Research report

Long-term assessment of motor and cognitive behaviours in the intraluminal perforation model of subarachnoid hemorrhage in rats

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ABSTRACT

The endovascular perforation model of subarachnoid hemorrhage (SAH) is a commonly used model in rats as it is performed without a craniotomy and accurately mimics the physiological effects of SAH in humans. The long-term behavioural profile of the model, however, has not been characterized. Given that humans often have cognitive deficits following SAH, we set out to characterize the behavioural profile as well as the spontaneous temperature changes of rats following intraluminal perforation. Rats were pre-trained on three motor tasks (tapered beam, limb-use asymmetry and the horizontal ladder tasks) prior to receiving a SAH. The animals were then assessed on post-surgical days 3, 7, 14 and 21 on these tasks. At the completion of motor testing, the rats were assessed on a moving platform version of the Morris water task. Despite significant mortality (33%), SAH did not result in lasting motor deficits on any of the tasks examined. However, the SAH group did show a minor cognitive impairment in the Morris water task. In addition, SAH produced a slight, but significant elevation in body temperature (vs. sham operated rats) despite an acute decrease in general home cage activity. The majority of the animals did not have any observable infarcts and the SAH did not significantly affect cortical thickness. In summary, the endovascular perforation model of SAH results in no lasting motor deficits and only minor cognitive impairment in survivors, which alone would be difficult to evaluate in neuroprotection or rehabilitation studies.

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1. Introduction

Subarachnoid hemorrhage (SAH) is a particularly devastating form of stroke that is caused by a blood vessel bursting on the surface of the brain causing blood to rapidly enter the subarachnoid space. Approximately half of all patients die from the ensuing injury [17], and many of those that do survive have lasting cognitive deficits [2]. There are several rodent models of SAH, such as injecting blood into a cavernous cistern, or the puncture of a vessel on the surface of the brain [1,3,27]. The blood injection models allow the experimenter to control the size and severity of the injury by varying the volume of blood injected. These models, however, somewhat lack clinical relevance as they require a craniotomy, and because the surface vessels are left intact. These are important distinctions as a craniotomy can relieve the increase in intracranial pressure (ICP) that follows SAH. Furthermore, a puncture in the wall of a vessel may induce hypoperfusion in the brain region irrigated by that vessel, an effect lacking in injection models.

SAH pathophysiology may be more accurately replicated in rats by perforating a blood vessel on the ventral surface of the brain without performing a craniotomy. The most commonly used perforation model of SAH is the endovascular filament model [3], where a nylon suture is advanced into the cranium through the carotid artery, and a vessel along the circle of Willis is punctured. This procedure allows one to create an SAH without the need for a craniotomy. Although there is significant variability in the size and location of the bleed [36], acute physiological measurements confirmed that the model is clinically relevant as it is characterized by a sharp increase in ICP as well as reduced cerebral perfusion due to cerebral vasospasm [34]. More delayed pathological processes also occur. For example, microvascular platelet aggregation peaks at 24 h post-SAH [35], and cerebral edema remains elevated for at least 72 h [7]. Thus, one would expect these physiological abnormalities to result in abnormal brain functioning and neurological impairment. Several studies have now examined behavioural outcome following both injection [14,19,37] and perforation models of SAH [18]; however, a comprehensive, long-term assessment of rats following SAH has not been completed.

Based on the recommendations of the STAIR report [29] long-term functional assessments should be used to gauge the efficacy of potential stroke treatments. Therefore it is essential that each model be evaluated in terms of the range and degree of functional...
deficits. To this end we chose to examine both the motor and cognitive behaviour of rats for several weeks following the intraluminal perforation model of SAH. Motor function was assessed on the tapered beam, limb-use asymmetry and the horizontal ladder tasks, while cognitive function was tested in the Morris water task (MWT).

