the reduction in flow has abated to ~60–75% and it remains at this reduced level for at least several months [27,32]. Several reports indicate that after 2VO, rats are impaired on visually guided tasks that are typically used to assess spatial learning/memory. For example, after 1 week of 2VO, they cannot perform the Morris water maze task, which requires that the rat locate a hidden submerged platform in a water tank by using extra-maze visual cues to spatially navigate [29]. A more slowly emerging impairment occurs on the radial arm

maze (RAM) task which requires that the rat forage to find food rewards that are located at the end of arms that radiate from a central hub [20,29]. On this task also, foraging is guided by extra-maze visual cues.

Evidence suggests that 2VO compromises the visual system. After seven days of 2VO, retinal morphology is not affected, though the b-wave of the electroretinogram (a function of bipolar cell depolarization and the resulting potassium efflux from Müller cell endfeet) is eliminated, suggesting that 2VO affects retinal signal transmission [1]. This effect begins shortly after ligation. Shorter duration (20–60 min) 2VO significantly reduced the magnitude of the b-wave and as well, lengthened its implicit time [1,4,5]. Relatedly, others have reported that chronic 2VO causes atrophy of the optic nerve at 16 weeks post-occlusion [30].

During the early course of an experiment, which we intended would examine the post-2VO emergence of deficits on the RAM task, we noticed that some 2VO rats showed a loss of the pupillary reflex. Accordingly, in this

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study, we systematically explored the relationship between the loss of the pupillary reflex on performance of the RAM. Additionally, we assessed the ability of 2VO rats in a visually cued water escape task and in another RAM task, which allowed for the simultaneous assessment of visual and tactile discrimination, to determine if sensory impairment was selective to the visual modality. Lastly, we histologically examined the optic tract and retinas of these rats and related these findings to their behavior and their pupillary reflex.

The results indicate that a chronic, moderate decrease in cerebral blood flow elicits significant retinopathy, characterized by reduction of thickness of the outer nuclear and plexiform layers, reduced cell density in the ganglion cell layer and by astrogliosis and degeneration of the optic tract in about half of the rats. These rats lost the pupillary reflex and showed impairment of visually guided behavior. The 2VO rat may provide an experimental model of the permanent blindness which is a frequent result of carotid artery disease in humans [6,10,25]. The data also emphasize the necessity to directly assess visual function in other stroke models.

2. Materials and methods

2.1. Animals

Approximately 10-month-old male retired breeder Sprague–Dawley rats (510–711 g) were obtained from Charles River (Montreal, Canada). Animals were singly housed with free access to water, in a 12-h reversed light cycle (on at 20:00). All procedures were approved by the Animal Care Committee of Carleton University and conformed to the guidelines of The Canadian Council of Animal Care and the ARVO statement for use of animals in ophthalmic and vision research.

Pupillary constriction was examined by illuminating the rats' dark adapted eye with an otoscope. Since the observation that the pupillary reflex might be affected by 2VO arose while RAM testing was already under way in experiment one, 12 of 27 animals had their reflex observed beginning after surgery and onwards. The rats were examined weekly for 6 weeks and then before any new behavioral task and before cardiac perfusion. The pupillary reflex of the remaining 15 animals was first examined 118 ± 14 days after surgery.

2.2. Behavioral testing

Prior to surgery, the 27 rats of experiment one were trained on a 10-arm RAM to a performance criterion. The maze consisted of a 75-cm diameter, grey plastic hub from which radiated 10 equally spaced arms that were 66 cm long, 12 cm wide, with 25 cm high clear side walls. Remotely operated clear plastic doors were placed 21 cm down the length of each arm. A recessed food well at the

end of each arm could be baited with one half of a Honey Nut Cheerio. The middle of the hub also contained a recessed well into which 1.0 ml of 10% liquid sucrose could be injected by a remotely controlled pump. The maze was situated in a room that contained a variety of visual cues including wall-mounted patterns.

The rats were food-deprived to $85\% \pm 2.5\%$ of their original body weight and trained to consume the liquid sucrose in the hub before the experimenter remotely opened all arms. The rationale for this was to prevent animals from adopting a simple non-visually guided algorithm for finding rewards (such as entering adjacent arms) by forcing them to return to the hub's center. Eight of the 10 arms were baited with reward and two adjacent arms were never baited. The baited arms were consistent for each rat but varied across rats. When an animal exited an arm, all the doors were closed and the rat was required to consume sucrose before the doors were re-opened. Animals were given 15 min to find all eight rewards.

Animals were trained for 5 consecutive days each week until they met a strict performance criterion after which they underwent surgery. RAM testing resumed 3–4 days later for 16 weeks, ceased for 7 weeks and then resumed for 1 week.

