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Neurobehavioral and Imaging Correlates of Hippocampal Atrophy in a Mouse Model of Vascular Cognitive Impairment

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Abstract

Vascular cognitive impairment (VCI) is the second most common cause of dementia. Reduced cerebral blood flow is thought to play a major role in the etiology of VCI. Therefore, chronic cerebral hypoperfusion has been used to model VCI in rodents. The goal of the current study was to determine the histopathological and neuroimaging substrates of neurocognitive impairments in a mouse model of chronic cerebral hypoperfusion induced by unilateral common carotid artery occlusion (UCCAO). Mice were subjected to sham or right UCCAO (VCI) surgeries. Three months later, neurocognitive function was evaluated using the novel object recognition task, Morris water maze, and contextual and cued fear conditioning tests. Next, cerebral perfusion was evaluated with dynamic susceptibility contrast magnetic resonance imaging (MRI) using an ultra-high fieild (11.75 Tesla) animal MRI system. Finally, brain pathology was evaluated using

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COMPLIANCE WITH ETHICAL STANDARDS

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

histology and T_2 weighted MRI (magnetic resonance imaging). VCI, but not sham, mice had significantly reduced cerebral blood flow in the right vs. left cerebral cortex. VCI mice showed deficits in object recognition. T_2 weighted MRI of VCI brains revealed enlargement of lateral ventricles, which corresponded to areas of hippocampal atrophy upon histological analysis. In conclusion, our data demonstrate that the UCCAO model of chronic hypoperfusion induces hippocampal atrophy and ventricular enlargement, resulting in neurocognitive deficits characteristic of VCI.

Keywords

Vascular dementia; cerebral blood flow; cognitive impairment; chronic cerebral hypoperfusion

INTRODUCTION

Vascular cognitive impairment (VCI) is the second most common cause dementia [1, 2]. While the pathogenesis of VCI is not completely understood, it is thought to be linked to cerebral microvascular dysfunction, which leads to cerebral hypoperfusion, brain tissue damage and subsequent cognitive impairment[1, 2]. VCI is diagnosed clinically by a combination of cognitive assessment and magnetic resonance imaging (MRI), which identifies evidence of vascular injury such as white matter lesions, infarcts or brain atrophy. However, MRI has rarely been used to assess disease progression in animal models of VCI.

Several rodent models of VCI have been developed, most of which rely on induction of chronic cerebral hypoperfusion to induce VCI-related pathology[3]. In mice, commonly used models include unilateral common carotid artery occlusion (UCCAO) [4] or bilateral carotid artery stenosis [5]. Both models have been shown to produce a variety of VCI pathology including white matter damage, inflammation, neuronal degeneration, and cognitive deficits [4, 6, 7]. One advantage of the UCCAO model is that damage to the endothelium in the carotids is avoided, in contrast to the stenosis model which involves inserting coils into the carotids. Another advantage of the UCCAO is the 100% survival rate following surgery[4], in contrast to the bilateral carotid artery stenosis model which has survival rates ranging from 25% to 85% [7] based on size of coil used to induce stenosis. Studies have shown that relative cerebral perfusion is acutely reduced following UCCAO using non-quantitative methods such as laser Doppler flowmetry [4]. However, using confocal imaging through a cranial window it has also been reported that blood flow is restored over time [8]. Both methods estimate changes in cortical perfusion in only a small region of the brain. Global changes in cerebral blood flow have been reported for other rodent models of VCI using magnetic resonance imaging (MRI) [9, 10], but not for the UCCAO mouse model. Therefore, in the current study, we used an ultra-high field (11.75 Tesla) animal MRI system to measure cerebral blood flow at 4 months after UCCAO via dynamic susceptibility contrast (DSC) MRI, in addition to evaluating T_2 weighted structural images. We also characterized long-term neurocognitive deficits resulting from UCCAO and confirmed T_2 weighted MRI findings using histology. To our knowledge, long-term effects (greater than 30 days) of chronic cerebral hypoperfusion on behavioral and cognitive performance and cerebral perfusion have not previously been examined in mouse models of

VCI. Therefore, we also assessed behavioral and cognitive changes at 3 months post-VCI surgery and subsequently conducted MRI. Our results show that UCCAO reduces CBF in the ipsilateral hemisphere, which leads to hippocampal atrophy, ventricular enlargement and long-term impairments in non-spatial memory behaviors.

MATERIALS AND METHODS

This study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and protocols were approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University, Portland, OR, USA.

Animals

Male C57BL/6J mice were purchased from Jackson laboratories (Bar Harbor, Maine) and group-housed until sham or UCCAO surgery was performed, at which point they were singly housed and remained singly housed until the end of the study.

UCCAO

Right UCCAO or sham surgery was performed in 3 month old male mice (n = 10 mice per group) using aseptic techniques. Mice were anesthetized with 2% isoflurane and kept warm with water pads. After midline cervical incision, the right common carotid artery was isolated and two 6-0 silk sutures were placed under the carotid. For VCI mice, the two ties were tightly tied and the carotid was cauterized between the two ties and cut. For sham mice, the ties were removed from under the carotid arteries without being tied and vessels were not cauterized. For all mice, incisions were closed and mice were allowed to recover. The survival rate was 100%. Mice were singly housed after surgery.

Behavioral and cognitive testing

Behavioral testing was conducted 3 months after VCI or sham surgery. Mice were behaviorally tested over 14 days. Mice were tested for exploratory activity and measures of anxiety in the open field (day 1) and elevated zero maze (day 2). Mice were further habituated to the open field on days 2 and 3 and tested for novel object recognition on days 4 and 5. Mice were tested for sensorimotor function on the rotarod, over three trials per day, in the afternoon on days 2, 3, and 4. Mice were subsequently tested for spatial learning and memory in the water maze on days 8–12. Finally, mice were tested for contextual and cued fear conditioning on days 13 and 14. The tests were performed as described below in detail.