In addition, we confirm the presence of early cell death through Fluoro Jade labeling, and also describe the acute changes in temperature and activity following SAH. Given that even slight changes in temperature can influence cell survival following stroke [6], the temperature profile of a given model may provide insight into the mechanisms of cell death, and the potential to influence the severity of an injury by modulating temperature [21]. Thus, post-SAH body temperature was measured via telemetry probes.

2. Materials and methods

2.1. Subjects

Sixty-one male Sprague–Dawley rats (Biosciences breeding colony, University of Alberta; weighing ~300 g) were used in this study. The animals were group housed in cages of 4 (except for rats with telemetry probe implants) and had water and food available ad lib. All procedures were in accordance with the Biosciences Animal Care and Use Committee at the University of Alberta and aseptic techniques were used during surgery. Additional step-by-step details of the methods used may be obtained from http://www.psych.ualberta.ca/~fcolbour/sops.htm.

2.2. Experiment 1: short-term outcome

2.2.1. Activity and temperature measurement

Rats \( (n = 21) \) were anesthetized with isoflurane (2% in 60% \( \mathrm{N}_2 \mathrm{O} \) and 40% \( \mathrm{O}_2 \)) and a telemetry probe measuring home cage activity as well as core temperature was implanted in the abdominal cavity [10]. Briefly, a 2–3 cm incision was made through the skin and the muscle wall of the abdomen. The sterilized telemetry probe (model TA10TA-F40, Transoma Medical, St. Paul, MN) was then inserted into the abdominal cavity and the incision was sutured shut and infiltrated with Marcaine (Sanofi Canada, Markham, Ont., Canada). Following recovery from the surgery rats were singly housed in individual cages and temperature was monitored for 4 days (with the last day serving as baseline) prior to inducing either an SAH \( (n = 14) \) or performing a sham surgery \( (n = 7) \); see below for methods). Temperature was monitored for 3 days post-surgery, at which point the rats were euthanized to examine the brain.

2.2.2. Fluoro Jade and Nissl staining

Fluoro Jade staining [32] was used to investigate the pattern of early cell death following SAH. An additional series of sections was used for Nissl staining in both the SAH and the sham groups. Brains were sectioned on a cryostat (40 μm), mounted on gelatin-subbed slides and allowed to dry overnight. For Fluoro Jade staining the slides were re-hydrated in ethanol, incubated in \( \mathrm{K MnO}_4 \) for 15 min followed by a 30-min incubation in a 0.001% solution of Fluoro Jade B (Chemicon, Temecula, CA, USA). The sections were examined on an epifluorescence microscope using an FITC filter.

2.3. Experiment 2: behavioural outcome following SAH

2.3.1. Behavioural training and testing

Rats were trained on the tapered beam, limb-use asymmetry and the horizontal ladder tasks prior to receiving a SAH \( (n = 32) \) or a sham surgery \( (n = 8) \). The animals were allowed to recover from the surgery and were subsequently assessed on the above motor tasks on post-surgical days 3, 7, 14 and 21. At the completion of motor testing, the rats were assessed on a cognitive test, the MWT, for 2 weeks before being euthanized at approximately post-lesion day 40.

2.3.2. Tapered beam

Rats were trained to traverse a 1.6 m long tapered beam that was 6 cm wide at the start and tapered evenly to a final width of 1.5 cm [41]. A piece of wood that was 2 cm wider than the beam (on both sides) was attached to the under surface of the beam, thus serving as a ledge that the rats could choose to step down onto if they were unable to balance on the top surface of the beam. Normal rats are able to traverse most of the length of the beam without having to step down onto the ledge, whereas the apparatus encourages injured rats to display their deficits by stepping down onto the ledge on the side contralateral to the injury. Rats were trained for 5 days (3–5 trials per day) at which point baseline performance was video recorded. A mirror placed behind the beam allowed for both sides of the rat to be filmed simultaneously with a single camera. The number of impaired steps with the contralateral hindpaw, defined as the limb making contact with the supporting ledge, was quantified for each testing session.