For the simultaneous visual/tactile discrimination, 13 rats had their pupillary reflexes examined on every post-surgery day for the first week and once a week thereafter. They were trained on a 12-arm RAM described elsewhere [18], that was modified such that the hub was enclosed by a 30-cm wall with remotely controlled, sliding guillotine-style doors allowing entry into each arm. Each arm could be cued as black or white by means of a suspended cardboard swinging door located 10 cm into each arm. The end wall of each arm contained a 28 cm high plastic panel, also either black or white. To provide the tactile cue, the floor of each arm was lined with an interchangeable strip of polyurethane matting, either rough or smooth surfaced, extending 4 cm into the centre chamber and 5 cm from the end or the arm.

The rats received a food pellet (Noyes Precision A/1 Rodent Pellets) for entering only arms that contained the combination of correct visual and correct tactile cue. The rough floor lining was the correct tactile cue for all the rats. Black visual cues were the correct visual cues for half of the rats and white for the other half. For each rat, 6 arms containing the combination of both correct tactile and visual cues, were baited. Three arms containing the correct tactile cue but the incorrect visual cue, were unbaited as were 3 arms that contained the correct visual cue combined with an incorrect tactile cue. Hence, the rat could commit either a visual cue error (e.g., enter black cued arms when only white-cued arms contained reward) or a tactile error (enter smooth-floored arms).

Training sessions began with the animal in the centre chamber of the RAM. All 12 doors were opened simultaneously to allow access to the 6 baited arms, 3 non-baited

visual error arms, and 3 non-baited tactile error arms. After an animal retrieved the pellet from a baited arm, the door to that arm remained shut for the rest of that test session. Conversely, unbaited arms always remained accessible. The session ended when all 6 pellets were retrieved or 10 min elapsed. Rats were trained to a strict performance criterion in order to qualify for 2VO surgery. Three days after surgery, RAM testing resumed. The rats were trained every 2nd–3rd day until post-surgical day 62.

Animals from both experiments were also tested in a water maze described elsewhere [29]. This maze task is often used for functional assessment in rat ischemia models [17,21,23,24,29]. The rats from experiment one were tested at about 220 days post-surgery. The maze procedure was intended to assess the rats' ability for visual discrimination. Two 12-cm diameter cylinders, one black and one yellow, were suspended above the water with the black cylinder always marking the location of a submerged platform. The platform location was randomly changed from trial to trial to one of two different sites, with the cuing cylinders also appropriately interchanged. The animals were tested 9 trials per day for 10 days. The latency to swim to the correct location was recorded.

The rats from the visual/tactile discrimination experiment were also trained at about 100 days post-surgery in the conventional spatial navigation version of the task described elsewhere [17,29], which required that they locate the submerged platform by using extra-maze cues. The rats underwent one daily session for 4 consecutive days.

2.3. Surgery

2VO (and sham) surgery was carried out under ketamine hydrochloride (100 mg/kg i.m.) and methohexital sodium (40 mg/kg) anaesthesia for all rats with the exception that the 12 rats that were tested in the visual-tactile RAM task did not receive atropine pre-medication. This was eliminated to test the possibility that it may have contributed to the loss of the pupillary reflex. In fact, it did not contribute, as 7/16 rats that received atropine lost the reflex while 7/9 rats that did not, also lost the reflex.

The surgery has been previously described [29]. Briefly, a ventral mid-line incision was made, the common carotid arteries were exposed bilaterally and gently separated from the carotid sheath and vagus nerve. Animals assigned to the 2VO surgery had both carotids doubly ligated with 5-0 silk suture 8–10 mm inferior to the origin of the external carotid. During surgery, the animal's body temperature was maintained at 37°C with a heat lamp connected to a rectal thermistor probe.

2.4. Histology

At about 300 days post-surgery, the rats from experiment one were perfused and their brains and eyes subse-

quently removed, post-fixed and paraffin embedded as described elsewhere [29]. The hippocampus was coronally sectioned (4 μm) and stained with Palmgren's silver stain [28]. To examine the optic tract, 8 μm coronal brain sections at -2.8 , -3.3 , -3.8 mm from bregma were stained with the Gallyas silver stain for degenerating neurons [13]. Eight-micrometer cross-sections from approximately the same eccentricity of the central retina of the left eye were also prepared and stained with hematoxylin and eosin (H and E). Four-micrometer sections of brain (-2.8 , -3.3 , -3.8 , -4.3 mm from bregma) and central retina were stained immunohistochemically, with a monoclonal antibody to glial fibrillary acidic protein (GFAP, Sigma), a marker for astrocytic infiltration/proliferation [22].

All hippocampal measures were derived from 2 sections at each of -3.3 , -3.8 and -4.3 mm from bregma. Optic tract measures were derived from two sections at each of -2.8 , -3.3 , -3.8 mm from bregma. Averages across sections and plates were calculated. All microscopic investigations were carried out by an observer blind to group membership on an Olympus BH-2 microscope. The total number as well as the number of damaged hippocampal CA1 and CA4 pyramidal cells of both hemispheres were counted manually from silver stained sections. Damaged cells were deemed to have smaller, darker nuclei with an irregular shape. GFAP immunoreactivity (percent area) was measured in the oriens layer of both hippocampi (CA1 and CA4 sectors), and in the left and right optic tract with commercially available image analysis software (Imaging Research, St. Catherines, ON) at $200\times$ magnification. Briefly, the experimenter defined target staining (manually eliminating artefact) and the computer determined the areal density of target in systematically sampled areas. These densities were then averaged for the two slides. Gallyas staining of the optic tract was similarly analysed.