Open field—Mice were placed into a square arena. The total open field is 16 × 16 inches. The center square is 8 × 8 inches. Mice were allowed to explore for 10 minutes. Behavioral performance was tracked and scored using an automated video system (Ethovision 7.0 XT, Noldus, Sterling VA). Exploratory behavior was analyzed using total distance moved (cm) as outcome measure. Time spent in the more anxiety-provoking center of the open field was analyzed as well.

Elevated zero maze—Measures of anxiety were also assessed in the elevated zero maze. The enclosure (Kinder Scientific, Poway, CA) consisted of four sections (6 cm wide), alternating between open and closed sections. Mice were placed into an open area of the maze and allowed to explore for 10 minutes. An automated photobeam detection method (Kinder Motor-Monitor software, Kinder Scientific, Poway, CA) was used to track mouse movements: distance moved (cm), time spent in the open and closed areas as well as crossings between the open and closed areas.

Novel object recognition—Mice were habituated to the open field described above over three days, one ten-minute trial per day. On day 4, the mice were exposed to the arena containing two identical objects. On day 5, one of the objects (henceforth “familiar”) was replaced by a novel object. Performance of the mice was video recorded. Orientation to the object, within 2 cm proximity, as well as interaction with the object (climbing, sniffing, pushing) was defined as exploring the object. Novel object recognition and discrimination was calculated as the percent time spent exploring the novel object out of the total time spent exploring both objects. Distance moved was analyzed using automated multiple body point video tracking (Ethovision XT 7.0, Noldus Information Technology, Wageningen, the Netherlands), using parameters previously described and validated (Benice, et al., 2008).

Watermaze—Hippocampus-dependent spatial learning and memory was assessed in the water maze. The maze consisted of a circular pool (diameter 140 cm), filled with opaque water (24°C), divided conceptually into four quadrants. Mice were first trained to locate an “escape” platform (plexiglass circle, 6 cm radius) submerged 2 cm below the surface of the water and made visible by the use of a cue (a colored cylinder, 2.5 cm radius, 8 cm height) during the “visible” trials (days 1 and 2). For the visible platform training days, there were two daily sessions, morning and afternoon, which were separated by an intersession interval of 2 hours. Each session consisted of three trials, with 10-min inter-trial intervals. Mice were placed into the water facing the edge of the pool in one of nine randomized locations (consistent for each mouse). A trial ended when the mouse located the platform. Mice that failed to locate the platform within 60 s were led to the platform by placing a finger in front of their swim path. Mice were taken out of the pool after they remained on the platform for a minimum of 10 s. During the visible platform sessions, the location of the platform was moved between each of the four quadrants to avoid procedural biases in task learning. Subsequent to the visual trials, mice were trained to locate a hidden platform, requiring the mice to rely on extra maze cues for spatial reference and orientation. The platform was not rotated during the hidden platform trials and remained in the same location. One hour after the last trial on each day of hidden platform training, spatial memory retention of the mice was assessed in a “probe” trial (no platform) that lasted for 60 seconds. During the probe trials, mice were placed into the water in the quadrant opposite of the target quadrant. The time spent in the target quadrant compared to the time spent in the three non-target quadrants was analyzed. The swimming patterns of the mice were recorded with Noldus Ethovision video tracking software (Ethovision XT, Noldus Information Technology, Wageningen, Netherlands) set at six samples/s. The time to locate the platform (latency) was used as a measure of performance for the visible and hidden platform sessions. Latency to reach the target was measured in seconds, and was calculated for each day by averaging

values from the six daily trials. Because swim speeds can influence the time it takes to reach the platform, they were also analyzed.

Rotarod—Sensorimotor performance was assessed on a rotarod. Mice were placed on an elevated rotating rod (diameter: 3 cm, elevated: 45 cm, Rotamex-5, Columbus Instruments, Columbus, OH, USA), initially rotating at 5.0 rpm. The rod accelerated 1.0 rpm every 3 seconds. A line of photobeams beneath the rod recorded the latency to fall (seconds). Each mouse received three trials per day, with no delay between trials, on three consecutive days.

Fear Conditioning—In this task, mice learn to associate a conditioned stimulus (CS, e.g. the environmental context, or a discrete cue) with a mild foot shock (unconditioned stimulus, US). CS-US pairings are preceded by a short habituation period, during which a baseline measure of locomotor activity is analyzed. Contextual fear conditioning is considered to be hippocampus- and amygdala-dependent, while cued fear conditioning is considered to be hippocampus independent. Freezing, defined as immobility with the exception of respiration, is considered a post-exposure fear response, and is a widely used indicator of conditioned fear. Freezing depends on the threshold settings. In our study, we used a relatively stringent threshold setting and as a result the freezing levels calculated by the software might be lower than in studies using less stringent thresholds. Mice were trained and tested using a Med Associates mouse fear conditioning system containing VideoFreeze automated scoring system (Med Associates, St. Albans, Vermont), as previously described in detail and validated against traditional hand scoring methods (Anagnostaras et al., 2010). On day 1, the mice were placed inside a dark fear-conditioning chamber. Chamber lights (at 100 lux) turned on at zero seconds, followed by a 90-second habituation period and a subsequent 30-second (2800 hz, 80 dB) tone (cue). A 2-second 0.7 mA footshock was administered at 28 seconds, co-terminating with the tone at 30 seconds. After a 30-second inter-stimulus-interval the tone-shock pairing were repeated for a total of five tone-shock pairings. On day 2, hippocampus dependent associative learning was assessed during re-exposure to the training environment for 300 seconds. Three hours later, mice were exposed to a modified environment (scented with vanilla extract, cleaned with 10% isopropanol instead of 0.5% glacial acetic acid, novel floor texture covering the shock-grid, and rounded walls). They were allowed to habituate for 90 seconds, and then exposed to the cue for a second period of 180 seconds. Associative learning was measured as the percent time spent freezing in response to the contextual environment or the tone. Immediate acquisition of conditioned fear was measured following CS-US pairings. Motion during shock (proprietary index, Med Associates) was measured to assess potential differences in response to the shock during training.