2.3.3. Horizontal rung walk

A 1 m long horizontal ladder made from 2 clear Plexiglass walls and irregularly spaced metal rungs (2 mm in diameter) was elevated over a countertop with a neutral start cage at one end and the rat’s home cage at the other end [24]. The rats were given 3–5 training trials for 3 days, before filming baseline performance. Post-operatively, the number of slips from all limbs was measured, which were when the paw slipped between rungs. While the test apparatus did not change, the rats were placed in slightly different start locations to reduce the likelihood that rats would learn the exact stepping pattern. This protocol has been found to be sensitive to multiple forms of sensorimotor injury in our lab [8,22].

2.3.4. Limb use asymmetry task

Rats were placed individually inside a transparent cylinder (20 cm in diameter and 30 cm in height) that was set on a transparent surface. A mirror placed at an angle below the cylinder allowed for the video recording of each animal’s vertical exploration from a ventral view. When placed inside a cylinder rats spontaneously rear and explore the walls of the cylinder through tactile paw placements. A paw preference ratio was determined for each testing session by counting the number of weight-bearing touches that were performed with the affected, the unaffected, or both paws simultaneously. As has been previously reported [31], Sprague–Dawley rats are not as active in the cylinder as other rat strains; therefore, to increase accuracy of this test we excluded those rats that made fewer than 30 wall touches in a testing session. An asymmetry score was calculated using the formula: \( \% \) asymmetry = \( \frac{100 \times ( \text{contralateral touches} + 1/2 \text{bilateral touches})}{(\text{total touches})} \). This scoring system has been shown to enhance sensitivity and reduce variability [41].

Fig. 1. Photographs depicting brains after SAH. The size of the bleed varied between rats. Animals that died during or shortly after surgery had large hemorrhages (A) as well as herniation of the cerebellum in all cases (C). More moderate-sized hemorrhages (B) were characterized by a pool of coagulated blood at the base of the brain, around the circle of Willis.
2.3.5. Morris water task

Rats were tested on a modified version of the MWT in which the platform was moved to a novel location within the pool on every second testing day[11]. For the first two days the platform remained in the same position, but then it was moved to a new location on days 3 and 4. On day 5 the platform was moved again, and this cycle was continued for 14 days of testing. The swimming pool (1.5 m in diameter, 60 cm deep) was painted black on the inside and was filled with water (21–22 °C) to 20 cm below the top of the pool wall. A clear, Plexiglass platform was submerged 1.5 cm deep (2% in 60% N2O and 40% O2) and kept at 37 °C via a rectal thermometer probe and a heated water blanket placed underneath the animal. This probe’s accuracy, as with the telemetry probes, was established to a laboratory standard—a calibration-grade glass thermometer. A 2–3 cm midline skin incision was made at the neck to allow access to the left common carotid artery (CCA). The internal and external carotid arteries (ICA and ECA) were further isolated in the anterior direction, along with all of their extra-cranial branches. The ECA was electrocautereagulated and bisected distally from the CCA bifurcation, leaving a 3 mm stump on the CCA. The CCA was temporarily occluded using a micro-arterial clip. A nylon monofilament (3 cm long), with one end cut at a bevel, was inserted into the ECA stump and advanced through the ICA until a slight resistance was felt (18–20 mm from CCA bifurcation). The suture was then further advanced another 4 mm to puncture the vessel wall and then immediately withdrawn, leaving a single perforation in the vessel wall. The ECA stump was sealed through electrocautery and the CCA clip was removed. The duration of CCA occlusion ranged from 3 to 5 min. The ICA was checked for re-perfusion and the skin incision was sutured shut and infiltrated with Marcaine. Sham animals underwent the same procedures except that the suture was not advanced sufficiently to occlude the vessel or to perforate it. Following termination of anesthesia, animals recovered in a clean cage and were monitored closely over the next 24 h.