The same software was used to measure the thickness of the different layers of central retina as well as cell density in the RGC layer. Cell density values (cells/mm) were formulated by counting the number of RGC layer cells across a fixed length of retina. Retinal GFAP immunoreactivity in the different layers of central retina was assessed along a scale where 0 represented no immunoreactivity and 4 represented extensive immunoreactivity. All retinal measures were conducted on the medial, nasal and temporal aspects of the central retina. Nasal and temporal aspects were defined as the section of the retina closest to the ciliary body where retinal thickness appeared stable.

3. Results

3.1. Behavioural results suggested vision deficit

Of the 27 animals trained to criterion on the RAM in experiment one, 11 underwent sham surgery and formed

the control group (CON). Sixteen animals underwent 2VO. Of these rats, 7 lost their pupillary reflex and formed the 2VOnoflx group. The remaining 9 2VO animals with the intact pupillary reflex formed the 2VOflx group. All CON animals retained the reflex.

RAM errors were defined as (i) baited arm re-entries (BAR), where an animal entered a baited arm from which it had previously retrieved the food reward and (ii) unbaited arm entries (UAE), where an animal entered an arm that never contains a food reward. It has been argued that BARs reflect working memory (for arms recently visited during a session on the maze) while UAEs reflect reference memory (for arms that on all sessions on the maze, have never been associated with food reward) [25].

The post-surgical performance of these three groups on the RAM can be seen in Fig. 1, upper panel, which shows BARs averaged over each week of post-surgical testing. ANOVA of these data indicated that the groups differed significantly ($p < 0.01$). Scheffé's post-hoc analysis indicated that the 2VOnoflx group committed more BARs than the CON group ($p < 0.05$). Similar results were found by

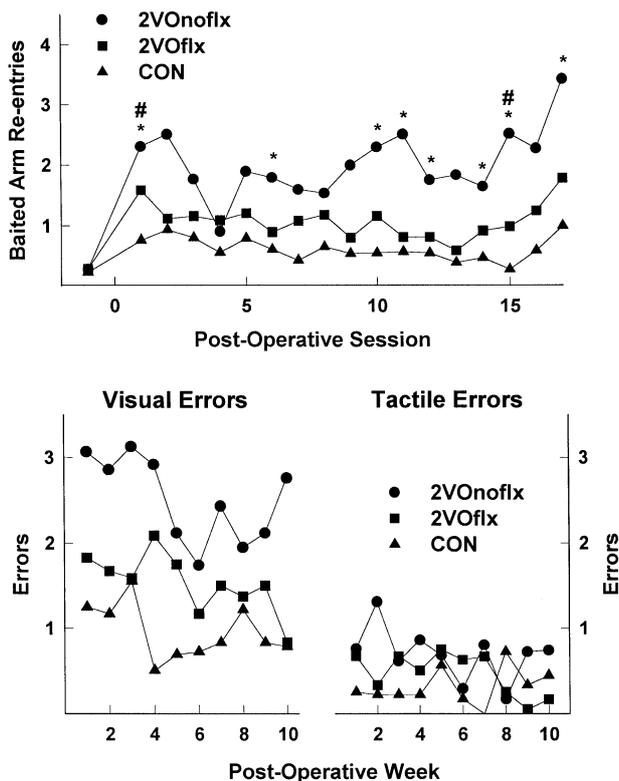


Fig. 1. The upper panel shows baited arm re-entry errors on the radial arm maze task of experiment one. The 2VOnoflx rats ($n = 7$) but not the 2VOflx rats ($n = 9$) committed more errors than the CON rats ($n = 11$). The lower panel shows visual (left side) and tactile (right side) errors on the visual/tactile radial maze task of experiment two. The 2VOnoflx rats ($n = 7$) showed significantly increased visual but not tactile errors when compared to the combined 2VOflx ($n = 2$) and CON ($n = 3$) group. (*: significantly different from CON group; #: significantly different from 2VOflx group).

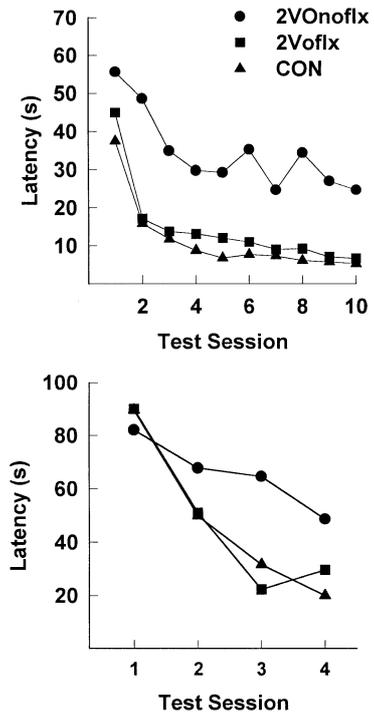


Fig. 2. The upper panel shows the mean group latencies to swim to the visually cued platform in the 2-cue version of the Morris water maze. The 2VOnoflx rats ($n = 7$) were significantly impaired at discriminating between the correct and incorrect cues. The lower panel shows performance on the spatial navigation version of the water maze in experiment two. The 2VOnoflx rats were impaired at navigating to the location of the hidden, submerged platform.

