

# Laser-Doppler Scanning of Local Cerebral Blood Flow and Reserve Capacity and Testing of Motor and Memory Functions in a Chronic 2-Vessel Occlusion Model in Rats

Peter T. Ulrich, MD; Stefan Kroppenstedt, MD; Axel Heimann, DVM; Oliver Kempfski, PhD, MD

**Background and Purpose**—An animal model of incomplete forebrain ischemia resembling human hemodynamic insufficiency was established. The model allows examination of acute and chronic changes of local cerebral blood flow (ICBF) and reserve capacity in correlation with behavioral parameters.

**Methods**—Anesthetized male Wistar-Kyoto rats underwent bilateral carotid occlusion (BCO). Laser-Doppler scanning of ICBF at baseline conditions and after acetazolamide was done 30 minutes after BCO, motor and memory function tests were administered after 1 and 2 days, and both investigations were repeated after 1, 2, 4, and 6 weeks. A sham-operated and a control group without any vessel manipulation served as controls.

**Results**—ICBF dropped within 60 minutes after surgery by 62% ( $P < 0.001$ ) in 10 animals surviving BCO (BCO<sub>survival</sub>) and by 69% in 5 rats that died within 9 days (BCO<sub>lethal</sub>). Acetazolamide increased ICBF to 142.33% in controls, to 136.66% in sham-operated rats (both significant), and to 104.80% in BCO<sub>survival</sub> (not significant), and it decreased flow by 23.1% in BCO<sub>lethal</sub> rats ( $P < 0.001$ ). Baseline ICBF normalized within 4 weeks. Total motor function scores were significantly reduced from 9 points preoperatively to  $5.80 \pm 0.65$  in BCO<sub>lethal</sub> and  $6.68 \pm 0.54$  points in BCO<sub>survival</sub> rats 1 day after occlusion. Memory retention function remained impaired after BCO, as did the acetazolamide response, which correlated with motor score and was inversely related to maze exploration time.

**Conclusions**—This model allows long-term follow-up of cerebral function, ICBF, and reserve capacity in a pathophysiological setting similar to hemodynamic insufficiency in humans. (*Stroke*. 1998;29:2412-2420.)

**Key Words:** acetazolamide ■ cerebral blood flow ■ cerebral ischemia ■ cerebrovascular circulation ■ rats

The assessment of cerebrovascular reserve capacity has become a frequently used tool in clinical practice.<sup>1-6</sup> Impaired vasoreactivity has been used as an indicator of an increased stroke risk due to hemodynamic compromise in cases of obliterative arterial disease.<sup>7,8</sup> Measurements of cerebral blood flow (CBF) and reserve capacity<sup>9,10</sup> revealed a considerable variability of results in healthy individuals and even more in stroke patients. Little is known about the natural history of impaired cerebrovascular reserve capacity after hemodynamic stroke. Experimental results of Coyle and Panzenbeck<sup>11</sup> in rats showed an increase of the luminal diameter of basilar carotid anastomoses to 186% 6 weeks after ipsilateral carotid artery ligation but an insufficient recovery of local CBF (ICBF) compared with controls within that period. However, persisting alterations of reserve capacity with permanently diminished or restored basal CBF are well known in humans after cerebrovascular insults. It has been postulated that those individuals should be prone to cerebral ischemia<sup>12</sup> in cases of hemodynamic stress situations such as hypotensive episodes.<sup>8</sup>

## See Editorial Comment, page 2420

The goal of the present study was to establish a rat model of chronic incomplete forebrain ischemia suitable to frequently monitor ICBF and reserve capacity. To achieve this, bilateral carotid artery occlusion (BCO) was induced in rats as a model of hemodynamic insufficiency, and reserve capacity was assessed repeatedly for 6 weeks together with motor and memory functions.

## Materials and Methods

Twenty-nine male Wistar-Kyoto rats weighing 304 to 445 g (mean,  $353 \pm 12.09$  g) were used (Charles River Wiga Company, Sulzfeld, Germany). The animals were housed in cages with a 12-hour light/dark cycle and a temperature of 22°C. They had unlimited access to food pellets and water except for 1 day before memory retention testing (see below). All procedures were in accordance with institutional and governmental guidelines.