2.5. Histological analyses

Following the completion of behavioural testing, rats were given an overdose of sodium pentobarbital and perfused transcardially with saline followed by 10% formalin. Brains were cryoprotected in sucrose and sectioned at 40 μm on a cryostat. Digital images captured of the slides were then used for morphometric analyses of cortical thickness using the ImageJ computer program (http://rsb.info.nih.gov/ij). Cortical thickness was measured at five different cortical planes, by measuring the distance from the dorsal edge of the corpus callosum to the outermost cell layer of the cortex (layer II) [9].

3. Results

Acutely following the SAH surgery most rats were lethargic, and were in-coordinated when moving about. A small number of rats (about 20%) had visible signs of seizures including whole body writhing movements or stereotyped limb movements. The seizures did not continue beyond 2–3 h after SAH in those that survived until the scheduled euthanasia time. The mortality rate was 15 out of 46 rats (33%), with all but 2 of these rats dying within the first 12 h after the initial SAH bleed. Animals that died prematurely had visibly larger hemorrhages than those killed at the 72 h period (Fig. 1A and B). In addition, all rats that died prematurely had noticeable herniation of the cerebellum (Fig. 1C), presumably compressing brainstem breathing centers.

3.1. Short-term telemetry measurements

3.1.1. Temperature

Following SAH rats showed a transient increase in body temperature, lasting approximately 6 h, while sham rats showed a slight decrease in temperature that only lasted for 2 h. The temperature profile of the two groups was otherwise similar for the remainder of the experiment (Fig. 2A). For statistical analyses temperature was averaged over 6 h blocks for the 4 days when measurements were taken. A repeated measures ANOVA indicated a non-significant effect of Group \((F(1,10)=0.524, P=0.486)\), however the effect of Time \((F(15,150)=6.333, P<0.001)\), as well as the Time × Group interaction \((F(15,150)=3.786, P<0.001)\) were significant. A follow-up analysis (one-way ANOVA) showed that the only significant time point between the two groups was the first 6 h block after surgery \((F(1,11)=7.824, P<0.019)\).
and limb-use asymmetry (C). The SAH group did not have behavioural impairments

3.1.2. Activity

During the baseline measurements the activity of the rats followed a normal circadian cycle characterized by a spike in activity at the start of the dark cycle. Following surgery, both the SAH and sham groups showed a disruption in the circadian cycle for the first post-operative day only (Fig. 2B). A repeated measures ANOVA of mean activity during the 6 h blocks showed that there was a significant effect of Time \((F(15,150) = 8.122, P < 0.001)\), however the Group effect \((F(1,110) = 0.189, P = 0.673)\), and the Time × Group interaction were not significant \((F(15,150) = 1.228, P = 0.257)\).

3.2. Long-term behavioural outcome following SAH

3.2.1. Beam walking

During baseline training rats were able to traverse the beam without any difficulty, mostly making errors at the very narrow end of the beam. During post-surgical testing there were no significant differences between the groups on any of the testing days (Fig. 3A). An ANOVA of baseline performance indicated no Group differences \((F(1,29) = 0.027, P = 0.872)\), and a repeated measures ANOVA of the post-lesion testing days indicated non-significant effects of Group \((F(1,28) = 2.179, P = 0.151)\), Testing Session \((F(3,84) = 1.151, P = 0.333)\), or the Group × Testing Session interaction \((F(3,84) = 0.752, P = 0.524)\). We also analyzed the total number of steps required to cross the beam and a one-way ANOVA showed that there were no significant Group differences \((F(1,30) = 0.007, P = 0.935)\).

3.2.2. Horizontal rung walk

Rats successfully crossed the apparatus during baseline training, and after SAH there were no significant deficits at any of the testing sessions (Fig. 3B). An ANOVA on baseline performance showed that the two groups did not differ significantly \((F(1,29) = 0.05, P = 0.946)\) before the SAH. A repeated measures ANOVA on the number of post-lesion slips from all limbs indicated a non-significant effect of Group \((F(1,29) = 1.872, P = 0.182)\), however the effect of Testing Session \((F(1,29) = 4.174, P = 0.008)\) and the Testing Session × Group interaction \((F(1,29) = 0.020, P = 0.889)\) were both significant. Follow-up investigation of the individual testing days showed that although there was a trend for impairment in the SAH group on post-lesion days 3 and 21, neither of these comparisons reached significance \((P = 0.080\) and \(P = 0.052\) respectively).

