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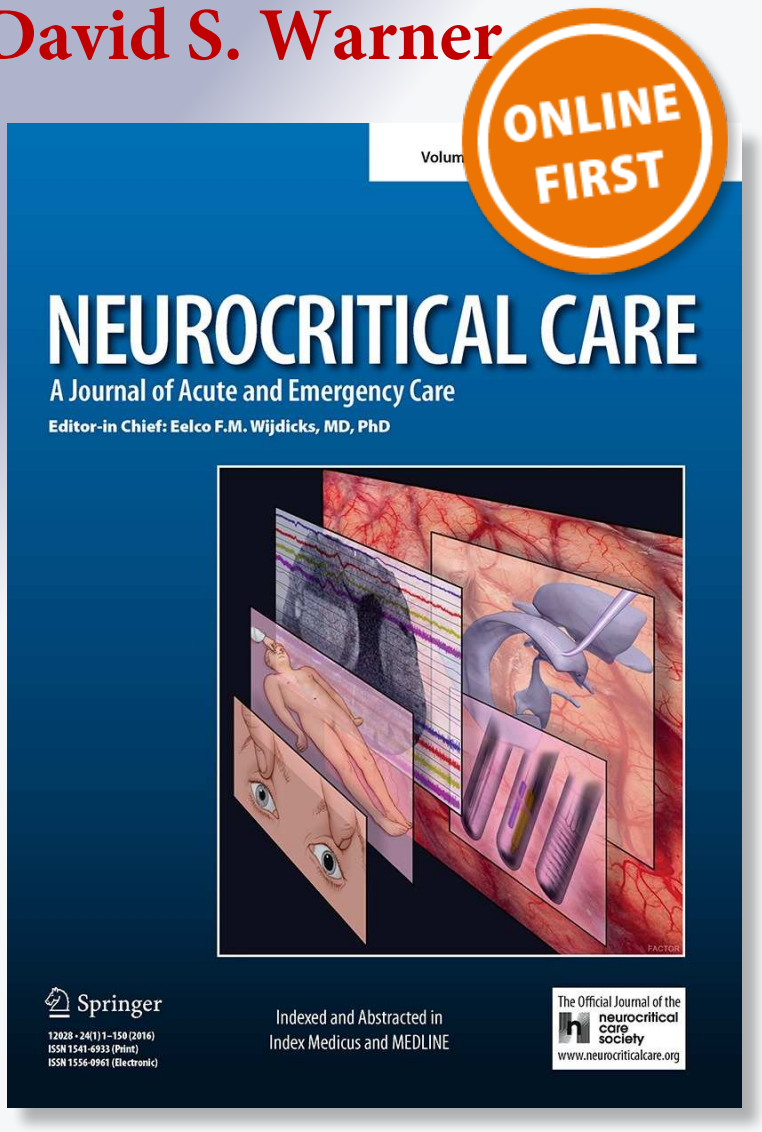

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TRANSLATIONAL RESEARCH

Long-Term Cognitive Deficits After Subarachnoid Hemorrhage in Rats

Toshihiro Sasaki[1] · Ulrike Hoffmann[1] · Motomu Kobayashi[1] · Huaxin Sheng[1] · Abdelkader Ennaceur[2] · Frederick W. Lombard[1] · David S. Warner[1]

Abstract

Background Cognitive dysfunction can be a long-term complication following subarachnoid hemorrhage (SAH). Preclinical models have been variously characterized to emulate this disorder. This study was designed to directly compare long-term cognitive deficits in the context of similar levels of insult severity in the cisterna magna double-blood (DB) injection versus prechiasmatic blood (PB) injection SAH models.

Methods Pilot work identified blood injectate volumes necessary to provide similar mortality rates (20–25 %). Rats were then randomly assigned to DB or PB insults. Saline injection and naïve rats were used as controls. Functional and cognitive outcome was assessed over 35 days.

Results DB and PB caused similar transient rotarod deficits. PB rats exhibited decreased anxiety behavior on the elevated plus maze, while anxiety was increased in DB. DB and PB caused differential deficits in the novel object recognition and novel object location tasks. Morris water maze performance was similarly altered in both models (decreased escape latency and increased swimming speed). SAH caused histologic damage in the medial prefrontal cortex, perirhinal cortex, and hippocampal CA1, although severity of injury in the respective regions differed between DB and PB.

Conclusion Both SAH models caused long-term cognitive deficits in the context of similar insult severity. Cognitive deficits differed between the two models, as did distribution of histologic injury. Each model offers unique properties and both models may be useful for study of SAH-induced cognitive deficits.

Keywords Subarachnoid hemorrhage · Cognitive dysfunction · Prechiasmatic blood injection model · Cisterna magna double blood injection model · Rat

Introduction

Cognitive dysfunction affects up to 50–60 % of aneurysmal subarachnoid hemorrhage (SAH) survivors and is a major cause of disability [1, 2]. Patients may experience a broad range of deficits involving memory, learning, attention, psychomotor speed, and emotional health [3–5]. Short-term rodent recovery models are often used to explore post-SAH mechanisms that result in neurologic deficits [6–8]. Only recently has this work been extended to examine cognitive deficits [9–13]. Because post-SAH cognitive and memory deficits may persist for months to years in humans [3, 14, 15], there is need to optimize preclinical modeling to facilitate investigation of putative injury mechanisms and therapeutic interventions.

There are three commonly employed rodent SAH models. Endovascular perforation of the middle cerebral artery produces vasospasm and neurologic deficit. Existing data indicate the presence of sustained cognitive deficit in rats [16, 17], but not mice [18]. The double-blood (DB)

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injection model, created by injection of autologous blood into the dorsal posterior fossa on two separate days, consistently causes long-term cognitive deficits [9, 10, 19]. The prechiasmatic blood (PB) model, which requires a single stereotactic injection of autologous blood into the anterior fossa, has been shown to produce both short- and long-term cognitive deficits [11, 20].

The purpose of this investigation was to directly compare and contrast long-term cognitive deficits in the DB and PB SAH models, representing anatomically posterior and anterior hemorrhages, respectively. We hypothesized that long-term cognitive and histologic effects would differ between the two models.

Materials and Methods

All animal procedures were approved by the Duke University Animal Care and Use Committee, Durham, NC.

Surgical Procedures

Male Wistar rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 280–320 g were used. Animals were fasted overnight with free access to water. On day 0, anesthesia was induced with 5 % isoflurane in 50 % O_2 balanced with N_2 and maintained with 1.5–1.8 % isoflurane in 30 % O_2 balanced with N_2. Following tracheal intubation, the lungs were mechanically ventilated to maintain normocapnia. A tail artery catheter was positioned to monitor blood pressure and sample blood. Arterial blood gases (pH, $PaCO_2$, and PaO_2), hematocrit, and blood glucose were measured before SAH induction. Pericranial temperature was monitored continuously by placing a thermocouple beneath the right musculus temporalis. An automated temperature control system maintained pericranial temperature at 37.0–37.5 °C.

Prechiasmatic Blood Model

On day 0, animals were secured in the prone position in a stereotaxic apparatus with the cervical spine in a neutral position. Through a midline scalp incision, a 1-mm midline burr hole was drilled 7.5 mm anterior to bregma. Through this burr hole a 25G Quincke tip spinal needle was inserted $\approx$10 mm into the brain at an angle of 30° caudal until contact was made with bone. The needle then was withdrawn 0.5 mm and 15-min physiologic stabilization was allowed. Fresh autologous blood aspirated from the tail artery or saline was then injected through the needle over 30 s using a syringe pump, creating PB or prechiasmatic saline (PS) groups, respectively. The rat was then titled into a 15° head down position for 45 min. Then, the intracranial needle and tail artery catheter were removed. Wounds were infiltrated with 0.25 % bupivacaine, closed with suture, and covered with Neosporin® ointment. Animals were allowed to recover from anesthesia, the tracheas were extubated, and they were returned to their cages.