## Surgical Preparation

### Anesthesia

The rats were anesthetized with chloral hydrate (36 mg/100 g IP) and supplemented as needed. A 20-gauge catheter filled with heparinized

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From the Neurosurgical Department, Municipal Hospital, Offenbach (P.T.U.), and the Institute of Neurosurgical Pathophysiology, University Hospital, Mainz, Germany.

Correspondence to Oliver Kempfski, PhD, MD, Institute for Neurosurgical Pathophysiology, Johannes Gutenberg University Mainz, Langenbeckstrasse 1, 55131 Mainz, Germany. E-mail kempfski@nc-patho.klinik.uni-mainz.de

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saline (100 U/mL) was inserted into the tail artery for continuous monitoring of mean arterial blood pressure and arterial blood sampling. Normothermia (37.4°C) was maintained by a rectal thermistor probe connected to a feedback-regulated heating blanket.

### **Surgery**

The sham-operated controls (n=9) and the rats that were to undergo carotid occlusion (n=15) were placed in supine position on the heating blanket. Both common carotid arteries (CCAs) were exposed over a midline incision, and a dissection was made between the sternocleidomastoid and the sternohyoid muscles parallel to the trachea. Each CCA was freed from its adventitial sheath and vagus nerve, which was carefully separated and maintained. In 5 control animals the carotid arteries were not exposed.

In 15 rats BCO was initiated (BCO group) with a 7-0 monofilament suture (Ethilon) circled around the left and the right arteries and guided outside through 5-cm-long elastic tubing. The snares on both sides were fixed together with the silicone elastomer tubes by clamps. The same preparation was performed in 9 rats of the sham group. Before the animals were turned and the heads were fixed in a stereotaxic frame, the skin was closed with stitches, sparing the tubes with the sutures around the CCA for later carotid artery occlusion.

The epicranium was exposed by a parieto-occipital midline skin incision in all animals. With the use of microsurgical technique, the periosteum was pushed back, and biparietal parasagittal groove-shaped trephinations (1.5×4 mm) were performed with a microdrill (Mikroton, Aesculap) during continuous irrigation with saline to prevent heating of the tissue. Special care was taken not to penetrate the dura mater. Therefore, a thin layer of the tabula interna of the calvarium was spared.

## **ICBF Measurement**

### **Baseline ICBF**

To monitor the cortical microcirculation, a laser-Doppler (LD) flow probe (needle-shaped, 0.8 mm), mounted on a micromanipulator and connected to a laser-flow blood perfusion monitor (Laserflo 403A, Vasamedics), was used.

ICBF data were collected from 25 locations on each side by moving the LD probe in 0.1-mm steps over the brain surface. Care was taken to obtain flow readings only from areas free of large pial vessels. ICBF was expressed in LD units (LDU). Scanned flow data were used to calculate frequency histograms with a width of the flow classes of 5 LDU and a range between 0 and 150 LDU. Observation frequency was mathematically normalized to 100% and plotted. Details of the technique have been published earlier.<sup>13</sup>

After a stabilization period of 15 minutes, a baseline scan was performed on both hemispheres. Flow data were saved on-line on a PC. Each scan took ≈7 minutes. Then the left and shortly thereafter the right CCA were occluded by pulling the sutures tight. During this procedure the head remained fixed in the stereotaxic frame, thereby securing the identical position of the scanning points for the subsequent measurements. In the sham group, sutures were not pulled tight and were removed at the end of the experiment. Fifteen minutes after carotid occlusion, a second scan on both hemispheres was performed. In the control group without exposure of the carotid arteries, both basal scans were performed without vessel manipulation.

### **Determination of Cerebrovascular Reserve Capacity by Acetazolamide**

The cerebrovascular reserve capacity is defined as change of ICBF after application of the inhibitor of carbonic anhydrase, acetazolamide, expressed in percentage of baseline flow.<sup>14</sup> According to experimental studies with LD monitoring of the cortical microcirculation in cats<sup>15</sup> and rats,<sup>16</sup> the vasodilatory effect of acetazolamide begins 4 to 8 minutes after intravenous or intraperitoneal application, reaches a maximum after 20 minutes, and lasts ≈60 minutes.