MRI

Imaging was conducted following behavioral and cognitive testing, 4 months after VCI or sham surgery. Femoral vein cannulation was performed under isoflurane anesthesia prior to MRI studies using a 30-gauge needle attached to PE10 tubing filled with sterile heparinized saline. Catheters were temporarily placed under the skin, which was closed with surgical glue, and mice were allowed to awaken. For MRI studies, mice were then anesthetized with a ketamine/xylazine mixture (1.5 mg xylazine/10 mg ketamine/100g), and catheters were

accessed. MR imaging employed a Bruker-Biospin 11.75T small animal MR system with a Paravision 4.0 software platform, 9cm inner diameter gradient set (750 mT/m), and a mouse head (20 mm ID) quadrature RF transceiver coil (M2M Imaging Corp.) The mice were positioned with their heads immobilized with a specially designed head holder with adjustable ear pieces. Body temperature of the mice was monitored and maintained at 37°C using a warm air temperature control system (SA instruments). Isoflurane (0.5–2%) in 100% oxygen was administered and adjusted while monitoring respiration. A coronal 25-slice T_2 weighted image set was obtained (Paravision spin echo RARE, 256×256 matrix, 98 μm in-plane resolution, 0.5 mm slice width, TR 4000 msec, $TE_{effective}$ 32 msec, RARE factor 8, 1 average). Dynamic Susceptibility Contrast (DSC)-MRI was then implemented (300 T_2* weighted images, Paravision FLASH, TR 11ms, TE 5.0 ms, FA 6°, 128×128 matrix, 1 slice, 1 mm slice thickness, 195 μm in-plane resolution, 0.7 s/image, zero fill acceleration =2) employing Prohance (0.5 mmole/kg, 3× diluted). Injections occurred at 30s after initiation of the image series, at 1 ml/min, using a 150 μl saline/heparin chase. The DSC-MRI sequence was implemented to obtain axial CBF maps.

Image processing

Calculation of DSC-MRI perfusion parameters followed the model-independent method described in Ostergaard L. *et al*.[11] using the Jim software package (Xinapse Systems LTD, Northants, UK). The arterial input function (AIF) was determined from 12 normal brain pixels identified by an automatic scanning and selection routine, which targeted brain parenchymal arterial microvessels[12]. DSC data analysis was restricted to the first 50 post-contrast images in the series to bracket the susceptibility bolus intensity changes. Parametric maps were generated for cerebral blood flow (CBF in ml blood/100g tissue/ minute). Analysis of the DSC-MRI parametric maps and T_2 multi-slice images employed the Jim software package. The DSC-MRI parameter of rCBF was reported relative to the neck muscle to reduce measurement error, and minimize effects from alterations in blood pressure and depth of anesthesia. Ventricular and brain volumes were calculated from sixteen 0.5 mm brain image slice by tracing regions of interest (ROIs). For calculation of brain volume without ventricles included, ventricles were masked using Jim software.

Histology

Mice were sacrificed 7 months after UCCAO surgery, after having undergone behavior testing and MRI. The animals were perfused with 0.9% saline followed by 4% paraformaldehyde. After removal from the skull, the brains were fixed for 24 hours in 4% paraformaldehyde then dehydrated and cleared (using Prosoft and Propar, Anatech Ltd, Battle Creek, MI) for paraffin embedding. Serial six micron thick sections were cut through the entire extent of the hippocampus and amygdala. Sections at 0.33 mm intervals were stained with hematoxylin and eosin(H& E). The sections were dehydrated, cleared and coverslipped with Permount, and observed and imaged with a light microscope. For quantification of changes in hippocampal size, an observer blinded to treatment traced the hippocampus of the ischemic and non-ischemic hemisphere and measured the area of each region of interest using Image J. Hippocampal size was expressed as a ratio of right compared to left within a given brain section.

Statistical Analysis

Data are expressed as mean ± SEM. Groups were compared by t-test for two groups or ANOVA with Holm-Sidak's *post hoc* test for multiple measures using Prism (GraphPad Software, La Jolla, CA). Differences were considered significant at $p<0.05$. We expected a priori hemisphere differences as only the right carotid was occluded causing a unilateral injury; therefore data for the two hemispheres were analyzed separately. Novel object preference was first analyzed using two-way ANOVA comparing the novel and familiar object and genotype, followed by t-tests to compare against a hypothetical value (no preference, 50%). Water maze and fear conditioning learning curves and rotarod performance were analyzed using repeated measures two-way ANOVA with Bonferroni post hoc test. Probe trial analyses utilized one-way ANOVAs with a Dunnet's post-hoc test to compare non-target quadrants against the target quadrant.