the ANOVA for UAEs (data not shown). In this instance however, Scheffé's test revealed that the 2VOnoflx group committed significantly more UAEs than both the CON and the 2VOflx groups ($p < 0.05$). Again the 2VOflx and CON rats did not differ.

Of the 12 rats trained on the simultaneous visual/tactile discrimination version of the RAM, 3 rats underwent sham surgery, 9 underwent 2VO surgery and 7 of these lost the pupillary reflex. Some rats appeared to lose the reflex in both eyes simultaneously; others lost the reflex first in one eye followed by the second within a few days. In one instance, only one eye functioned properly. That is, exposure of the functioning eye to light elicited a consensual reflex in the damaged eye, while exposure of the damaged eye to light, failed to elicit any pupillary reaction in either eye. The 2VOnoflx rats lost the pupillary reflex in both eyes either immediately, or within 5 days post-surgery. One rat temporarily lost the pupillary reflex in both eyes immediately after surgery, regained it in one eye 5 days later, and regained it in the remaining eye about two weeks later.

ANOVAs comparing the 2VOnoflx group against the combined 2VOflx and CON groups, both of whom retained the pupillary reflex, indicated that the 2VOnoflx rats committed more visual ($p < 0.001$) but not tactile errors

than the other two groups. The results are shown in the lower panel of Fig. 1.

As shown in the upper panel of Fig. 2, when tested for their ability to locate the hidden platform in the water maze by discriminating between light and dark cue location markers, the three groups of experiment one were found to differ by ANOVA ($p > 0.001$). Post-hoc analysis indicated that the 2VOnoflx group required significantly more time than either the CON or the 2VOflx group ($p < 0.05$). This was not due to differing swim speeds as shown by analysis of videotapes of the first session. Consistent with these data for the visually cued water maze task, the 2VOnoflx rats of experiment two were clearly impaired on the spatial navigation version of the water maze. These data are shown in the bottom panel of Fig. 2.

3.2. Histological analysis revealed visual system pathology

Histological analyses of CA1 and CA4 cell fields of the hippocampus indicated that the three groups from experiment one did not differ in total numbers of cells, number of damaged cells, or the percentage of area in each sector

that displayed GFAP immunoreactivity (all F 's < 1.0 , ANOVA, data not shown).

Fibre degeneration and reactive astrocytosis in the optic tract as shown by the percentage area stained with the Gallyas silver technique and for GFAP respectively, indicated significant group differences ($p < 0.001$, ANOVA). For both measures, the 2VOnoflx group had higher percent area staining than both the CON and 2VOflx groups ($p < 0.01$; Fig. 3A–F).

ANOVAs of the results of morphometric analyses of H and E stained retinal cross-sections indicated that the groups differed overall in cell density in the RGC layer and in thickness of both the outer plexiform and outer nuclear layers ($p < 0.001$; Fig. 3G–I). Post-hoc comparisons indicated that 2VOnoflx rats showed reduced overall RGC layer cell density and reduced overall thickness of the outer plexiform and nuclear layers when compared to the control and 2VOflx rats (p 's < 0.05), who did not differ from each other. The data for thickness of the temporal retina are shown in Fig. 4. Similar effects were observed for medial and temporal aspects with the one exception that the 2VOnoflx and control rats did not differ