3.2.3. Forelimb asymmetry task

Forelimb asymmetry, as assessed in the cylinder task, was not affected by the SAH surgery (Fig. 3C). Rats in both the sham and SAH groups made an equal number of wall touches with the paw contralateral to the hemisphere where the SAH was induced. At each testing session, 1–8 rats had to be excluded due to insufficient rearing (appeared to be randomly distributed between groups and among test sessions); however if these rats performed a sufficient number of rears in a subsequent testing session those data were included in our analyses. Given the missing values at some of the time points we were unable to perform a repeated measures ANOVA; however, an ANOVA on each of the testing days indicated no significant difference between groups \((F(1,26) = 0.195, P = 0.662)\). Furthermore, an ANOVA of the total post-lesion limb use (all data points considered) also indicated a non-significant Group difference \((F(1,29) = 0.113, P = 0.740)\).

3.2.4. Morris water task

Rats were assessed on a moving-platform version of the Morris water task where the platform was moved to a novel location for every second day of testing. Both the sham and the SAH groups learned within each testing day (Fig. 4A and B), but the SAH group was significantly impaired in this task on days when the platform was moved to a novel location (Fig. 4C and D). When the mean swim distance from all of the testing days was separated into the four daily trials, a repeated measures ANOVA indicated a significant effect of Trial \((F(3,87) = 75.654, P < 0.001)\), but not Group \((F(1,29) = 2.756, P = 0.108)\). The Group × Trial interaction was also non-significant \((F(3,87) = 0.757, P = 0.521)\). An ANOVA of average swim distance on days when the platform was moved to a novel location indicated that the SAH group took a significantly longer path to find the platform relative to the sham Group \((F(1,29) = 6.666, P = 0.015)\). An analysis of the mean latency to find the hidden platform showed a similar trend it decreased over trials \((F(3,84) = 62.14, P < 0.001)\), but neither the effect of Group \((F(1,28) = 1.247, P = 0.274)\), nor the Group × Trial interaction were significant \((F(3,84) = 0.426, P = 0.735)\). The SAH group however, took significantly longer to find the platform on days when it was moved to a novel location \((F(1,29) = 4.863, P = 0.036)\). A repeated measures ANOVA of swim speed showed that although there was a significant change in swim speed over the testing days \((F(13,377) = 4.591, P < 0.001)\), neither...
the effect of Group ($F(1,29) = 0.484, P = 0.492$), nor the Group × Day interaction were significant ($F(13,377) = 0.946, P = 0.505$).

3.3. Histological analyses

3.3.1. Fluoro Jade staining at 3 day survival

Fluoro Jade positive cells were present in 4 of 5 rats examined following SAH. The majority of labeled cells were present in the hemisphere in which the SAH was induced; however, animals with moderate sized hemorrhages also had some labeling in the contralateral hemisphere (Fig. 5A). Notably, orbital as well as the cingulate (Fig. 5C) prefrontal cortical regions contained a significant number of Fluoro Jade positive cells. In more posterior sections (Fig. 5B), labeled cells could be found in the striatum (Fig. 5D) as well as anterior hypothalamic nuclei (Fig. 5E).

3.3.2. Cresyl violet staining at long-term survival

The majority of the brains did not have any frank lesions 6 weeks after the insult (2 brains had small cavities on the ventral surface, at the site of the SAH). Fifteen of the 23 SAH brains had obvious discoloration on either the dorsal or ventral surface, likely caused by degraded blood products following the SAH. A careful examination of cresyl violet stained sections showed that there were no obvious areas of focal infarcts or any other observable pathology. An ANOVA showed that there was no significant difference in cortical thickness between the hemispheres ($F(1,29) = 1.053, P = 0.313$) or between groups ($F(1,29) = 0.306, P = 0.584$; Fig. 6).