RESULTS

VCI surgery does not cause behavioral alternations in locomotor activity or anxiety-related behavior

Young (3 months old) male mice were subjected to right UCCAO, a mouse model of VCI, or sham surgery. Three months after surgery, mice underwent behavior testing. Deficits in locomotor activity or increased anxiety can confound results of cognitive behavior tests, such as novel object recognition test (NORT) or Morris water maze (MWM). Therefore, prior to cognitive testing, mice locomotor activity and anxiety-related behavior were assessed. General locomotor activity was assessed via open field test, rotarod, and zero maze. All three tests showed no significant differences in activity between sham and VCI mice (Figure 1). Both sham and VCI mice remained longer on rotarod before falling on day 3 compared to day 1. Anxiety-related behavior was also assessed using open field test and zero maze to determine the amount of time spent in the center of the field or open arms, respectively. Increased time spent in the center field or open arms is indicative of decreased anxiety-related behavior. No differences in time spent in the center field (Figure 1B) or open arms (Figure 1E) were detected between groups.

VCI mice show impairments in non-spatial memory

Cognitive function was assessed using both non-spatial and spatial memory tests. First, the NORT was used to assess non-spatial memory in sham and VCI mice. The sham mice showed no impairments in object recognition, as evidenced by their clear preference for a novel compared to familiar object ($p<0.05$). In contrast, VCI mice showed no preference for the novel object, indicating a memory deficit (Figure 2A). Next, spatial memory was examined using the MWM. During the visible and hidden learning sessions, mice from both groups improved their ability to locate the platform over time, as measured by latency to reach the target ($F(4,72)$ 13.65, $p<0.0001$, data not shown). However, there were no differences between groups in these measures. Over three probe trials, both groups showed similar strong trends towards improved performance over the probe trials as percent time spent in the target quadrant ($p=0.07$, Figure 2B) or measured by cumulative distance from the target ($p=0.05$, Figure 2C). Finally, mice were subjected to both cued and contextual fear conditioning tasks. No differences in motion were detected between groups at baseline.

Mice from both groups showed an increase in percent time spent freezing during training for the fear conditioning task ($F(3,54)$ 15.48, $p<.0001$, data not shown). No differences were detected between groups on either task (Figure 2D).

UCCAO Causes a Long-Term Decrease in Cerebral Blood Flow

Short-term (30 days or less) alterations in cerebral blood flow (CBF) have previously been reported following UCCAO, with some reports showing prolonged hypoperfusion[4] and others showing an initial hypoperfusion followed by a restoration of flow over two weeks[8]. To clarify the long-term changes in global CBF, CBF was measured in sham and VCI mice 4 months after surgery using dynamic susceptibility contrast-MRI perfusion. Whole brain CBF, relative to the neck muscle, was not altered by VCI surgery (Figure 3A–B). However, CBF in the right cerebral cortex was significantly reduced in VCI, but not sham mice, compared to the left cortex after occlusion of the right common carotid artery ($p<0.05$, Figure 3C).

VCI Mice Display Hippocampal Atrophy and Enlargement of the Lateral Ventricles

Clinically, brain atrophy and a corresponding expansion of lateral ventricles is associated with vascular dementia [13], with hippocampal atrophy in particular being associated with cognitive decline [14]. To determine if similar pathology occurs in the UCCAO model of VCI, ventricular volumes were measured in sham and VCI mice 4 months after surgery using T_2 weighted MRI images. VCI mice exhibited a significant increase in ventricular volume compared to sham mice ($p<0.05$, Figure 4B), this difference between groups was more pronounced in the right/ischemic hemisphere ($p<0.05$, Figure 4C). Histological analysis revealed that the cause of the right lateral ventricular enlargement was likely due to hippocampal atrophy in the right hemisphere of VCI mice compared to sham mice ($p<0.05$, Figure 5). Additionally, out of 8 mice analyzed histologically, we observed evidence of focal infarction in only one of the VCI mice, which was surrounded by reactive astrocytes and activated microglia (data not shown).

DISCUSSION

The goal of the current study was to elucidate the pathological and neuroimaging substrates of long-term cognitive decline in VCI using a mouse model of chronic hypoperfusion induced by UCCAO. First, we confirmed that the mice that received the VCI surgery indeed had chronic brain perfusion using DSC-MRI. We also characterized long-term (4 months) changes in T_2 weighted structural MRI, and confirmed neuroimaging findings using histology. Finally, we showed long-term impairments in non-spatial memory. Results show that VCI mice with chronic hypoperfusion have long-term pathology in the ischemic hemisphere resulting in hippocampal atrophy, ventricular enlargement and long-term deficits in cognitive function.

Our current study shows that the UCCAO model of chronic cerebral hypoperfusion causes impairments in non-spatial memory. This is in agreement with other studies in which chronic cerebral hypoperfusion induced by UCCAO mice, bilateral common carotid artery stenosis in mice, or triple vessel occlusion in rats has been associated with cognitive deficits

[6, 4, 7]. Specifically, we detected deficits in non-spatial memory using the novel object recognition test, which have been previously reported following UCCAO in mice [4, 6]. We did not observe a deficit in spatial memory performance in the MWM. Spatial memory deficits have previously been reported in the bilateral carotid stenosis mouse model of VCI [15] and, a mild (not statistically significant) spatial memory impairment has been shown 1 month after UCCAO in mice [6]. We did not observe significant differences in cognitive function in the cued or contextual fear conditioning tests. Importantly, when behavior test were conducted 3 months after UCCAO there were no differences in activity or anxiety between groups. This is important since these differences can be confounding variables in the behavior tests used and have been reported one month post-UCCAO [6], although others have also found no deficits at the same time point [4]. Nevertheless, the long-term cognitive effects in our study are not confounded by these variables, as we observed no differences in locomotor activity or anxiety-related behavior.