Cisterna Magna Double-Blood Injection Model

Previously reported procedures were followed [9, 10]. On day 0, rats were anesthetized as described above and positioned prone in the stereotaxic apparatus. The atlanto-occipital membrane was exposed through a midline incision. Rats were tilted 30° head down and a 30-gauge needle was inserted into the cisterna magna. Following withdrawal of 0.05 ml of cerebrospinal fluid, 0.5 ml of autologous arterial blood or 0.9 % NaCl (saline) was injected over 5 min by syringe pump. The rat remained in the head down position for 30 min. The intracisternal needle and arterial catheter were then removed. Wound closure and anesthesia recovery were as above. Two days later (day 2), the rats were re-anesthetized and 0.4 ml of fresh autologous arterial blood or saline was injected intracisternally, as described above, creating DB or double-saline (DS) injection groups, respectively.

Post-SAH, all animals were housed 2 per cage in a dedicated temperature and noise-controlled rodent vivarium with a normal 12-h day/night circadian rhythm. Animals had free access to drinking water and standard rat chow. Post-surgical animals received 2 ml of 0.9 % NaCl subcutaneously twice per day to prevent dehydration and soft food for the first 48 h.

Pilot Study

Given the different anatomic locations of blood injection and total blood volume injected in the two models, we chose mortality rate to define a comparable level of insult severity. Previous work with the DB model showed a long-term mortality of 20–25 % [9, 10]. Accordingly, a dose-escalation study was performed with the PB model to determine the volume of autologous blood injectate that would result in 20–25 % mortality over the anticipated study duration. The blood injection volume was increased in a stepwise fashion: 350 µl ($n = 9$), 400 µl ($n = 10$), and 450 µl ($n = 14$). An additional nine rats underwent 450 µl saline injection. Rotarod performance was tested on days 0, 1, 7, 14, and 21. The Morris Water maze (MWM) learning set task was performed on days 21–24 and hippocampal CA1 neuronal count was obtained on day 28. Based on these results (see below), we selected a 450-µl prechiasmatic injectate volume for further study.

Experimental Design for DB Versus PB Model Comparison

Rats were randomly assigned (drawing numbers from a box) to blood injection (DB = 20, PB = 20) or 0.9 % NaCl (DS = 15, PS = 15) groups. Twelve rats (naïve controls) were contemporaneously exposed to identical environmental and testing conditions but did not undergo anesthesia and surgery.

All rats were allowed to survive 35 days during which functional and histologic outcomes were assessed as depicted in Fig. 1. Animals were transported in their cages to a dedicated noise-controlled room 2 h prior to testing for acclimation during the light phase of the circadian cycle.

A single observer, blinded to group assignment, conducted all behavioral tests.

Rotarod

All rats underwent training on the rotarod for 2 days prior to randomization. Testing was continued on post-SAH days 0, 1, 7, and 35. The rod rotation rate was increased from 4 to 40 rpm over 5 min. The duration (s) rats remained on the rod was recorded. On each day, three trials were performed with an inter-trial interval of 15 min. The best daily performance was used for analysis.

Elevated Plus Maze

The elevated plus maze was performed on day 20 to test for anxiety-like behavior [21]. The apparatus consisted of a plus-shaped maze (each arm 50 cm long and 10 cm wide) elevated 50 cm above the floor. Black wooden walls (15 cm high) enclosed two opposing arms (closed arms). The remaining two arms were devoid of walls (open arms). Each rat was placed in the center of the maze facing an open arm, after which the cumulative time spent in each arm and the number of entries into the open or closed arms were recorded during a 10-min test session. An individual entry into the arm was defined as the animal placing all four paws in that arm. More time spent in the open arm indicates lesser anxiety [21]. Data were analyzed for both the first and last 5-min bins.

Novel Object Recognition and Location Tasks

These tests are based on rats' tendency to explore novel objects; this tendency has been shown to be a sensitive index of memory function [22, 23]. The apparatus consisted of an open field made of a matte black wooden box 100 × 100 × 50 cm high, and three different sets (three identical replicas per set) of similarly sized (approximate width = 7 cm, approximate height = 9 cm) simple shaped objects (closed box, sphere, cylinder). The open field was always positioned in the same location and orientation in the testing room. One corner was marked with orange tape from the bottom to the top to serve as an orientation visual cue. No objects were placed in this corner. For habituation, the rats were placed in the empty open field for 5 min each day on days 21–25 [22].

On day 26, novel object recognition was measured. Two identical objects (A1 and A2) were placed 10 cm away from the corners and adjacent walls of the box. Rats were allowed to explore the objects for 5 min. Time spent exploring each object was recorded (sample phase). After a 15-min delay, rats were re-exposed for 3 min to an identical object shown in the sample phase, now familiar (A3), and a novel object (B1) (choice phase). The objects were

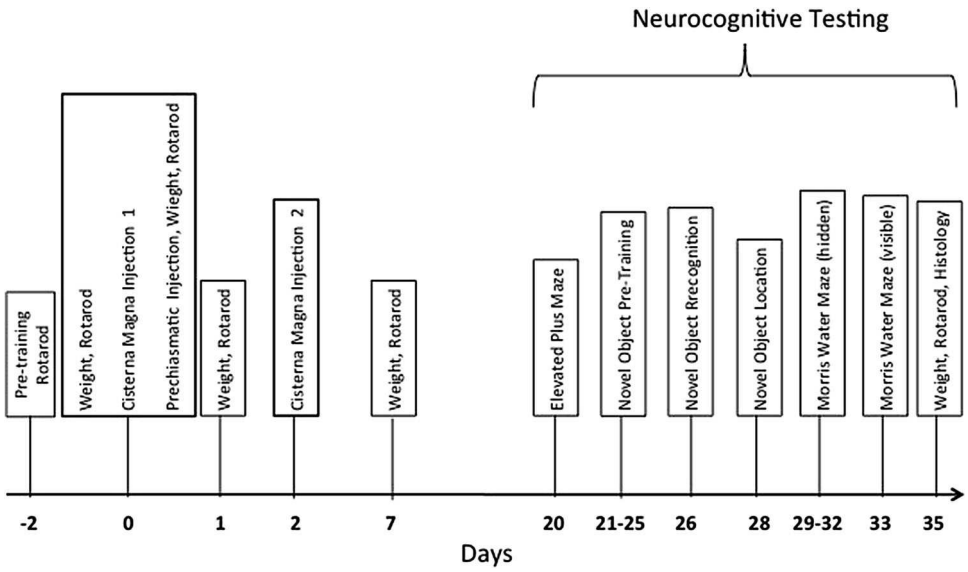

Fig. 1 Sequence of experimental events for comparison of cisterna magna double-blood versus prechiasmatic blood injection subarachnoid hemorrhage models

placed in the same location as in the sample phase. Once again, the time spent exploring each object was measured.

On day 28, the object location task was performed. During the sample phase, rats were allowed to explore two identical objects (C1 and C2) for 5 min. The objects were placed 10 cm away from the corners and adjacent walls of the box. After a 15-min delay, a 3-min choice phase began in which two identical copies of the objects presented in the sample phase were used. One object (C3) occupied either the C1 or C2 object location and the other object (C4) occupied a novel location. The amount of time spent exploring each object was recorded in the sample and choice phase.

The objects placed in the box were secured to prevent movement. Exploration of an object was defined as the rat having its nose within 2 cm of the object and actively sniffing or touching the object. Turning around or sitting on the object was not considered exploratory behavior. After each exposure, the objects and test chamber were cleansed with 70 % ethanol to control odor cues. Object preference was determined within each group by comparing the time difference between novel and familiar objects or novel and familiar location of objects.

Morris Water Maze

For the learning set task, a modified MWM was employed, using a black circular pool (having fixed visual cues) filled with water (27 °C), the surface of which was 3 cm above a submerged invisible escape platform [9, 10, 24]. Rats were tested for four consecutive days (days 29–32), with eight trials per day (inter-trial interval = 15 min). The quadrant containing the platform was changed daily. The rat was introduced, randomly, into the remaining three quadrants over the eight trials and was allowed to swim for a maximum of 60 s. If the platform was not found, the rat was placed by hand on the platform and left there for 30 s in the first trial and for 15 s on any subsequent trial. An automated video tracking system (Ethovision 2.2.14; Noldus Information Technology, Leesburg, VA) recorded the time taken to reach the hidden escape platform (escape latency), swimming speed, and total swim distance.