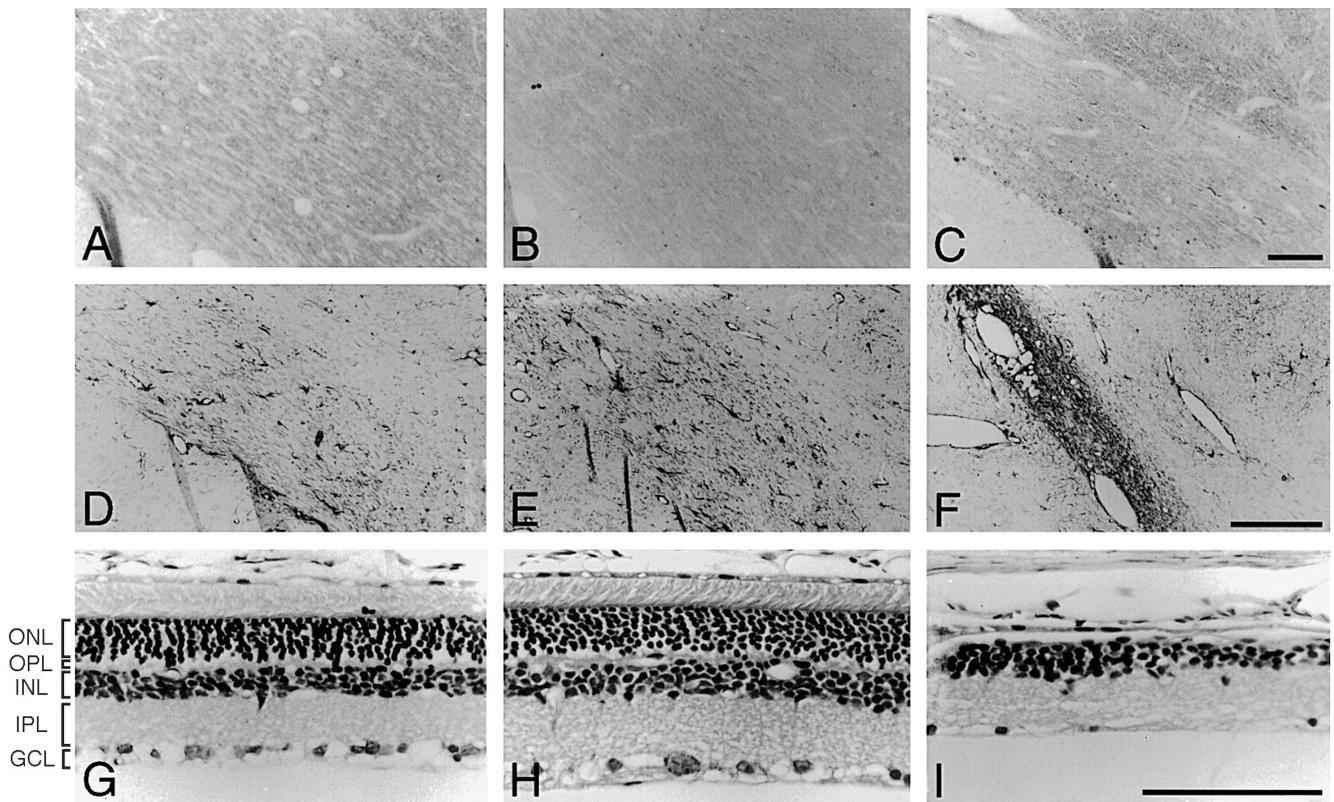


Fig. 3. Photomicrographs of optic tract (A–F) and central medial retina (G–I). Sections from the CON (A, D, G), 2VOflx (B, E, H), and 2VOnoflx (C, F, I) groups were stained with Gallyas silver (A, B, C), monoclonal anti-GFAP Ab (D, E, F), or hematoxylin and eosin (G, H, I). Optic tracts from 2VOnoflx animals had more intense silver (C), and GFAP staining (F) compared to animals with an intact pupillary reflex (A, B and C, D, respectively). Similarly, 2VOnoflx animals had thinner OPL and ONLs, and a lower GCL cell density (I) than did the other groups (G, H). In this instance, the OPL and ONL is not present in the 2VOnoflx retina (I). Scale Bar = 100 μ m.

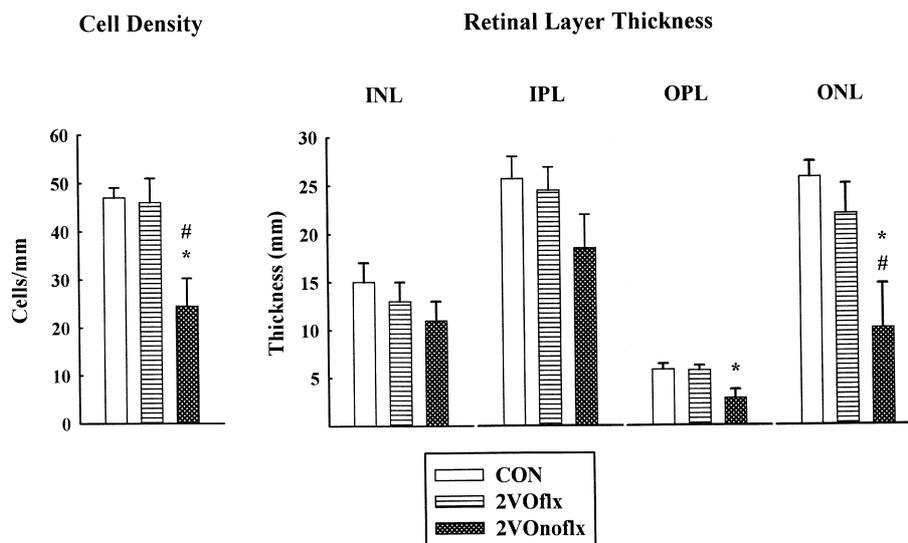


Fig. 4. The leftmost panel shows cell density in the RGC layer of the temporal retina. Cell density was significantly reduced in the 2VOnoflx rats ($n = 7$). The other four panels show the thicknesses of the inner nuclear layer (INL), inner plexiform layer (IPL), outer plexiform layer (OPL) and outer nuclear layer (ONL). Significant reductions in the thickness of the OPL and ONL were observed only for the 2VOnoflx rats, indicating the loss of photoreceptor cells. (*: significantly different from CON ($n = 11$) group; #: significantly different from 2VOflx ($n = 7$) group; error bars represent S.E.M.).

in RGC layer cell density at the nasal aspect. The thickness of neither the inner plexiform nor the inner nuclear layer differed among the three groups.