Thirty minutes after carotid occlusion or exposure, an intraperitoneal injection of 0.1 mg/g body wt acetazolamide (Diamox, Lederle)

was applied. Seventeen minutes after the injection, a third bilateral LD scan was initiated in all 3 groups.

After completion of these 3 LD scans, the animal was turned to the supine position, thereby strictly avoiding recirculation in the BCO group. Permanent occlusion of both CCAs by double ligation with 7-0 silk sutures concluded the acute phase of the experiment. Measurements of ICBF and acetazolamide response were repeated with the animals under chloral hydrate anesthesia after 1, 2, 4, and 6 weeks.

Readings from all single scan points of each animal were usually not normally distributed. Therefore, from the 50 scanning points of each animal a median flow was calculated. Median values of all animals were then averaged and presented ±SEM.

## **Test Battery**

The neurological test battery was always performed between 9 and 12 AM. The labyrinth test (memory retention test) was done first and the motor function tests second.

### **Memory Retention Test**

The rats were tested in a 4-arm wooden maze installed in a darkened, quiet room. Each roofless arm (650×180×160 mm) projected from a square central chamber (420×420×400 mm) with 4 openings (160×160 mm) to the arms. Each arm contained at its far end a 150×160×180-mm chamber formed by 2 pieces of wood (at an 80-mm distance) that fit in notches in each of the 2 walls. The plates overlapped each other, preventing the rats from directly looking from the inner part of the arm into the outer chamber but permitting the rats access to the arm of the maze. One of these chambers contained food pellets and was kept dark by a removable roof, while the other chambers and the central compartment were highly illuminated by lighting a 100-W bulb 1 m above it whenever the rat entered to set an averse stimulus and to establish a passive avoidance reaction. Maze adaptation of the rat started after 24 hours of food deprivation 2 days before the initial CBF measurement and was repeated 1 day thereafter to test memory retention. The rat was placed in the central chamber of the maze covered by an opaque box. When the cover was removed, the light source was switched on, and a stopwatch was started. Whenever the rat exploring the maze entered 1 of the 3 open chambers in the arms or the central part of the maze, that compartment was immediately illuminated. The trial ended as soon as the rat entered the dark compartment and remained there or after 300 seconds of unsuccessful exploration of the maze. Three compartments in each arm and the central chamber were defined as separate locations within the maze. Every change of location was counted, and a mean frequency of movements was computed from 3 trials. Exploration times from 3 trials were averaged.

### **Motor Performance Tests**

We examined motor performance with an inclined screen test, a balance beam test, and the prehensile traction test according to Combs and D'Alecy<sup>17</sup> with minor modifications.

In the inclined screen test, a 300×300-mm board covered with a cork pad was mounted on a pole and pivoted on the rims of a wooden case 700 mm above a thick sponge pad. The trial started after the rat was placed on the horizontal board. By rotation of the pole slowly but continuously to each side, the plane was inclined to a maximum angle of 60 degrees. The rat scored 3 points when spending 21 to 30 seconds on the board, 2 points for 11 to 20 seconds, 1 point for up to 10 seconds, and 0 points when it fell down immediately or within the first 3 seconds.

In the balance beam test, a wooden rod 700 mm long and 25 mm wide was positioned horizontally 700 mm above the sponge pad. The rat was placed at the center of the rod. The score was 0 if the rat lost hold within 3 seconds, 1 if the rat was able to stay on the beam for up to 10 seconds, 2 if the time on the rod was between 11 and 20 seconds, and 3 for spending ≥21 seconds on the beam.

In the prehensile traction test, a nylon rope, 700 mm long with a diameter of 5 mm, was stretched horizontally between the rims of the case with the sponge pad on its bottom. The rat was permitted to grab the rope with its forefoot pads, and the animal was released. The time

TABLE 1. Experimental Protocol

Wek	Day	Protocol
1	1	Maze test, motor function test battery
	2	Maze test, motor function test battery
	3	ICBF measurement
		Surgery: control, sham, BCO groups
		ICBF and reserve capacity measurement
2	4	Maze test, motor function test battery
	5	Maze test, motor function test battery
	9	Maze test, motor function test battery
3	10	ICBF and reserve capacity measurement
	11	Maze test, motor function test battery
	16	Maze test, motor function test battery
4	17	ICBF and reserve capacity measurement
	18	Maze test, motor function test battery
	30	Maze test, motor function test battery
6	31	ICBF and reserve capacity measurement
	32	Maze test, motor function test battery
	44	Maze test, motor function test battery
	45	ICBF and reserve capacity measurement

the rat remained on the rope was measured. The score was 0 for <2 seconds, 1 for 3 to 4 seconds, 2 for  $\geq 5$  seconds without bringing a third limb up to the rope, and 3 for  $\geq 5$  seconds bringing 1 or both hind paws up to the rope.