On day 33 (cueing session), the platform location was indicated by a flag, which extended 15 cm above the water surface (visible platform test). Room lighting was decreased so that the extra-maze cues were no longer available while the visible platform was illuminated. Each rat was allowed one trial in order to acclimate to the new set of conditions and locate the platform visually. Once the platform was located, the rat was left on the platform for 30 s. The rat was then immediately given four consecutive trials in the same manner and the latency to reach the platform was measured. The platform location was changed on each subsequent trial with the animal introduced into the diametrically opposite quadrant.

Histology

On day 35, animals were anesthetized with isoflurane and perfused in situ with heparinized saline and 10 % paraformaldehyde. The brains were removed and paraffin-embedded. Coronal sections (5 μm thickness) were obtained and stained with cresyl violet. The number of morphologically intact neurons in hippocampus CA1 and perirhinal cortex were counted ≈4 mm posterior to bregma, at ×400 magnification in rectangular areas (200 × 50 μm). The number of intact neurons in the medial prefrontal cortex (mPFC) was measured ≈2.5 mm anterior to bregma in rectangular areas (100 × 50 μm). Only neurons with normal morphology with distinct cytoplasmic and nuclear outlines and a visible nucleolus were counted. Neurons that had a shrunken cell body with vacuolizations were excluded. Counts were performed in both hemispheres. The pooled count for each region was averaged between hemispheres in each animal. An observer blinded to group assignment performed counting.

Statistical Analysis

Data were analyzed using SPSS, SAL, and Statistica for Windows, version 5.5. Samples size requirements were estimated on the basis of previous experience with the DB model that demonstrated sustained post-SAH MWM deficits [9, 10]. Mortality rates in the DB and PB models were compared with the Chi-square statistic. Rotarod, body weight, and MWM learning set data were analyzed with repeated-measures ANOVA using the Greenhouse–Geisser correction. Significant ANOVA interactions were explored using the Bonferroni post hoc test. All novel object recognition and elevated plus maze data were tested for differences among group mean values with one-way or two-way ANOVA and where appropriate with Newman–Keuls post hoc comparisons. The two-tailed paired Student t test was used for within group comparisons. Neuronal survival was analyzed using one-way ANOVA with Bonferroni correction. Results were considered significant when $p \leq 0.05$ and are presented as mean ± standard deviation.

Results

Prechiasmatic SAH Pilot Study

Mortality rates were 22 % for 350 μl ($n = 2/9$), 30 % for 400 μl ($n = 3/10$), and 28 % ($n = 4/14$) for 450 μl of

injected blood. No surviving animals were excluded. All saline injection animals survived. Over the 3-week rotarod testing period, there was a main effect for group ($p < 0.01$). Post hoc comparisons to the saline group showed a decrement for the 450 µl blood group ($p < 0.01$), but not for the other two blood volume groups. In all blood groups, rotarod latencies returned to saline control values by day 21. There was a main effect of group on MWM escape latency ($p < 0.001$). Compared to saline, escape latency was increased in the 450 µl blood group ($p < 0.01$), but not for the other blood volumes. The 450 µl group also showed decreased intact hippocampal CA1 neurons ($p < 0.006$) compared to saline injection. We did not see a significant effect on CA1 neuronal viability in the 350 and 400 µl groups.

DB Versus PB Model Comparisons

Mortality rates were similar ($p = 0.76$) in the DB ($n = 4/20$, 20 %) and in the PB ($n = 5/20$, 25 %) groups. Deaths in the DB group typically occurred during or after the second injection, whereas animals in the PB group died during the first 2 days after surgery. All saline injection animals, regardless of injection site, survived.

Physiological values (arterial blood gases/pH and glucose, mean arterial pressure, and pericranial temperature) were similar between groups prior to the first injection in both models (data not shown). However, plasma glucose concentrations were greater prior to the second versus first injection in the double injection model for both blood (158 ± 24 vs. 119 ± 9 mg/dl, $p < 0.001$) and saline (144 ± 14 vs. 116 ± 16 mg/dl, $p < 0.001$) groups. A transient increase in arterial blood pressure was observed after both the first and second cisterna magna injections and the prechiasmatic injection (blood > saline). Values returned to baseline within 10 min in all groups ($p < 0.001$), Fig. 2.

Prior to SAH there was no difference among groups for body weight. On day 1, weight was decreased in all treatment groups compared to naïve controls ($p < 0.01$). All animals returned to naïve control values by day 35 except DB animals, which gained less weight (DB vs. DS, PB, PS, and naïve $p < 0.01$), Fig. 3a.

Rotarod

Prior to SAH there was no difference among groups for rotarod latency to fall. Over the 5-week testing period, there was a main effect for group ($p < 0.01$). Post hoc comparisons showed differences between DB and DS ($p < 0.01$) and between PB and PS groups ($p < 0.01$) on day 1. Differences between DB and PB groups or between DS and PS groups were not detected. Latencies to fall recovered to naïve control values in all groups by day 35 (Fig. 3b).

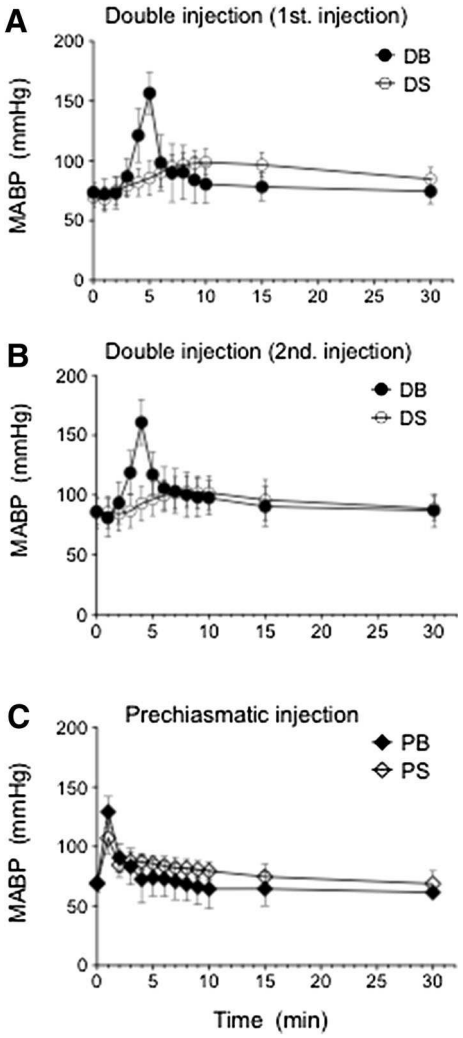

Fig. 2 Change in mean arterial blood pressure (MABP) during 30 min after **a** first injection of blood (DB) or saline (DS) into the cisterna magna, **b** second injection of blood or saline into the cisterna magna, or **c** blood (PB) or saline (PS) injection into the prechiasmatic cistern. Injection onset was at time 0. Values are mean ± SD

Elevated Plus Maze

Total Time Spent in Maze Arms

There were differences among groups ($F_{4,67} = 3.86$, $p < 0.007$), but no differences between 5-min bins ($F_{1,67} = 0.70$, $p > 0.10$). An interaction between groups and bins was present ($F_{4,67} = 2.94$, $p < 0.03$). Post hoc analysis revealed differences between groups in the last ($F_{4,67} = 5.35$, $p < 0.0008$), but not in the first ($F_{4,67} = 1.18$, $p > 0.10$) 5-min bins. The time spent in the arms was greater in DB versus naïve controls