In order to investigate whether retinal pathology was still ongoing, GFAP immunohistochemistry of retinal sections was rated by an experimenter blind to group membership on a 0–4, where 0 represented no visible staining. Differences between the 2VOnoflx and CON groups and between the 2VOnoflx and 2VOflx groups were analysed with Mann–Whitney U -tests. No differences in the density of GFAP were noted in any retinal layer (data not shown).

4. Discussion

The results of the behavioural testing of 2VO rats were clearly related to their pupillary reflex status. 2VO rats that lacked the pupillary reflex committed more errors on the RAM task of experiment one, committed more errors of visual but not tactile discrimination on the RAM task of experiment two and were significantly slower to locate the submerged platform in both the explicitly cued and the spatial navigation version of the water maze task. Conversely, 2VO rats that retained the pupillary reflex were not significantly different from the control rats on any of these tasks.

Histological analyses of the retinas indicated loss of photoreceptors and RGC layer cells and optic tract atrophy in 2VOnoflx rats. Current research from our laboratory which uses the Thy-1 antibody to selectively label retinal ganglion cells [2] has indicated that the cell loss in the RGC layer reflects the death of these cells rather than displaced amacrine cells (unpublished observations).

While the pupillary reflex was affected within several days after 2VO, retinal pathology was not assessed until many months later and so from these data, it is not possible to ascertain the temporal link between these two measures. However, we have observed elevated GFAP in the optic tract and in the lateral geniculate nucleus at 14 days and the Gallyas degeneration staining in the optic tract is also increased at this time (unpublished observations). Hence, it seems reasonable to conclude that visual system degeneration is contiguous with the loss of the pupillary reflex. The exact sequence and cause of these events remains unclear and is the subject of current investigation in our laboratory. At this time, it seems that the loss of the pupillary reflex likely does not reflect early degeneration of RGC layer cells. DNA fragmentation in photoreceptors is apparent 2 weeks after 2VO while DNA damage in the RGC cell layer is first observed at 10 weeks after 2VO and becomes increasingly more evident at subsequent time points, when substantial loss of ganglion but not displaced amacrine cells is detected (unpublished observations). Our results are supported by a recent report that 2VO caused marked suppression of the electroretinogram 3 and 9 months later with marked loss of photoreceptors evident at 9 but not 3 months [26]. Furthermore, thy-1 immunoreactivity was reduced at 9 months, indicating retinal ganglion cell loss at this time. The pupillary reflex was not measured in the rats of that study.

One explanation for the loss of the pupillary reflex is that it is caused by ischemic damage to the ciliary ganglion. Retinal and optic nerve degeneration may then result from light toxicity due to the collapse of the pupillary reflex. Alternatively, photoreceptor and then RGC degeneration, and/or optic tract degeneration may occur as a

result of ischemia, resulting in the loss of the pupillary reflex due to sensory deafferentation. Future experiments could determine the contribution of light toxicity by eliminating light access to the eye after 2VO.

A common symptom of occlusive carotid artery disease in humans is amaurosis fugax or transient ischemic blindness. This occurs in over one third of patients and may progress to permanent blindness if the occlusion is not reversed [10]. Exposure to bright light diminishes the visual evoked potential in patients with carotid disease probably because diminished retinal blood flow cannot meet the metabolic demands of photoreceptor re-pigmentation [7,9,11]. Retinal histopathologies from six patients with carotid disease who experienced visual symptoms, showed cell loss, which varied considerably even within the same retina and which was total for all retinal elements in some regions [15,16]. Atrophy of the optic nerve as well as dilated and non-reactive pupils had been observed in several of these patients. In parallel to our findings here for the rat, tonic pupil was noted for some of these patients as well as for other patients suffering retinal ischemia [33].

An important issue that remains to be resolved is why approximately half of our 2VO rats and apparently about the same percentage of humans who suffer carotid stenosis, in fact demonstrate visual disturbance and retinal pathology. The pterygopalatine artery, which is the principal source of retinal perfusion in the rat branches from the internal carotid and it seems reasonable to assume that when the common carotid is blocked, there would be reverse perfusion of the internal carotid and thence the pterygopalatine, via the circle of Willis. It seems to be the case that this reverse perfusion does not occur or is insufficient in about half of the 2VO rats. The reason(s) for this remain(s) to be determined.

These clinical findings and the results of this experiment underscore the vulnerability of the visual system to carotid occlusion. Furthermore, they suggest that the reduction of light exposure may be important to the preservation of retinal integrity prior to the surgical resolution of the carotid occlusion. Lastly, the possibility that the retinal degeneration is apoptotic suggests that prophylactic treatment be aimed at counteracting this mode of cellular demise.