The total motor score was calculated as the sum of the scores for the screen, balance beam, and prehensile traction tests. The scores of each test were averaged from 3 trials performed in sequence with a few minutes of rest between tests.

The test battery was repeated on days 4 and 5 of the experiment; 1 day before and 1 day after the ICBF follow-up measurements after 1, 2, and 4 weeks; and 1 day before the final reserve capacity test after 6 weeks (Table 1).

### Statistical Analysis

Descriptive statistics, tests for normal distribution, and correlation analyses were performed with Sigma Stat, and illustrations were done with Excel (Microsoft). For nonparametric tests, the Kruskal-Wallis 1-way ANOVA on ranks or the Friedman repeated-measures ANOVA on ranks and Dunn's method for multiple comparisons were used. Medians in collectives lacking a normal distribution are given; means are presented  $\pm$ SEM. Differences are considered significant at  $P < 0.05$ .

## Results

### Acute Stage

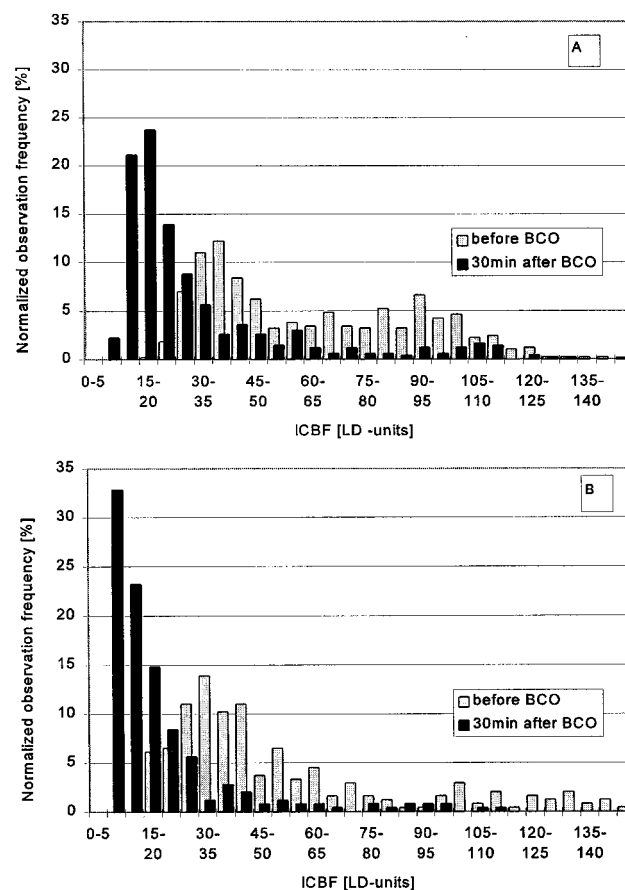
#### Basal ICBF and Acetazolamide Response in Control and Sham-Operated Groups

Fourteen rats served as control group, which consisted of 5 rats without exposure of the CCA and 9 rats that had undergone sham operation. The mean baseline ICBF averaged from the medians of 9 sham-operated animals with 450 individual scan points was  $48.00 \pm 2.23$  LDU. The value was  $47.00 \pm 1.84$  LDU in the control group with 150 individual scan points. In these 5 animals without exposure of the CCAs, the mean of the second measurement 30 minutes later was  $46.60 \pm 2.11$  LDU. In the sham-operated group ( $n=9$ ), the mean of the second measurement was  $37.50 \pm 1.83$  LDU.