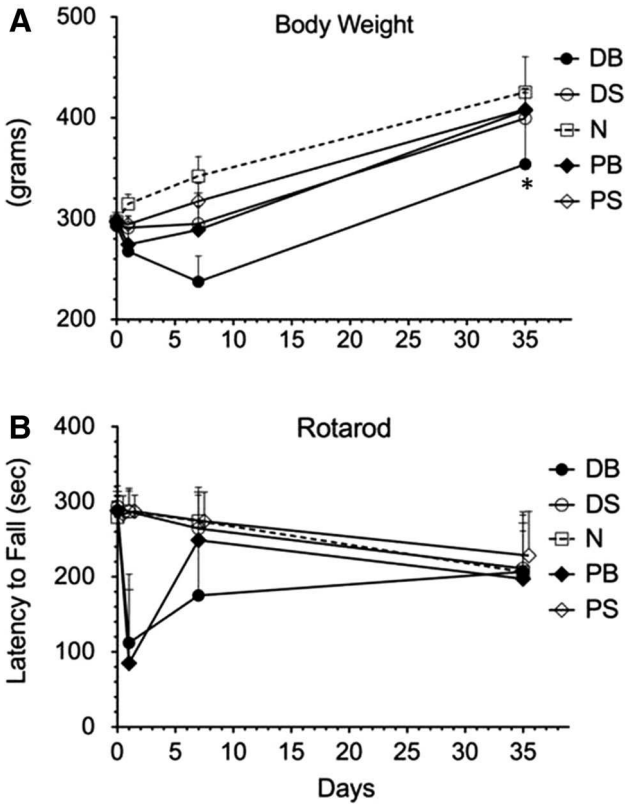

Fig. 3 **a** Weight change over 35 days post-subarachnoid hemorrhage (SAH). Prior to SAH there was no difference between groups for body weight. *$p < 0.01$ for DB versus DS, N, PB, and PS on day 35. **b** Rotarod latencies over 35 days post-SAH. No difference was detected among groups on day 35. *DB* double-blood, *DS* double-saline, *N* naïve, *PB* prechiasmatic blood, *PS* prechiasmatic saline

($p < 0.0006$) and PS ($p < 0.02$), but not versus DS ($p < 0.06$). It was also greater in PB compared to naïve ($p < 0.02$). For the total 10-min session, time spent in the arms was greater in DB versus naïve ($p < 0.004$) and PB ($p < 0.02$).

Total Number of Entries into Maze Arms

There were differences among groups ($F_{4,67} = 8.44$, $p < 0.0001$). Differences between 5-min bins ($F_{1,67} = 0.47$, $p > 0.10$) and interactions ($F_{4,67} = 1.39$, $p > 0.10$) were not detected. Post hoc analysis revealed differences between groups in the first ($F_{4,67} = 5.21$, $p < 0.001$) and last ($F_{4,67} = 8.93$, $p < 0.0001$) 5 min. The number of entries was fewer in DB compared to naïve ($p < 0.002$), DS ($p < 0.008$), PS ($p < 0.009$) groups in both the first and last 5 min. Entries were also fewer in DB compared to PB in the last ($p < 0.005$), but not in the first ($p > 0.10$) 5 min. For the total 10-min session, the number of entries was fewer in DB compared to naïve ($p < 0.0002$), DS ($p < 0.0005$), PS ($p < 0.0003$), and PB ($p < 0.02$).

Time Spent in Enclosed Arms (Table 1)

There were differences among groups ($F_{4,67} = 3.67$, $p < 0.01$) and between 5-min bins ($F_{1,67} = 77.22$, $p < 0.0001$). An interaction was not detected ($F_{4,67} = 1.81$, $p > 0.10$). Post hoc analysis revealed differences between groups in the last 5 min ($F_{4,67} = 4.99$, $p < 0.001$), but not in the first 5 min ($F_{4,67} = 1.98$, $p > 0.10$). DB rats spent more time in the enclosed arms than any other group ($p < 0.05$).

Number of Entries into Enclosed Arms (Table 1)

There were differences among groups ($F_{4,67} = 10.15$, $p < 0.0001$) and between 5-min bins ($F_{1,67} = 16.46$, $p < 0.0001$). An interaction was present (F4,67 = 2.45, $p < 0.05$). Post hoc analysis revealed differences between groups in the first ($F_{4,67} = 8.47$, $p < 0.0001$) and last 5 min ($F_{4,67} = 8.42$, $p < 0.0001$). DB rats made fewer entries into the enclosed arms than any other group ($p < 0.001$) in both test phases, except in the first 5 min compared to PB rats ($p > 0.10$). PB made fewer entries than naïve ($p < 0.0003$), PS ($p < 0.04$) and DS ($p < 0.03$) in the first 5 min.

Percent Time in the Open Arms (Table 1)

There were differences among groups ($F_{4,67} = 4.24$, $p < 0.005$) and between 5-min bins ($F_{1,67} = 77.24$, $p < 0.0001$). An interaction was not detected ($F_{4,67} = 0.22$, $p > 0.10$). Post hoc analysis revealed differences between groups in the first ($F_{4,67} = 2.70$, $p < 0.04$) and second ($F_{4,67} = 4.96$, $p < 0.002$) 5-min bins. PB had a higher percent open arm time compared to all other groups in the first 5 min ($p < 0.05$) except versus PS ($p < 0.09$) and compared to all other groups, including PS, in the last 5 min ($p < 0.006$). Differences between the other groups were not detected ($p > 0.10$). Paired comparisons of time spent confirmed that all groups showed preference for the enclosed arms ($p < 0.005$) except PB rats, which spent similar time in the open and enclosed arms ($p > 0.10$).

Percent Entries into Open Arms (Table 1)

There were differences among groups ($F_{4,67} = 4.29$, $p < 0.004$) and between 5-min bins ($F_{1,67} = 93.21$, $p < 0.0001$). An interaction was not detected ($F_{4,67} = 1.56$, $p > 0.10$). Post hoc analysis revealed

Table 1 Elevated plus maze performance

Group	Enclosed arms				Percent open arms			
	Entries		Time (s)		Entries		Time	
	Bin 1	Bin 2	Bin 1	Bin 2	Bin 1	Bin 2	Bin 1	Bin 2
N	9.7	10.4	165.9	196.6	15.6	3.5	15.9	1.8
	(3.9)	(4.7)	(54.3)	(67.9)	(11.8)	(5.4)	(4.2)	(2.9)
PS	7.3	10.3	161.5	208.0	31.0	8.5	22.1	4.2
	(3.6)	(3.7)	(53.8)	(49.5)	(12.2)	(10.9)	(3.4)	(9.9)
PB	4.3◆	6.9	118.1	170.6	47.7✖	16.4✖✖	39.9♣	24.6♣♣
	(2.8)	(5.1)	(87.2)	(101.8)	(33.9)	(27.0)	(9.0)	(36.3)
DS	6.9	9.2	159.6	218.6	32.7	8.1	22.9	3.8
	(3.3)	(3.4)	(66.2)	(74.25)	(17.9)	(10.2)	(4.3)	(5.2)
DB	3.4•	3.1•	181.8	263.1*	26.2	0	17.9	0
	(3.2)	(4.2)	(73.3)	(41.3)	(24.6)	(0)	(5.5)	(0)

Group means, (±s.d.)

N normal rats ($n = 12$), *PS* prechiasmatic saline ($n = 15$), *PB* prechiasmatic blood ($n = 15$), *DS* double-saline ($n = 15$), *DB* double-blood ($n = 16$)

• Compared to all other groups ($p < 0.001$), except PB ($p > 0.10$)

◆ Compared to N ($p < 0.0003$), PS ($p < 0.04$), DS ($p < 0.03$)

* Compared to all other groups ($p < 0.05$)

✖ Compared to N ($p < 0.002$), PS ($p > 0.10$), DS ($p < 0.08$), DB ($p < 0.06$)

✖✖ Compared to N ($p < 0.09$), PS ($p > 0.10$), DS ($p > 0.10$), DB ($p < 0.03$)

♣ Compared to all other groups ($p < 0.05$) except PS ($p < 0.09$)

♣♣ Compared to all other groups ($p < 0.006$)

differences between groups in the first ($F_{4,67} = 3.78$, $p < 0.008$) and last ($F_{4,67} = 2.76$, $p < 0.03$) 5-min bins. In the first 5 min, the percent entries into open arms were greater in PB compared to naïve ($p < 0.002$), but not PS ($p > 0.10$), DS ($p < 0.08$), or DB ($p < 0.06$). In the last 5 min, the percent open arm entries were greater in PB compared to DB ($p < 0.03$), but not naïve ($p < 0.09$), PS ($p > 0.10$), or DS ($p > 0.10$). Paired comparisons of the number of entries revealed that all groups demonstrated a preference for enclosed arms compared to open arms in both 5-min bins ($p < 0.009$), except PB in the first 5 min ($p > 0.10$). For the total 10-min session, PB had a higher percent open arm entries compared to naïve ($p < 0.008$), PS ($p < 0.01$), DS ($p < 0.03$), and DB ($p < 0.007$).