4. Discussion

The main finding of the current study is that rats do not exhibit significant motor deficits following the intraluminal perforation model of SAH. However, rats do show a mild, but statistically significant, impairment in the MWT, indicating that the early physiological changes that have been described for this model [4] do have functional consequences. These findings extend upon a recent description of the short-term behavioural sequelae of this model [38] in that we assessed both motor and cognitive performance using separate, well-established tasks in rats that survived for 5 weeks following the lesion. In addition, we show that despite an acute hypo-activity lasting 24 h, SAH rats have a significant increase in body temperature for several hours after the stroke. Similar to our current behavioural findings, a recent study found that the double injection rat model of SAH also results in significant cognitive, but no motor deficits [37]. We chose to describe the behavioural profile of the endovascular perforation model as it more closely resembles the clinical condition and would most likely be used for assessing potential treatments for SAH. This is the first study to investigate behavioural performance at 5 weeks post-lesion in the intraluminal perforation model of SAH.

The lack of motor deficits in our current study may be explained by the fact that this model does not directly injure the descending motor tracts, but rather produces more diffuse and often mild injury throughout the brain by several different mechanisms. First, the acute increase in ICP produces a transient period of global hypoperfusion due to decreased cerebral perfusion pressure [3]. Second, the accumulation of blood in the subarachnoid space will surround the remaining intact vessels, thus causing vasoconstriction and a further decrease in cerebral perfusion [30]. Finally, microvascular platelet aggregation and the formation of microemboli further contribute to perfusion impairments by blocking flow through capillaries [35]. Taken together these pathological processes would be unlikely to cause focal or diffuse injury to the motor system that would result in gross motor impairments on tasks such as skilled
walking or forepaw asymmetry. Rather, this model produced cognitive deficits in the Morris water task—a task dependent on the interaction of multiple cortical and sub-cortical brain regions [16]. Alternatively, it is possible that the lack of persistent motor deficits is due to the fact that the rats have an opportunity to compensate for any motor impairment [40]. Slowly progressing neural damage (such as that resulting from chronic hypoperfusion) is more likely to go undetected by behavioural measures as the animals have time to develop compensatory strategies, whereas testing following an acute injury would more clearly show the impaired behaviour. It may also be the case that our battery of motor tasks was not sensitive enough to detect slight motor impairments: although, these tests are all sensitive to other ischemic and hemorrhagic brain insults in our lab (e.g. [8,22]). Nonetheless, more demanding motor tasks (such as skilled reaching) or tasks that examine species typical behaviour (nest building and grooming) might reveal some motor impairments in this model. We should also emphasize that animals with SAH did appear lethargic and uncoordinated in the acute phase following SAH. Had we performed a gross neurological assessment within the first day after the surgery we would have likely seen impairments similar to those reported by others [7,33]. However, rats with the most severe impairments tend to die soon after the surgery, and our aim here was to quantify the behavioural profile of those rats that survive beyond the first couple of days after the SAH surgery.

In addition to the behavioural measures we also performed a systematic evaluation of the histological outcome in this model. We found no evidence of significant tissue necrosis, cell loss, or an effect on cortical thickness when animals were sacrificed at 40 days post-injury. Brains from SAH animals that were perfused at a short survival time (3 days) did have clusters of Fluoro Jade positive cells, but no such labeling was present at the long survival time or in sham animals (data not shown). A previous study used hematoxylin and eosin staining to investigate cell death 7 days following endovascular perforation and found that only 11% of rats showed any signs of neuronal damage [26]. It appears that this model is characterized by a transient period of cell death, which does not result in the formation of frank lesions when examined several weeks following the injury. This early period of cell death coincides with the period of early hypoperfusion, and suggests a causative relationship between these phenomena [28].