We characterized the changes in CBF via DSC-MRI following VCI or sham surgery. While global changes in CBF monitored by MRI had been assessed following bilateral carotid artery occlusion in rats [10], and more recently bilateral carotid artery stenosis in mice [9], effects of UCCAO on whole brain CBF in mice had not previously been reported. Our data show that CBF is impaired in the ischemic hemisphere for up to 4 months after VCI surgery. UCCAO is known to cause large decreases (50–70%) in CBF in the ischemic hemisphere acutely [8, 4]; however, there has been conflicting evidence in the literature over whether UCCAO leads to permanent reductions in CBF [8, 4]. Prior studies have used non-quantitative measures such as laser Doppler flowmetry [4] or methods that examine CBF in only a small region of the brain, such as confocal imaging through a cranial window [8]. Using laser Doppler flowmetry, it was shown that although CBF improves over time, it remains decreased by 20–37%, compared to baseline, 28 days after surgery [4, 16]. Using confocal imaging it was shown that CBF acutely decreases and then increases back to baseline due to capillary remodeling, pial arterial dilation, and collateralization [8]. The final time points analyzed in the above studies were 14–18 days. Longer time points following surgery had not previously been assessed. We show for the first time, using ultra-high field MR imaging of the entire brain, that UCCAO in mice causes long-term (4 months) decreases in CBF in the cortex of the ischemic hemisphere. However, our findings also show that total brain CBF is not significantly changed in the whole brain. Since prior studies have shown an increase in capillary remodeling, pial arterial dilation, and collateralization [8] following UCCAO, it is likely that these mechanisms contributed to the partial recovery of flow in the ischemic hemisphere. Therefore, the current data contribute to the current understanding of the UCCAO model of VCI by demonstrating a clear unilateral deficit in CBF that persists 4 months after surgery.

Finally, we demonstrated that the UCCAO model of cerebral hypoperfusion causes an expansion of the ventricles, particularly the lateral ventricle of the ischemic hemisphere. This effect is likely due to the observed hippocampal atrophy in the ischemic hemisphere. Studies by others did not observe hippocampal atrophy 1 – 2.5 months after UCCAO in mice, although they did observe cellular markers of neuronal degeneration and histological evidence of ischemic damage [6, 16]. Because ventricular enlargement and hippocampal atrophy are known clinically to be associated with aging and chronic cerebral perfusion, we

opted to delay the time between imaging/behavioral testing and terminal histology. We reasoned that while neuronal injury may be sufficient to account for the neurocognitive deficit observed at 3 months, delaying histology until mice reach the age of 10 months (7 months after UCCAO) would allow us to observe hippocampal atrophy. This observation suggests that brain damage in chronically hypoperfused brain is a not a static insult, and that aging will exacerbate brain damage and cognitive impairment. In addition, the current study provides first evidence of ventricular enlargement. Clinically, ventricular enlargement and brain atrophy increase with age and are associated with decreases in cerebral perfusion [13]. Those with vascular dementia show the greatest perfusion deficits, atrophy and ventricular enlargement [13]. Thus, our study has identified new aspects of VCI that are modeled by UCCAO. In addition to these findings, we observed an infarct in one out of the eight VCI mice we histologically examined. A similar rare incidence of infarcts has previously been reported in the UCCAO model [16]. Therefore, while overt infarction is possible in this model, it is an infrequent occurrence.

In summary, we have characterized global changes in brain structure and CBF in response to UCCAO using MRI. Our results show that UCCAO in mice causes long-term deficits in cognitive function, CBF, and leads to ventricular enlargement and hippocampal atrophy. These pathologies are also observed clinically. Therefore, the current data demonstrate that UCCAO is an informative mouse model for many clinical aspects of VCI and that this model elicits pathology that can be monitored via MRI. Use of MRI to monitor disease pathology and progression in the UCCAO mouse model of VCI may increase the translational potential of findings.

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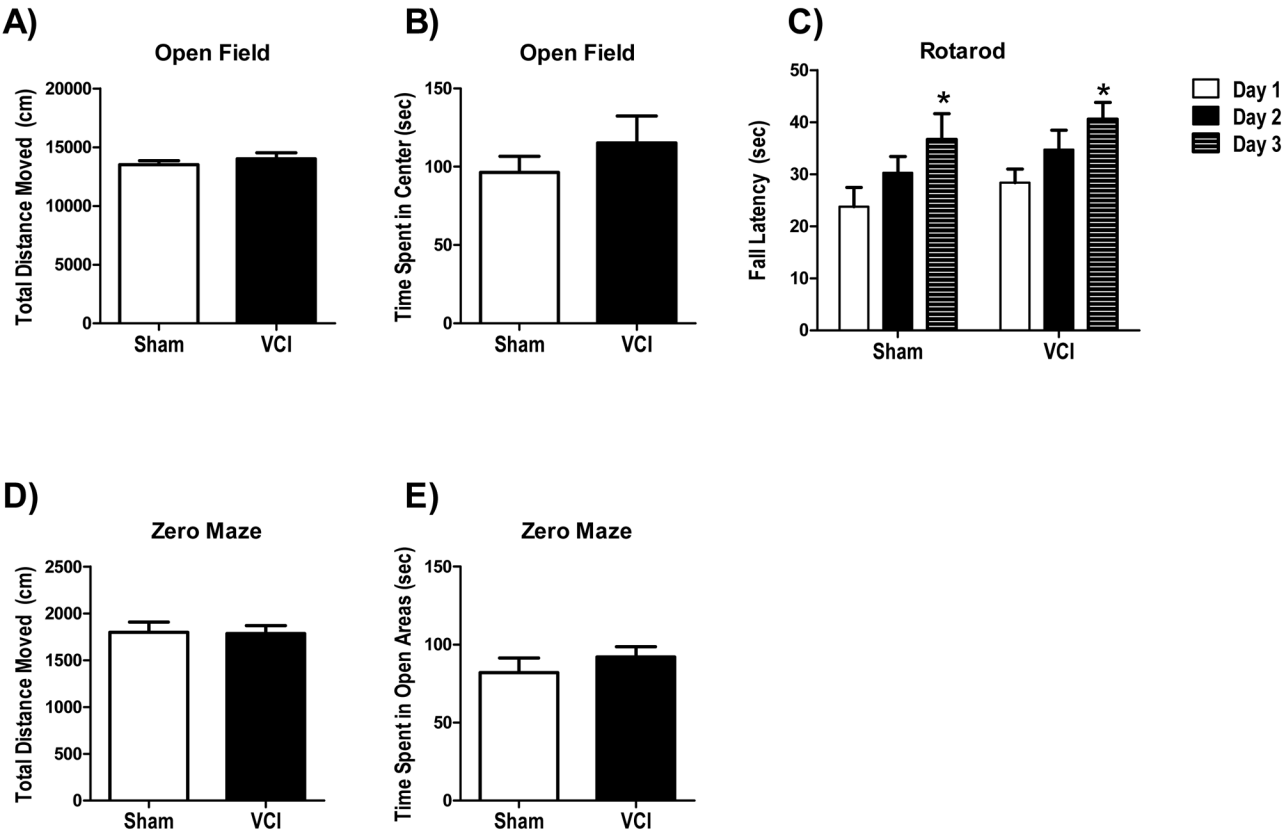