After intraperitoneal application of 0.1 mg/g body wt acetazolamide, the mean ICBF rose significantly to  $70.70 \pm 1.49$  LDU in the group without any vessel preparation and to  $61.00 \pm 1.33$  LDU in the sham-operated group. Therefore, the mean response of ICBF to acetazolamide challenge was an increase to  $142.33 \pm 4.73\%$  in the control group and to  $136.66 \pm 2.88\%$  in the sham group (Figure 3). In both groups the increases were statistically significant.

#### Basal ICBF and Acetazolamide Response in BCO Group

In 15 animals both CCAs were occluded (BCO group). In 3 rats ischemia was lethal within 12 hours after occlusion, and in 2 ischemia was lethal between days 7 and 9. These 5 rats are hereafter referred to as the BCO<sub>lethal</sub> subgroup, with the survivors referred to as the BCO<sub>survival</sub> subgroup. All animals in the BCO<sub>lethal</sub> subgroup had severely impaired neurological function on the first day after occlusion (see below). Thirty minutes after occlusion, the median ICBF in the BCO<sub>survival</sub>



**Figure 1.** A, BCO<sub>survival</sub> group: flow observation frequency histogram from calculated 500 scan points (50 locations in 10 rats) before and 30 minutes after BCO. The histogram is shifted to the left, and >50% of all measurements are <20 LDU, whereas under baseline conditions only 1% of measurements are <20 LDU. The drop of the median value by 62% is significant ( $P=0.001$ ). B, BCO<sub>lethal</sub> group: flow observation frequency histogram calculated from 250 scan points (50 locations in 5 rats) before and 30 minutes after BCO. The majority of all measurements is <10 LDU after CCA occlusion. The 69% drop of the median value is significant ( $P=0.001$ ). Differences between the 2 BCO subgroups are not significant (Mann-Whitney rank sum test) either before BCO ( $P=0.842$ ) or 30 minutes after BCO ( $P=0.187$ ).

**TABLE 2. Mean Arterial Blood Pressure and Biochemical Data From Arterial Blood Samples**

	Sham		BCO	
	Baseline	30-Min Sham	Baseline	30-Min BCO
MABP, mm Hg	85.87±4.17	92.13±2.68	87.06±2.86	98.18±3.76
pH	7.381±0.010	7.367±0.013	7.383±0.011	7.367±0.015
Pco <sub>2</sub> , mm Hg	45.41±2.31	44.22±1.65	44.16±1.80	44.11±2.06
Po <sub>2</sub> , mm Hg	79.84±3.08	79.29±2.41	75.53±2.67	70.11±1.65
Hematocrit	0.37±0.014	0.35±0.015	0.38±0.008	0.39±0.014
Glucose, mmol/L	143.79±9.78	142.37±11.28	146.00±8.66	143.25±17.76
HCO <sub>3</sub> , mmol/L	26.55±1.25	25.50±1.10	25.92±0.61	24.92±0.44
O <sub>2</sub> saturation	0.94±0.01	0.94±0.01	0.93±0.01	0.91±0.02

Values are mean±SEM. MABP indicates mean arterial blood pressure. Blood samples were taken during surgery from the tail artery in sham-operated controls (n=9) at baseline conditions and 30 minutes after sham operation and in the BCO group (n=10) at baseline conditions and 30 minutes after BCO. In both groups, changes between baseline and postoperative values failed to reach statistical significance. Differences between the sham and the BCP groups also failed to reach statistical significance.

subgroup had dropped significantly ( $P<0.001$ ) by 62% from  $55.50\pm 4.78$  to  $21.00\pm 1.2$  LDU (Figure 2). In the 5 rats with lethal ischemia, ICBF decreased also significantly ( $P<0.001$ ) by 69% from  $42.5\pm 2.22$  to  $13.00\pm 1.33$  LDU. Figure 1 presents the frequency histograms of ICBF before and 30 minutes after carotid occlusion. The frequency maximum in the BCO<sub>survival</sub> subgroup (Figure 1A) shifted after occlusion from the flow class 36 to 40 LDU to 16 to 20 LDU; in the BCO<sub>lethal</sub> subgroup (Figure 1B), the frequency maximum shifted from 31 to 35 LDU to 6 to 10 LDU. Fifty-six percent of the ICBF values in the BCO<sub>lethal</sub> subgroup were <15 LDU, compared with 0% before occlusion. The corresponding rates in the BCO<sub>survival</sub> subgroup are 23.28% and 0%, respectively.