Novel Object Recognition Task

Differences were present among groups ($F_{4,68} = 2.91$, $p < 0.03$) and between the sample and choice phases ($F_{1,68} = 28.95$, $p < 0.0001$) for object exploration (Table 2). An interaction was not detected ($F_{4,68} = 1.63$, $p > 0.10$). *Post hoc* comparisons revealed differences between groups in the sample ($F_{4,68} = 3.62$, $p < 0.01$), but not choice phase ($F_{4,68} = 1.07$, $p > 0.10$). Time spent exploring the object in the sample phase was less in DB versus naïve ($p < 0.01$) and PS ($p < 0.02$), but not versus DS ($p < 0.06$) or PB ($p > 0.10$).

Time spent exploring objects in the sample phase was greater than in the choice phase in naïve ($t_{11} = 4.3$, $p < 0.001$), PS ($t_{14} = 4.33$, $p < 0.0007$), and DS ($t_{14} = 3.08$, $p < 0.008$). A difference between the two test phases could not be detected in DB ($t_{15} = 0.77$, $p > 0.10$) or PB ($t_{14} = 1.07$, $p > 0.10$).

For novel object recognition (Table 2), there were no differences among groups ($F_{4,68} = 1.07$, $p > 0.10$) for object discrimination, but a difference was present between objects ($F_{1,68} = 39.93$, $p < 0.0001$). An interaction was not detected ($F_{4,68} = 1.83$, $p > 0.10$). Time spent exploring the novel object was greater than time spent exploring the familiar object in naïve ($t_{11} = 3.41$, $p < 0.006$), DS ($t_{14} = 4.62$, $p < 0.0004$), and PS ($t_{14} = 5.28$, $p < 0.0001$). A difference between the two objects could not be detected in DB ($t_{15} = 0.76$, $p > 0.10$) and PB ($t_{14} = 1.93$, $p < 0.07$).

Novel Object Location Task

For overall object exploration during the sample phase and the choice phase (Table 2), there were differences among groups ($F_{4,68} = 2.79$, $p < 0.03$) and between the two test

Table 2 Object recognition and object location performance

Group	Object recognition				Object location			
	e1	e2	FO	NO	e1	e2	FL	NL
N	35.3••	25.1	8.9✖	16.2	30.3♣♣	20	8.3*	11.8
	(12.5)	(11.7)	(5.8)	(7.9)	(18.2)	(10.8)	(3.8)	(8.0)
PS	34.1••	24.1	8.3✖	15.8	36.7♣♣	21.7	9*	12.7
	(8.1)	(7.5)	(5.2)	(4)	(9.2)	(10.4)	(6.5)	(5.4)
PB	26.7	22.7	8.6✖	14.1	32.1♣♣	21	9.9	11.1
	(12.3)	(11.5)	(7.3)	(8.6)	(13.9)	(12.1)	(6.7)	(6.2)
DS	30.9••	22.1	6.3✖	15.8	37.4♣♣	27.9	11.7*	16.1
	(13.2)	(10.3)	(4.6)	(8)	(9.4)	(10)	(4.3)	(6.7)
DB	20.2•	18	8.1	9.9	23.2♣	18.5	8.4	10.1
	(14.9)	(9.5)	(7)	(6.2)	(16.5)	(8.7)	(4.4)	(6)

Group means, (±s.d.)

e1 object contact time (s) in the sample phase, *e2* object contact time (s) in the choice phase, *FO* familiar object, *NO* novel object, *FL* familiar location, *NL* novel location, *N* normal rats ($n = 12$), *PS* prechiasmatic saline ($n = 15$), *PB* prechiasmatic blood ($n = 15$), *DS* double-saline ($n = 15$), *DB* double-blood ($n = 16$)

• Compared to N ($p < 0.01$), PS ($p < 0.02$), DS ($p < 0.06$), PB ($p > 0.10$)

•• e1 compared to e2: N ($p < 0.001$), PS ($p < 0.0007$), DS ($p < 0.008$)

✖ FO compared to NO: N ($p < 0.006$), PS ($p < 0.0001$), PB ($p < 0.07$), DS ($p < 0.0004$)

♣ compared to DS ($p < 0.05$) and PS ($p < 0.05$)

♣♣ e1 compared to e2: N ($p < 0.07$), PS ($p < 0.0001$), PB ($p < 0.002$), DS ($p < 0.02$)

* FL compared to NL: N ($p < 0.09$), DS ($p < 0.006$), PS ($p < 0.03$)

phases ($F_{1,68} = 44.92$, $p < 0.0001$). An interaction ($F_{4,68} = 1.30$, $p > 0.10$) was not detected. Post hoc comparisons revealed differences between groups in the sample ($F_{4,68} = 2.72$, $p < 0.04$), but not choice phase ($F_{4,68} = 1.79$, $p > 0.10$). Time spent exploring the object in the sample phase was less in DB versus DS ($p < 0.05$) and PS ($p < 0.05$), but not naïve ($p > 0.10$) or PB ($p > 0.10$).

The time spent exploring the objects was greater in the sample versus choice phase in DS ($t_{14} = 2.74$, $p < 0.02$), PS ($t_{14} = 8.89$, $p < 0.0001$), and PB ($t_{14} = 3.68$, $p < 0.002$). It was comparable between the two test phases in naïve ($t_{11} = 1.98$, $p < 0.07$) and DB ($t_{15} = 1.42$, $p > 0.10$).

In the choice phase (Table 2), a difference among groups was not present for object location discrimination ($F_{4,68} = 1.79$, $p > 0.10$), but there was a difference between objects ($F_{1,68} = 19.82$, $p < 0.0001$). An interaction was not detected ($F_{4,68} = 0.87$, $p > 0.10$). Time spent exploring the object was greater in a novel location than in a familiar location in DS ($t_{14} = 3.26$, $p < 0.006$) and PS ($t_{14} = 2.47$, $p < 0.03$). It was comparable between the two object locations in DB ($t_{15} = 1.17$, $p > 0.10$), PB ($t_{14} = 1.12$, $p > 0.10$), and naïve ($t_{11} = 1.87$, $p < 0.09$) rats.

Morris Water Maze

There was a main effect for group on escape latency ($p < 0.01$), swim distance ($p < 0.01$), and swim velocity ($p < 0.01$) in the hidden platform test (Fig. 4). Post hoc comparisons for escape latency showed differences between DB versus DS and naïve groups ($p < 0.05$) and between PB versus PS and naïve groups ($p < 0.01$). No differences were detected between DB and PB groups, or among the DS, PS, and naïve groups. For swim distance and swim velocity, there were differences between DB versus DS and naïve groups ($p < 0.05$). PB was different from the PS and naïve groups ($p < 0.01$). No differences were detected between DB and PB groups or between DS, PS, and N groups. There were no differences among groups for escape latency in the visible platform test ($p = 0.31$, data not shown).