Figure 1. VCI surgery does not cause behavioral alternations in locomotor activity or anxiety-related behavior

Young (3 month old) male mice were subjected to right UCCAO (VCI) or sham surgery. Three months after surgery, locomotor activity and anxiety-related behavior were assessed in the open field, rotarod, and zero maze. Open field test showed no differences in distance traveled (A) or time spent in the center of the field (B) between groups. C) Both sham and VCI mice improved their performance in the rotarod test over three trials ($F(2,36)$ 11.07, $p=0.002$). No differences were detected between groups. Zero maze showed no difference in total distanced moved (D) or time spent in the open arms (E) between groups. n = 10 per group. *$p<0.001$ vs. trial 1 of same group.

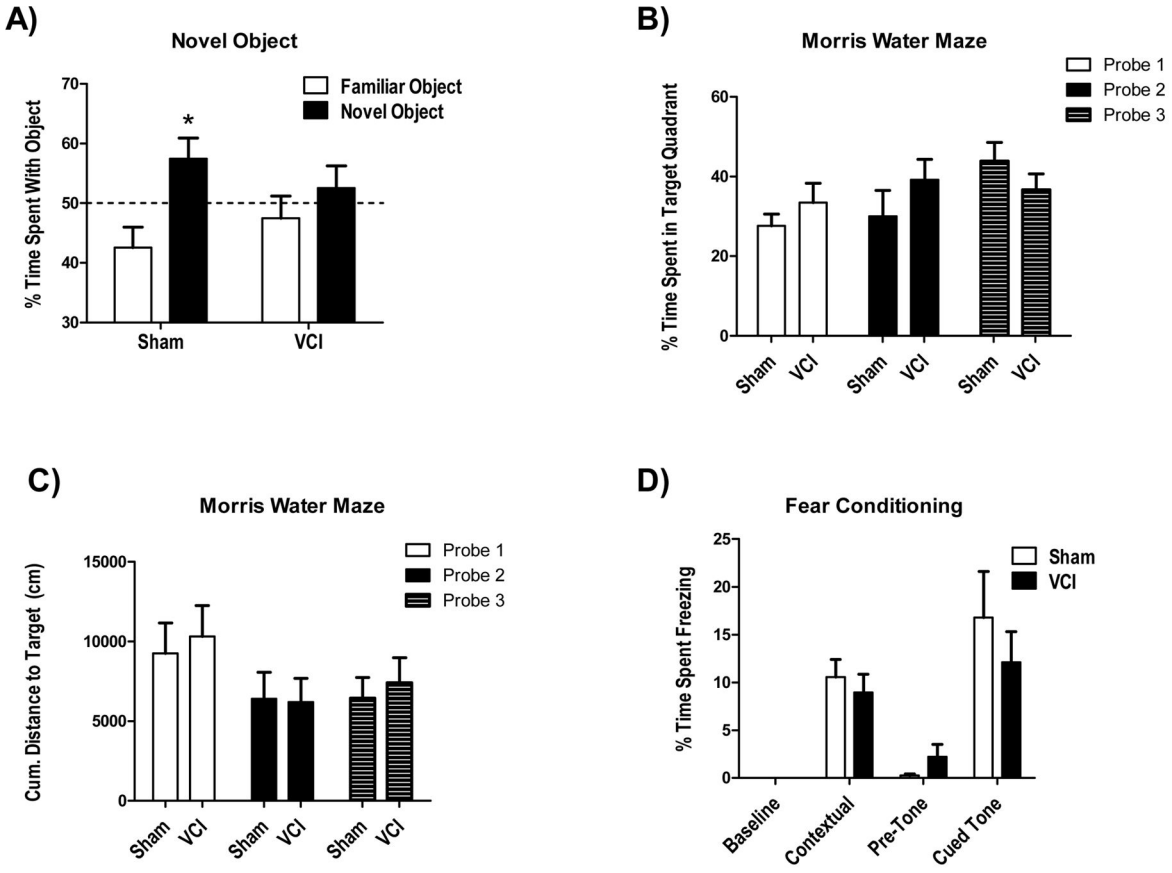

Figure 2. VCI mice show impairments in non-spatial memory

Young (3 month old) male mice were subjected to right UCCAO (VCI) or sham surgery. Three months after surgery, cognitive function was assessed in the novel object recognition test (NORT), Morris water maze (MWM), and cued and contextual fear conditioning tasks. A) Sham mice showed a clear preference for the novel object in the NORT, while VCI mice did not. *p<0.05 vs. familiar object. B) During the probe trials of the MWM, both sham and VCI mice showed a strong trend ($F(2,36)$ 2.76, $p = 0.07$) toward increased time spent in the target quadrant. C) During the probe trails of the MWM, both sham and VCI mice shown a strong trend ($F(2,36)$ 3.15, $p=0.05$) toward decreased cumulative distance from the target over the trials. DNo differences between groups were observed in either the cued or contextual fear conditioning tasks. n = 10 per group.