Acetazolamide application in the BCO<sub>survival</sub> subgroup produced a minimal change of the ICBF mean from  $21.00\pm 1.2$  to  $22.00\pm 1.81$  LDU, which is a nonsignificant 4.8% rise. In the BCO<sub>lethal</sub> subgroup, acetazolamide caused a significant ( $P<0.001$ ) 23.1% flow decline from  $13.00\pm 1.33$  to  $10.00\pm 1.57$  LDU.

Table 2 depicts mean arterial blood pressure, pH, gases, and glucose from arterial blood samples of all groups during surgery. There were no significant differences.

### Chronic Stage

Preparation of the skull grooves for follow-up ICBF measurements after reopening of the scalp wound required in most cases the removal of minor amounts of scar tissue on the

preserved thin layer of tabula interna. There never was any leakage of cerebrospinal fluid. In no case were signs of inflammation visible.

Table 3 shows the mean body weight of the control animals and the BCO group at the individual measurement dates. There was a significant weight loss in the sham group 2 weeks after surgery ( $P<0.05$ ) and in the BCO group 1 week after surgery ( $P<0.05$ ). The differences between the control, sham, and BCO groups did not reach significance at any time.

Figure 2 depicts the mean baseline ICBF at 30 minutes and 1, 2, 4, and 6 weeks after surgery of the control group and the BCO<sub>survival</sub> subgroup. The ICBF means of the BCO group show a gradual rise but decrease after 4 weeks, again differing at all measurements significantly ( $P<0.05$ ) from the values of the control group. The means of the sham-operated group were significantly lower than the means of the control group in baseline scans of the first measurement (day 3 of the experiment) and the last measurement 6 weeks thereafter.

The percent acetazolamide responses in the 3 groups at the various follow-up dates are shown in Figure 3. Values of the BCO group were reduced and significantly differed at 30 minutes and 2 and 4 weeks from both the control and the sham groups.

### Memory and Motor Function Test Battery

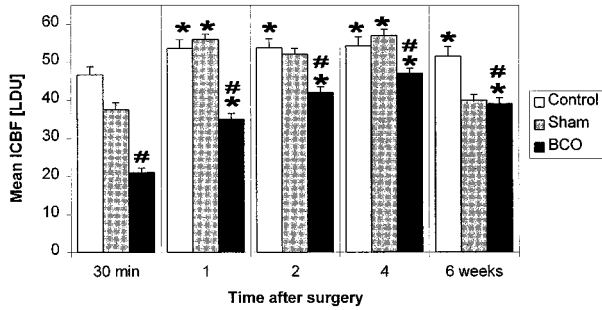
The learning effect reduced the labyrinth exploration time (Figure 4A) within the 2-day training phase before surgery

**TABLE 3. Body Weight of Control, Sham, and PCO Groups Before Surgery (Day 3) and at Follow-Up Measurements**

	Day of Experiment				
	3	9	16	30	44
Control	360.00±14.41	352.00±14.54	350.00±13.30	355.40±11.12	363.40±12.29
Sham	343.89±3.93	331.00±7.61	327.44±7.74*	346.56±9.03	350.78±6.48
BCO	357.70±12.09	322.30±8.96*	330.40±6.77	347.90±5.97	365.60±6.19

Values are means±SEM, expressed in grams. Differences between the groups did not reach significance at any time.

\* $P<0.05$  vs baseline values of the group.

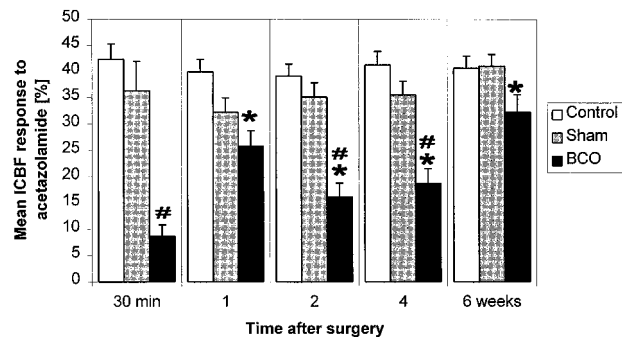


**Figure 2.** Baseline rCBF data (medians calculated from 50 measurements per animal) 30 minutes and 1, 2, 4, and 6 weeks after surgery of controls (no vessel manipulation, n=5), sham-operated rats (n=9), and animals with BCO (n=10). \**P*<0.05 vs 30-minute values; #*P*<0.05 vs sham and control groups.