Histology

Both models resulted in diffuse histopathological brain damage (Figs. 5, 6). Blood groups had fewer intact neurons in hippocampal CA1 (among groups, $p < 0.0001$; DB vs. DS, $p < 0.001$; DB vs. PB, $p < 0.001$; PB vs. PS, $p < 0.05$); in the mPFC (among groups, $p < 0.0001$; DB

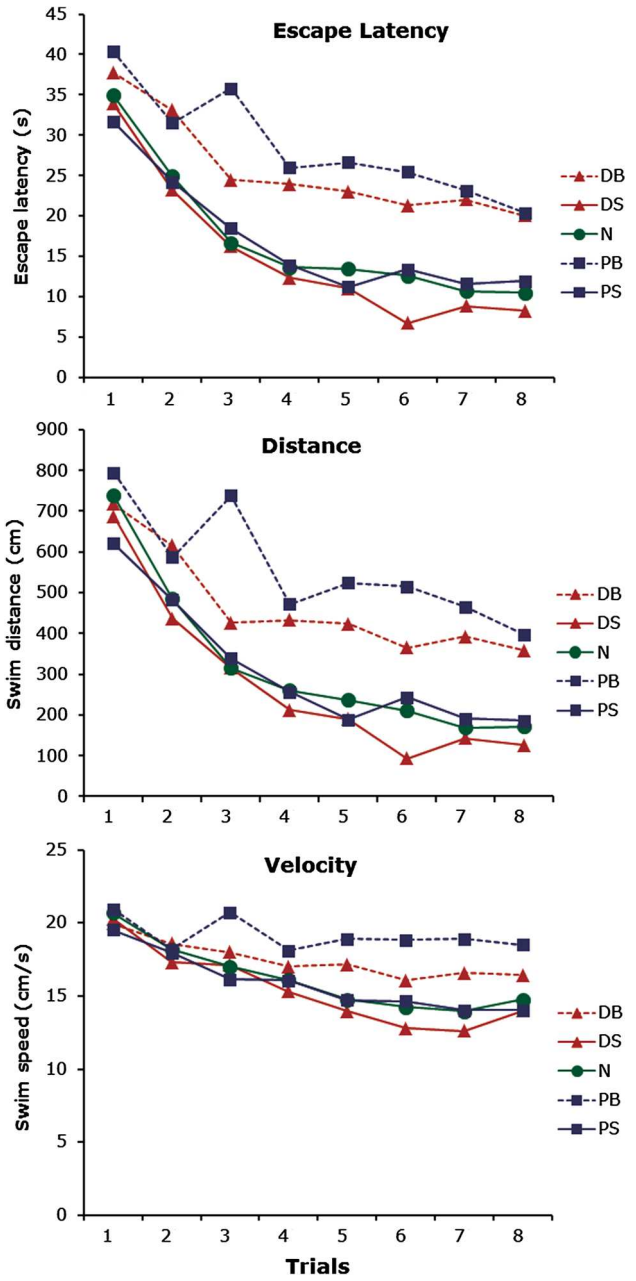

Fig. 4 Spatial memory, as determined by the Morris water maze learning set task, following cisterna magna or prechiasmatic injections of blood (DB, PB), saline (DS, PS), or in naïve (N) animals. Measurements were made on days 29–32 post-SAH. Both blood groups displayed increased escape latency, and greater swim distance and swim velocity compared to their saline-injected counterparts and naïve control animals. Values presented as group means. *Error bars* omitted for presentation clarity

vs. DS, $p < 0.01$; PB vs. PS, $p < 0.001$; PB vs. DB, $p < 0.05$); and fewer intact neurons in the PRC (among groups, $p < 0.002$; DB vs. DS, $p < 0.001$; DB vs. PB, $p > 0.10$; PB vs. PS, $p < 0.05$).

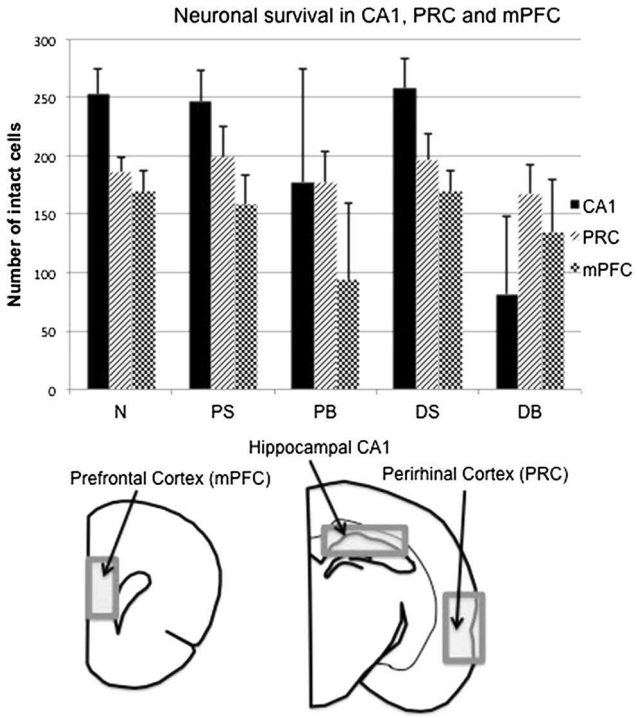

Fig. 5 The number of morphologically intact neurons in hippocampal CA1, perirhinal cortex (PRC), and medial prefrontal cortex (mPFC) in rats subjected to cisterna magna or prechiasmatic injections of blood (DB, PB), saline (DS, PS), or in naïve (N) animals. Values = mean ± standard deviation

Discussion

Humans surviving aneurysmal SAH may have persistent cognitive deficits. Similar sequelae are present in experimental SAH, but characterization of these deficits has been limited. The purpose of this study was to better define and contrast long-term cognitive deficits in two rat SAH models. In the DB and PB models, we observed sustained deficits in the elevated plus maze, novel object recognition and location tasks, and the MWM. Deficits varied in some tests, which may reflect the differential histologic damage we observed. These findings indicate both models are suitable for study of SAH-induced injury mechanisms and therapeutic intervention.

To directly compare the DB and PB models, we selected mortality as a marker of insult severity. In the pilot PB injectate-escalation study, 450 µl blood produced a mortality rate consistent with that previously observed in the rat DB model where sustained MWM deficits have been reported [9, 10]. Thus, 450 µl PB was selected for subsequent direct comparison with the DB model in the main study, in which mortality rates for the two models (20–25 %) did prove similar. In the pilot study, we also

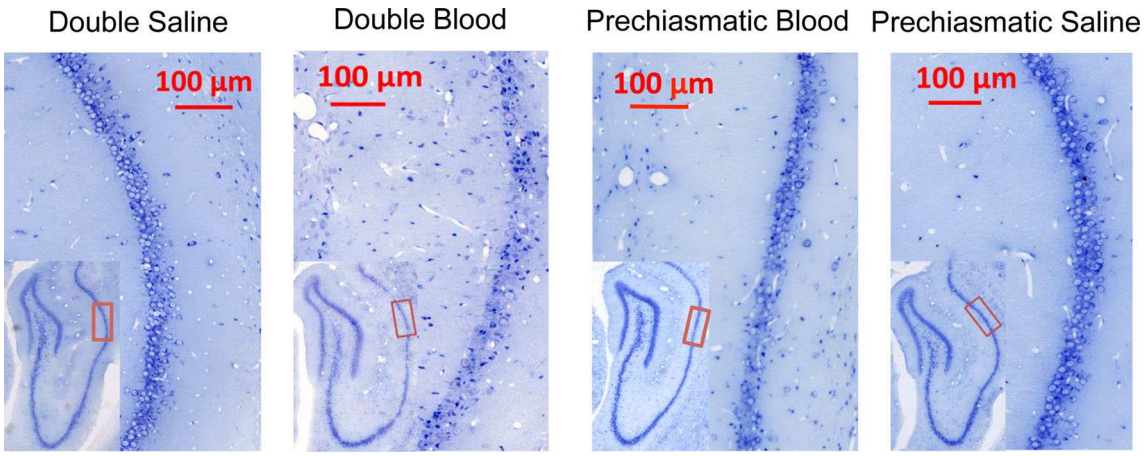

Fig. 6 Representative hippocampal CA1 photomicrographs from the experimental subarachnoid hemorrhage model groups and respective saline controls. The rat from each group having alive CA1 cell counts in closest approximation to that of the mean value for each respective group (see Fig. 5) was selected for illustration. Inset depicts coronal section taken ≈4 mm posterior to bregma

observed the magnitude of hippocampal CA1 neuronal viability and spatial memory (MWM) deficit to be associated with blood injectate volume.