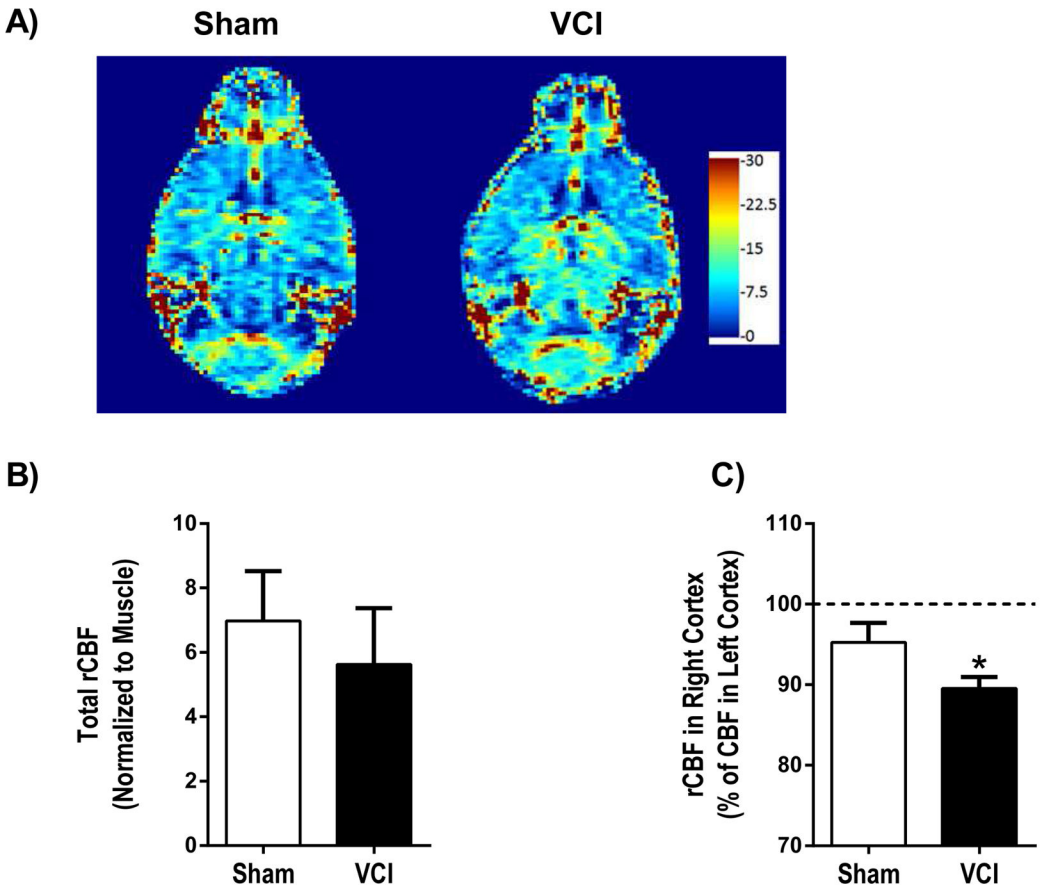

Figure 3. ULCAAO Causes a Long-Term Decrease in Cerebral Blood Flow

Cerebral blood flow (CBF) was measured in sham and VCI mice 4 months after surgery using dynamic susceptibility contrast-MRI perfusion, with bolus injection of Prohance. A) Representative axial views of CBF maps in sham and VCI mice. B) Whole brain CBF, relative to the neck muscle, was similar between groups. C) CBF in the right cortex was significantly reduced compared to CBF in the left cortex in VCI mice compared to sham mice ($p<0.05$). n = 3 per group.