It should be emphasized that markers of cell death are unable to detect more subtle forms of neuronal damage. For example, this injury model may have significant effects on the number of synapses, growth-factor levels or neuron morphology—factors that would influence brain function but have remained uninvestigated in this model. In addition, electrophysiological measurements show that SAH induces severe EEG abnormalities [18] as well as a decrease in the threshold of spreading depolarization [25]. These findings further suggest that the SAH injury produces abnormal brain functioning at the cellular level (at least transiently), but so far, the electrophysiological properties of individual neurons have not been investigated.

Our study is also the first to describe the spontaneous temperature profile of rats following SAH. We found that rats became hyperthermic immediately following the SAH injury. The sham surgery was found to induce a much shorter period of very mild hypothermia, which can be attributed to the use of isoflurane anaesthesia [20]. Based on the time-course of the temperature increase in the SAH group, as well as the fact that we performed our surgeries aseptically, it is unlikely that the hyperthermia was caused by an infection. Rather, a transient functional disruption of anterior hypothalamic nuclei may have resulted in the observed hyperthermia, as has been reported for other stroke models [23]. Given that even a slight increase in temperature affects stroke outcome [6], the spontaneous temperature increase in this model is a factor that should be considered when testing future therapeutic agents. In those studies in which surgery was not performed aseptically, the rats may have had a prolonged infection and fever, which might then exacerbate injury and behavioural impairment. Directly relat-

Fig. 5. Fluoro Jade labeling in a brain 3 days following SAH. A composite picture of an anterior section (A) shows Fluoro Jade positive cells in medial as well as lateral frontal regions (cingulate and orbital cortices) and olfactory nuclei. The SAH was induced in the right hemisphere, but some labeling is also present in the orbital region of the opposite (left) hemisphere. A more posterior section from the same brain (B) shows additional labeling in the striatum (D), and the anterior hypothalamus (E).
patients were febrile following their injury, and with every 1 °C increase to this issue, a recent clinical study has shown that 72% of SAH left (B) hemispheres.

Cortical thickness measurements at 5 different rostro-caudal levels showed that there was no significant difference between groups in either the right (A) or the left (B) hemispheres.

Fig. 6. Cortical thickness measurements at 5 different rostro-caudal levels showed that there was no significant difference between groups in either the right (A) or the left (B) hemispheres.

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et al. [36] classified their post-SAH rats as mild, moderate or severe based on a simple grading system that evaluates the amount of blood on the surface of the brain. Similar to our current behavioural findings they found that when the mean impairment of all of the SAH rats was compared to a sham group the SAH group did not have a significant motor deficit at 24 h post-surgery. However, additional comparisons showed that both the moderate and severe groups were significantly impaired, whereas the mild group had no significant deficits. In our current study we are unable to segregate our rats based on hemorrhage size as we have no way of judging the original size of the hemorrhage at the time of euthanasia. A large number of the brains in our study had visible discoloration on the ventral surface, presumably caused by the degradation of blood products after the SAH, however the degree of discoloration (as judged by a blinded experimenter) did not correlate with functional outcome (data not shown). A more sensitive measure of hemorrhage size can be attained through MR imaging [39], therefore future studies may use such techniques to better predict outcome.

When comparing across studies, it becomes obvious that the severity of the motor impairment acutely after an SAH varies significantly [5,7,42]. Given that the impairments are transient most authors attribute it to the severe increase in ICP and edema following SAH. Treatments that reduce edema in other models of hemorrhagic stroke (ICH) do not necessarily translate to functional improvement [13]; therefore it is possible that treatments that target brain edema in the acute phase will not provide functional improvement in this model of SAH.

In summary, our findings show that the endovascular perforation model of SAH produces no observable motor deficits, and a slight cognitive impairment in rats that survived for 5 weeks following an SAH. SAH patients often have cognitive impairments [2]; therefore, it is important to have cognitive measures as part of a test battery in animal models. Given the slight impairments found in our current study, it would be difficult to use functional outcome as a gauge for treatment efficacy in the endovascular perforation model.