Human SAH results in a wide range of sustained cognitive dysfunction [1, 2, 25]. Visual and spatial memory is commonly affected. Animal studies also confirm the presence of SAH-induced spatial memory impairment that cannot be attributed to motor dysfunction [9–11, 16]. Our study confirmed the sustained spatial memory deficit previously reported for rats subjected to endovascular perforation (3 weeks post-SAH) [16] and the DB model (5 weeks post-SAH) [9, 10]. Previous work has only shown that PB causes MWM deficits at 3–8 days post-SAH [16, 17, 20]. A major impetus for the current investigation was to determine whether sustained cognitive deficits are also present in the PB model. Sustained deficits were present and we believe this to be of importance.

The PB model may more closely resemble human SAH than the DB model. A single hemorrhagic event in the ventral brain corresponds to typical anatomical sites of clinical aneurysmal rupture. The PB model, with defined long-term cognitive deficits, may provide a more anatomically relevant scenario, is technically simpler to execute, and offers a discrete time point of pathology onset to be investigated. This is contrasted with the DB model, in which blood is injected over the dorsal hindbrain to be gravitationally dispersed into the basal cisterns. The DB model has been advocated for its clinical relevance due to resemblance of delayed responses to SAH seen in humans [26]. However, the sequential double injection paradigm does not anatomically reflect most examples of singular clinical aneurysm rupture and leaves discrete time points of onset of pathologic events ambiguous surrounding the two injection intervals. The DB model also requires two exposures to anesthetic agents, the effects of which are undefined. Because both models produce sustained, diverse, and measurable cognitive deficits when employed with similar levels of mortality, use of either model appears valuable for investigation. Further, because both models produce sustained deficits, definition of purported therapeutic robustness could be enhanced by confirmation in both models and use of an array of testing paradigms to assess cognitive dysfunction.

Both models caused substantial hippocampal CA1 and mPFC damage. Damage was less pronounced in the perirhinal cortex. The patterns of neuronal injury were different between the two models, presumably due to the anatomical distribution of subarachnoid blood. DB animals (posterior hemorrhage) displayed more pronounced CA1 damage than PB. Hippocampal blood flow is largely derived from the posterior cerebral artery [27], and post-DB hippocampal hypoperfusion has been documented in this model [9]. In contrast, PB animals (anterior hemorrhage) predominantly exhibited prefrontal cortical injury. These injury distributions may provide insight into the differential effects of the two models on cognitive behavior.

In the elevated plus maze, an increase in open arm entries has been associated with lesions of both the mPFC [28] and dorsal hippocampus [29]. Thus, SAH-induced injury in either region would predict decreased anxiety behavior. However, we could find no reports on maze performance when both structures are damaged, which complicates interpretation of our data. The two models differentially injured these regions, with greater CA1 injury in the DB model versus greater mPFC injury in the PB model. PB rats demonstrated a higher percent of open arm entries and percent time in the open arms compared to the

other groups in both 5-min exposure bins, consistent with decreased anxiety. In contrast, DB rats spent more time in the enclosed arms than any other group, consistent with increased anxiety. Boyko et al. [19] reported that plus maze responses were dependent upon insult severity in the cisterna magna injection model. Single-blood injection caused a major decrease in open arm entries and time spent in the open arms at 3 weeks, consistent with our observation. But, DB injection had no effect. Time spent in closed arms was not reported.

Clinical reports vary markedly in description of sustained anxiety disorders in SAH patients, with incidence estimates ranging from 0 to 40 % [30–33]. While the wide range in anxiety disorder prevalence may be due to methods of assessing anxiety and patient age [34], preclinical data from this study and other reports indicate that both severity and location of hemorrhage also may be contributory. The finding of increased open arm entries in the PB group and worsened mPFC injury may be consistent with reports that SAH patients can exhibit poor decision-making and increased risk taking [35–37]. At the same time, increased time spent in the closed arms in the DB group is inconsistent with worsened CA1 injury. Hence, SAH-induced effects on elevated plus maze performance appear model and insult severity dependent. Better understanding of interactions between regional injuries may be necessary to explain the anatomical basis of our observations.

The novel object recognition/location task performed on post-SAH days 21–28 detected cognitive deficits in both models. DB and PB both impaired memory for object location. PB and DB did not discriminate between displaced and non-displaced objects like their saline-treated counterparts. However, naïve controls were also unable to discriminate between objects ($p = 0.09$). In the object recognition task, the DS, PS, and naïve groups did discriminate between novel and familiar objects while PB and DB did not. This indicates impaired memory of the familiar object.

Finally, both models produced similar deficits in the MWM. Both models similarly affected escape latency and swimming velocity. It is unlikely that decreased escape latency was due to motor or visual function. Both the DB and PB groups had recovered to normal rotarod performance by the time of MWM testing and there was no effect of either SAH model on escape latency in the visual platform test. Similarly, Boykin et al. [19] found no motor deficits in the DB model at 3 weeks post-SAH. MWM deficits have largely been associated with CA1 injury, which was present in both models. The role of the mPFC in MWM performance appears complex. In the absence of SAH, rats with selective mPFC lesions were subjected to a MWM test similar to that used in this study and no deficits were observed [38]. Others have reported selective mPFC lesions can alter MWM performance when spatial cues are minimized or task demands are changed, which indicates an interaction between hippocampal and mPFC function in MWM performance [39, 40]. Because our lesions were not selective, further speculation regarding the neuroanatomical substrate for the cognitive deficits in each model is necessarily limited, but consistent with histologic damage to these structures.

There are several limitations to this study. The respective cognitive function tests were performed serially (Fig. 1) to prevent fatigue and to minimize interactions between testing conditions. It is plausible that different results for the respective measures would have been obtained if testing was performed at simultaneous intervals post-SAH. We have previously shown that cerebral blood flow deficits persist in the DB model for at least 21 days [9, 10]. To our knowledge, blood flow has not been investigated in the PB model beyond the first few hours post-SAH. Intracranial pressure was not measured in this study. Thus, the effects of any sustained intracranial hypertension or hydrocephalus on long-term cognitive function are unknown. Regardless, it is evident that SAH presents a pathologic evolution that may not have been completed at initiation of testing in our study. We also did not study a range of insult severities. Our primary goal was to determine if both models would present sustained cognitive deficits under conditions of similar insult severity, as defined by mortality rate. Indeed, this was found. However, it is likely that the spectrum of deficit and relative effects of the two models will likely differ with different severities of insult.

Conclusion

Cognitive deficits in the DB and PB rat SAH models were compared after sustained recovery from insults standardized on the basis of mortality rate. Both models produced measurable long-term deficits compared to saline-injected and naïve control rats, although the patten of injury differed between models. Both DB and PB caused transient rotarod deficits that normalized over 5 weeks. In the elevated plus maze, PB rats showed decreased anxiety with greater time spent in the open arms and more open arm entries. DB rats spent more time in the closed arms indicating increased anxiety. For novel object recognition, neither DB nor PB rats distinguished novel objects, in contrast to control groups, which did. For novel object location, both DB and PB rats spent less time exploring an object in a novel location compared to animals injected with saline. Both DB and PB caused histologic damage in hippocampal CA1, the perirhinal cortex, hippocampal CA1, and medial prefrontal cortex, but severities of injury

in respective regions differed between models. Cumulatively, this work demonstrates that both the DB and PB models produce sustained cognitive deficits. Each model offers unique properties and both models may be useful for study of SAH-induced cognitive deficits.

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Compliance with Ethical Standards

Conflicts of Interest None.

Human and Animal Rights All applicable institutional and/or national guidelines for the care and use of animals were followed.