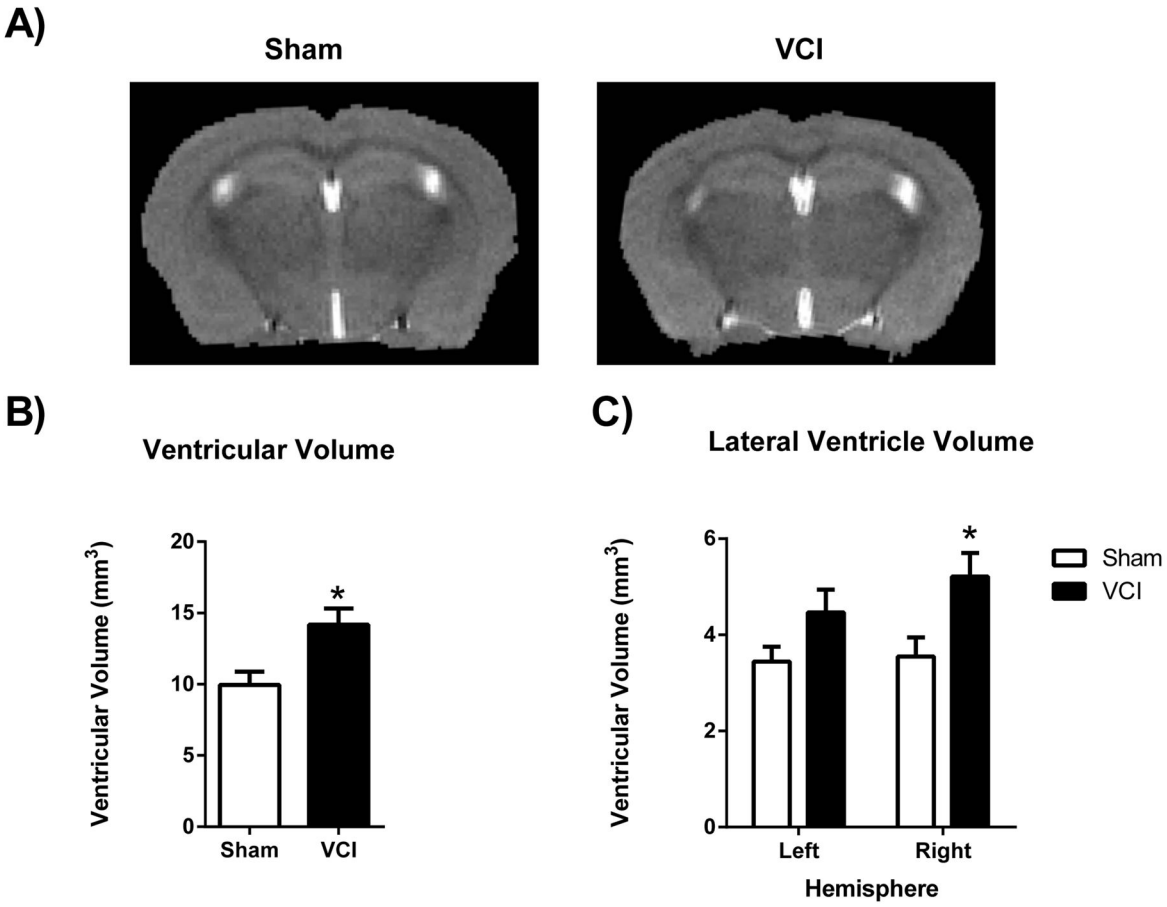

Figure 4. VCI Mice Display Ventricular Enlargement

Ventricular and brain volumes were measured in sham and VCI mice 4 months after surgery using T_2 weighted MRI images. A) Representative T_2 weighted MRI images of a coronal section of brain in a sham and a VCI mouse. B) Ventricular volume was increased in VCI mice compared to sham mice ($p<0.05$). C) Volume analysis of lateral ventricles showed increased ventricular volume specifically in the right/ischemic hemisphere of VCI mice compared to sham mice ($p<0.05$) n = 4–5 per group.

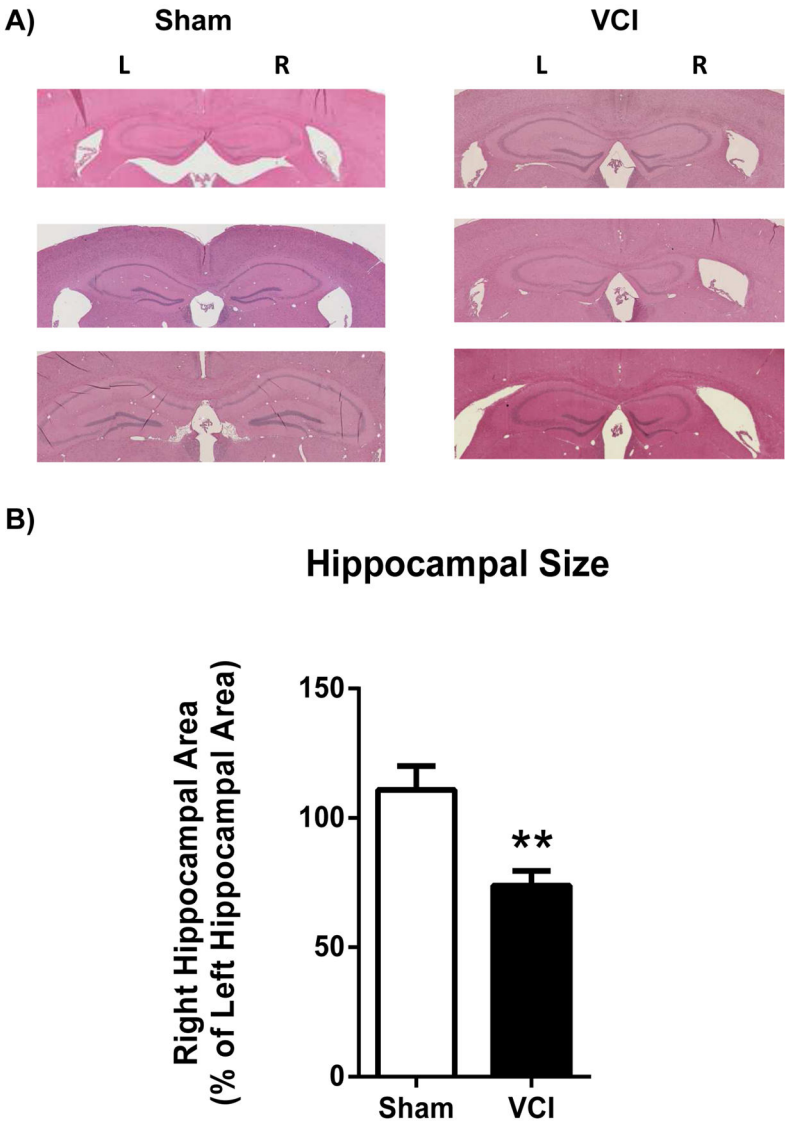

Figure 5. VCI Mice Display Hippocampal Atrophy

Brains of sham and VCI mice were stained with H & E and hippocampal sizes were measured. A) Representative images of brain sections stained with H & E in sham and VCI mice. B) Hippocampal size was decreased in the right hemisphere of VCI mice compared to sham mice ($p<0.01$). n = 6–8 per group.