Most, if not all, of the experiments concerning the behavioural effects of 2VO in rats have based their conclusions of learning/memory deficits in 2VO rats on tests that are sensitive to visual dysfunction [14,19,20,29,31,34]. The deficits have been attributed to brain and notably hippocampal ischemia. Indeed, there is substantial evidence that the hippocampus is dysfunctional in 2VO rats. Cytochrome oxidase activity is reduced and there is low-level, apparently apoptotic death of pyramidal cells [3,8]. Late-appearing pyramidal cell loss and elevated GFAP may occur, although as suggested by the current experiment, they may not always be evident [20,29]. Our current findings from the application of unbiased stereological cell

counting techniques indicates pyramidal cell loss after 2VO (unpublished observations). Hence, the discrepant results regarding pyramidal cell loss is probably due to inadequacies of cell counting techniques. However, the degree of pyramidal cell loss is relatively modest and the functional assessment of metabolically dysfunctional but not degenerate hippocampal pyramidal cells in 2VO rats and as well the determination of factors that might push these cells to extinction, remain as future research challenges.

Lastly, it is important to note that retinal blood supply may also be compromised in other rat ischemia models (e.g., carotid occlusion plus hypotension, occlusion of vertebral and carotid arteries etc.). It has been reported that 15 min of global ischemia achieved by the 4-vessel occlusion technique, causes degeneration of the optic tract and neuronal damage in the superior colliculus although surprisingly, this is not manifested until sometime between 5 and 60 days post-surgically [12]. Thus, visual dysfunction could also contribute to the effect of transient global ischemia on behaviour. This effect has frequently been assessed by performance in the Morris maze which relies upon visually-guided spatial navigation. This could account for the poor correlation between performance of this task and measures of ischemic brain damage, most notably CA1 pyramidal cell loss [21,23,24]. Furthermore, even when CA1 damage has been found to correlate with impaired water maze performance, it is possible that visual dysfunction could be a causative factor.

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References

- [1] N.L. Barnett, N.N. Osborne, Prolonged bilateral carotid artery occlusion induces electrophysiological and immunohistochemical changes to the rat retina without causing histological damage, *Exp. Eye Res.* 61 (1995) 83–90.
- [2] C.J. Barnstable, U.C. Drager, Thy-1 antigen: a ganglion cell specific marker in rodent retina, *Neuroscience* 11 (1984) 847–855.
- [3] S.A.L. Bennett, M.P.R. Tenniswood, T. Fortin, C.M. Davidson, M.T. Keyes, B.A. Pappas, Chronic cerebral hypoperfusion elicits neuronal apoptosis and behavioural impairment, *NeuroReport* 9 (1997) 161–166.
- [4] F. Block, K.-H. Sontag, Differential effects of transient occlusion of common carotid arteries in normotensive rats on the somatosensory and visual system, *Brain Res. Bull.* 33 (1994) 589–593.