References

1. Mayer SA, Kreiter KT, Copeland D, Bernardini GL, Bates JE, Peery S, et al. Global and domain-specific cognitive impairment and outcome after subarachnoid hemorrhage. Neurology. 2002;59(11):1750–8.
2. Kreiter KT, Copeland D, Bernardini GL, Bates JE, Peery S, Claassen J, et al. Predictors of cognitive dysfunction after subarachnoid hemorrhage. Stroke. 2002;33(1):200–8.
3. Sheldon S, Macdonald RL, Cusimano M, Spears J, Schweizer TA. Long-term consequences of subarachnoid hemorrhage: examining working memory. J Neurol Sci. 2013;332(1–2):145–7.
4. Latimer SF, Wilson FC, McCusker CG, Caldwell SB, Rennie I. Subarachnoid haemorrhage (SAH): long-term cognitive outcome in patients treated with surgical clipping or endovascular coiling. Disabil Rehabil. 2013;35(10):845–50.
5. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. Stroke. 2010;41(8):e519–36.
6. Prunell GF, Mathiesen T, Diemer NH, Svendgaard NA. Experimental subarachnoid hemorrhage: subarachnoid blood volume, mortality rate, neuronal death, cerebral blood flow, and perfusion pressure in three different rat models. Neurosurgery. 2003;52(1):165–75 (**discussion 75–6**).
7. Lee JY, Huang DL, Keep R, Sagher O. Characterization of an improved double hemorrhage rat model for the study of delayed cerebral vasospasm. J Neurosci Methods. 2008;168(2):358–66.
8. Lee JY, Sagher O, Keep R, Hua Y, Xi G. Comparison of experimental rat models of early brain injury after subarachnoid hemorrhage. Neurosurgery. 2009;65(2):331–43 (**discussion 43**).
9. Takata K, Sheng H, Borel CO, Laskowitz DT, Warner DS, Lombard FW. Long-term cognitive dysfunction following experimental subarachnoid hemorrhage: new perspectives. Exp Neurol. 2008;213(2):336–44.
10. Takata K, Sheng H, Borel CO, Laskowitz DT, Warner DS, Lombard FW. Simvastatin treatment duration and cognitive preservation in experimental subarachnoid hemorrhage. J Neurosurg Anesthesiol. 2009;21(4):326–33.
11. Jeon H, Ai J, Sabri M, Tariq A, Macdonald RL. Learning deficits after experimental subarachnoid hemorrhage in rats. Neuroscience. 2010;169(4):1805–14.
12. Liu Y, Li J, Wang Z, Yu Z, Chen G. Attenuation of early brain injury and learning deficits following experimental subarachnoid hemorrhage secondary to cystatin C: possible involvement of the autophagy pathway. Mol Neurobiol. 2014;49(2):1043–54.
13. Liu Y, Qiu J, Wang Z, You W, Wu L, Ji C, et al. Dimethylfumarate alleviates early brain injury and secondary cognitive deficits after experimental subarachnoid hemorrhage via activation of Keap1-Nrf2-ARE system. J Neurosurg. 2015;123(4):915–23.
14. Han SM, Wan H, Kudo G, Foltz WD, Vines DC, Green DE, et al. Molecular alterations in the hippocampus after experimental subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2014;34(1):108–17.
15. Macdonald RL. Subarachnoid hemorrhage and outcome. J Neurosurg. 2013;119(3):603–4.
16. Silasi G, Colbourne F. Long-term assessment of motor and cognitive behaviours in the intraluminal perforation model of subarachnoid hemorrhage in rats. Behav Brain Res. 2009;198(2):380–7.
17. Xie Y, Liu W, Zhang X, Wang L, Xu L, Xiong Y, et al. Human albumin improves long-term behavioral sequelae after subarachnoid hemorrhage through neurovascular remodeling. Crit Care Med. 2015;43(10):e440–9.
18. Milner E, Holtzman JC, Friess S, Hartman RE, Brody DL, Han BH, et al. Endovascular perforation subarachnoid hemorrhage fails to cause Morris water maze deficits in the mouse. J Cereb Blood Flow Metab. 2014;34:e1–e9. doi:10.1038/jcbfm.2014.108.
19. Boyko M, Azab AN, Kuts R, Gruenbaum BF, Gruenbaum SE, Melamed I, et al. The neuro-behavioral profile in rats after subarachnoid hemorrhage. Brain Res. 2013;1491:109–16.
20. Shen H, Chen Z, Wang Y, Gao A, Li H, Cui Y, et al. Role of neurexin-1beta and neuroligin-1 in cognitive dysfunction after subarachnoid hemorrhage in rats. Stroke. 2015;46(9):2607–15.
21. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn Schmiedebergs Arch Pharmacol. 1984;327(1):1–5.
22. Ennaceur A. One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res. 2010;215(2):244–54.
23. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process. 2012;13(2):93–110.
24. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 1984;11(1):47–60.
25. Samra SK, Giordani B, Caveney AF, Clarke WR, Scott PA, Anderson S, et al. Recovery of cognitive function after surgery for aneurysmal subarachnoid hemorrhage. Stroke. 2007;38(6):1864–72.
26. Guresir E, Schuss P, Borger V, Vatter H. Experimental subarachnoid hemorrhage: double cisterna magna injection rat model–assessment of delayed pathological effects of cerebral vasospasm. Transl Stroke Res. 2015;6(3):242–51.
27. Coyle P. Vascular patterns of the rat hippocampal formation. Exp Neurol. 1976;52(3):447–58.
28. Shah AA, Treit D. Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests. Brain Res. 2003;969(1–2):183–94.
29. Bertoglio LJ, Joca SR, Guimaraes FS. Further evidence that anxiety and memory are regionally dissociated within the hippocampus. Behav Brain Res. 2006;175(1):183–8.
30. Morris PG, Wilson JT, Dunn L. Anxiety and depression after spontaneous subarachnoid hemorrhage. Neurosurgery. 2004;54(1):47–52 (**discussion 52–4**).
31. Visser-Meily JM, Rhebergen ML, Rinkel GJ, van Zandvoort MJ, Post MW. Long-term health-related quality of life after aneurysmal subarachnoid hemorrhage: relationship with psychological symptoms and personality characteristics. Stroke. 2009;40(4):1526–9.

32. Fontanella M, Perozzo P, Ursone R, Garbossa D, Bergui M. Neuropsychological assessment after microsurgical clipping or endovascular treatment for anterior communicating artery aneurysm. Acta Neurochir (Wien). 2003;145(10):867–72 **(discussion 72)**.
33. von Vogelsang AC, Forsberg C, Svensson M, Wengstrom Y. Patients experience high levels of anxiety 2 years following aneurysmal subarachnoid hemorrhage. World Neurosurg. 2015;83(6):1090–7.
34. Ronne-Engstrom E, Enblad P, Lundstrom E. Outcome after spontaneous subarachnoid hemorrhage measured with the EQ-5D. Stroke. 2011;42(11):3284–6.
35. Al-Khindi T, Macdonald RL, Schweizer TA. Decision-making deficits persist after aneurysmal subarachnoid hemorrhage. Neuropsychology. 2014;28(1):68–74.
36. Escartin G, Junque C, Juncadella M, Gabarros A, de Miquel MA, Rubio F. Decision-making impairment on the Iowa Gambling Task after endovascular coiling or neurosurgical clipping for ruptured anterior communicating artery aneurysm. Neuropsychology. 2012;26(2):172–80.
37. Mavaddat N, Kirkpatrick PJ, Rogers RD, Sahakian BJ. Deficits in decision-making in patients with aneurysms of the anterior communicating artery. Brain. 2000;123(Pt 10):2109–17.
38. de Bruin JP, Sanchez-Santed F, Heinsbroek RP, Donker A, Postmes P. A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. Brain Res. 1994;652(2):323–33.
39. Compton DM, Griffith HR, McDaniel WF, Foster RA, Davis BK. The flexible use of multiple cue relationships in spatial navigation: a comparison of water maze performance following hippocampal, medial septal, prefrontal cortex, or posterior parietal cortex lesions. Neurobiol Learn Mem. 1997;68(2):117–32.
40. Jo YS, Park EH, Kim IH, Park SK, Kim H, Kim HT, et al. The medial prefrontal cortex is involved in spatial memory retrieval under partial-cue conditions. J Neurosci. 2007;27(49):13567–78.