- [5] F. Block, M. Schwarz, K.-H. Sontag, Retinal ischemia induced by occlusion of both common carotid arteries in rats as demonstrated by electroretinography, *Neurosci. Lett.* 144 (1992) 124–126.
- [6] L.A. Breen, Atherosclerotic carotid disease and the eye, *Neurol. Clin.* 9 (1991) 131–145.
- [7] R.A. Brigham, J.R. Youkey, G.P. Clagett, M.A. Walton, M.A. Lary, D.F. Fischer, G. Wind, Bright-light amaurosis fugax: an unusual symptom of retinal hypoperfusion corrected by external carotid revascularisation, *Surgery* 97 (1985) 363–368.
- [8] J.C. de la Torre, A. Čada, G. Davis, R.J. Sutherland, F. Gonzalez-Lima, Reduced cytochrome oxidase and memory dysfunction after chronic brain ischemia in aged rats, *Neurosci. Lett.* 223 (1997) 165–168.
- [9] G.A. Donnan, F.W. Sharbrough, J.P. Whisnant, Carotid occlusive disease. Effect of bright light on visual evoked response, *Arch. Neurol.* 39 (1982) 687–689.
- [10] J. Duggan, W.R. Green, Ophthalmologic manifestations of carotid occlusive disease, *Eye* 5 (1991) 226–238.
- [11] A.J. Furlan, J.P. Whisnant, T.P. Kearns, Unilateral visual loss in bright light. An unusual symptom of carotid occlusive disease, *Arch. Neurol.* 36 (1979) 675–680.
- [12] F. Gallyas, M. Hsu, G. Buzsaki, Delayed degeneration of the optic tract and neurons in the superior colliculus after forebrain ischemia, *Neurosci. Lett.* 144 (1992) 177–179.
- [13] F. Gallyas, F.H. Güldner, G. Zoltay, J.R. Wolff, Golgi-like demonstration of “dark” neurons with an argyrophil III method for experimental neuropathology, *Acta Neuropathol.* 79 (1990) 620–628.
- [14] R.M.A. Jaspers, F. Block, C. Heim, K.-H. Sontag, Spatial learning is affected by transient occlusion of common carotid arteries (2VO): comparison of behavioural and histopathological changes after ‘2VO’ and ‘four-vessel-occlusion’ in rats, *Neurosci. Lett.* 117 (1990) 149–153.
- [15] M. Kahn, D.L. Knox, W.R. Green, Clinicopathologic studies of a case of aortic arch syndrome, *Retina* 6 (1986) 228–233.
- [16] M. Kahn, W.R. Green, D.L. Knox, N.R. Miller, Ocular features of carotid occlusive disease, *Retina* 6 (1986) 239–252.
- [17] R.G.M. Morris, P. Garrud, J.N.P. Rawlins, J. O’Keefe, Place navigation impaired in rats with hippocampal lesions, *Nature* 297 (1982) 681–683.
- [18] S.J.E. Murtha, B.A. Pappas, Neurochemical, histopathological and mnemonic effects of combined lesions of the medial septal and serotonin afferents to the hippocampus, *Brain Res.* 651 (1994) 16–26.
- [19] J.-W. Ni, K. Matsumoto, H. Watanabe, Tetramethylpyrazine improves spatial cognitive impairment induced by permanent occlusion of bilateral common carotid arteries or scopolamine in rats, *Jpn. J. Pharmacol.* 67 (1995) 137–141.
- [20] J.-W. Ni, K. Ohta, K. Matsumoto, Y. Murakami, H. Watanabe, Progressive cognitive impairment following chronic cerebral hypoperfusion induced by permanent occlusion of bilateral carotid arteries in rats, *Brain Res.* 653 (1994) 231–236.
- [21] J.A. Nunn, E. Le Peillet, C.A. Netto, H. Hodges, J.A. Gray, B.S. Meldrum, Global ischaemia: hippocampal pathology and spatial deficits in the water maze, *Behav. Brain Res.* 62 (1994) 41–54.
- [22] J.P. O’Callaghan, Neurotypic and gliotypic proteins as biochemical markers of neurotoxicity, *Neurotoxicol. Teratol.* 10 (1988) 445–452.
- [23] G.M. Olsen, J. Scheel-Krüger, A. Møller, L.H. Jensen, Does neuronal damage of CA1 relate to spatial memory performance of rats subjected to transient forebrain ischemia?, *Acta Neurol. Scand.* 89 (1994) 204–209.
- [24] G.M. Olsen, J. Scheel-Krüger, A. Møller, L.H. Jensen, Relation of spatial learning of rats in the Morris water maze task to the number of viable CA1 neurons following four-vessel occlusion, *Behav. Neurosci.* 108 (1994) 681–690.
- [25] D.S. Olton, B.C. Papas, Spatial memory and hippocampal function, *Neuropsychologia* 17 (1979) 669–682.
- [26] N.N. Osborne, S. Rukhsana, M.S. Nash, Photoreceptors are preferentially affected in the rat retina following permanent occlusion of the carotid arteries, *Vis. Res.* 39 (1999) 3995–4002.
- [27] T. Otori, T. Katsumata, Y. Katayama, A. Terashi, Measurement of regional cerebral blood flow and glucose utilization in rat brain under chronic hypoperfusion conditions following bilateral carotid artery occlusion. Analysed by autoradiographical methods, *Nippon Ika Daigaku Zasshi* 64 (1997) 428–439.
- [28] K.M. Page, Histological methods for peripheral nerves: II, *Med. Lab. Technol.* 28 (1971) 44–58.
- [29] B.A. Pappas, J.C. de la Torre, C.M. Davidson, M.T. Keyes, T. Fortin, Chronic reduction of cerebral blood flow in the adult rat: late-emerging CA1 cell loss and memory dysfunction, *Brain Res.* 708 (1996) 50–58.
- [30] J. Takamatsu, A. Hirano, D. Levy, P. Henkind, Experimental bilateral carotid artery occlusion: a study of the optic nerve in the rat, *Neuropathol. Appl. Neurobiol.* 10 (1984) 423–428.
- [31] K.-I. Tanaka, N. Ogawa, M. Asanuma, Y. Kondo, M. Nomura, Relationship between cholinergic dysfunction and discrimination learning disabilities in Wistar rats following chronic cerebral hypoperfusion, *Brain Res.* 729 (1996) 55–65.
- [32] M. Tsuchiya, K. Sako, S. Yura, Y. Yonemasu, Cerebral blood flow and histopathological changes following permanent bilateral carotid artery ligation in wistar rats, *Exp. Brain Res.* 89 (1992) 87–92.
- [33] H. Wilhelm, Tonic pupil caused by ischemia, *Fortschr. Ophthalmol.* 86 (1989) 380–382.
- [34] Y. Yamamuro, H. Iwano, N. Sensui, K. Hori, M. Nomura, Acetylcholine in the hippocampus during the discrimination learning performance in a rat model of chronic cerebral ischaemia, *NeuroReport* 7 (1996) 1837–1840.