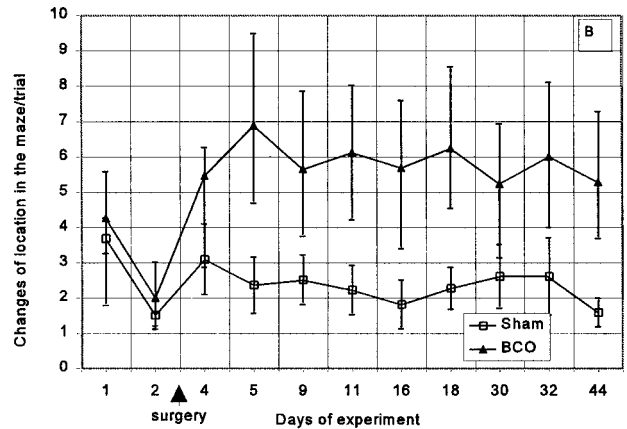
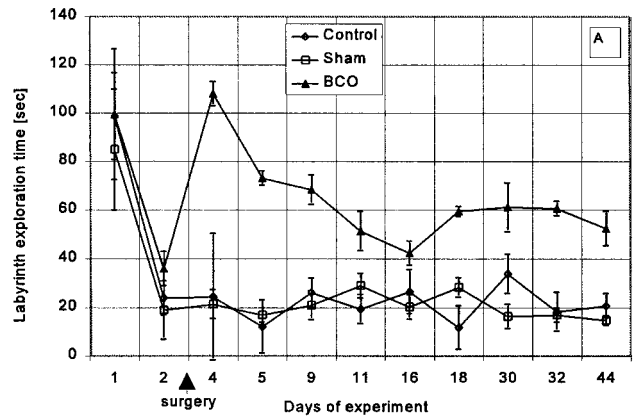
from  $98.80 \pm 27.12$  to  $24.00 \pm 4.89$  seconds in control rats, from  $109.78 \pm 24.84$  to  $50.33 \pm 11.95$  seconds in the sham group, and from  $99.60 \pm 18.07$  to  $36.00 \pm 7.00$  seconds in the BCO group. Differences between these groups were not significant at baseline conditions. The maze exploration time remained unchanged during the entire follow-up period in control and sham-operated rats, while it was significantly prolonged in the BCO group. Differences between controls or sham-operated animals versus the BCO group were significant 2 days after carotid occlusion, before the ICBF measurements after 1 and 6 weeks, and after the measurements 1, 2, and 4 weeks after BCO. There were no significant differences between the control and the sham groups at any time (Figure 4A). Animals of the BCO<sub>lethal</sub> subgroup after occlusion had an initial mean labyrinth time of  $116 \pm 46.29$  seconds, which did not differ significantly from that of the BCO<sub>survival</sub> subgroup at  $108 \pm 18.07$  seconds.

BCO rats showed significantly more futile tries in the maze and needed more time to find their way into the dark compartment than the sham-operated animals (Figure 4B).

The baseline total motor function score (maximum, 9.00 points) (Figure 5A) reached means of  $8.72 \pm 0.07$  points in controls,  $8.94 \pm 0.03$  in the sham group, and  $8.99 \pm 0.001$  in the BCO group. One day after BCO, the motor score dropped to  $5.80 \pm 0.65$  points in the BCO<sub>lethal</sub> subgroup and to  $6.68 \pm 0.54$  points in the BCO<sub>survival</sub> subgroup. The score of the BCO<sub>lethal</sub> subgroup was significantly reduced (*P*=0.008)



**Figure 3.** Averaged median ICBF responses to acetazolamide (medians calculated from 50 measurements per animal); time intervals and groups are as in Figure 2. \**P*<0.05 vs 30-minute values; #*P*<0.05 vs sham and control groups.

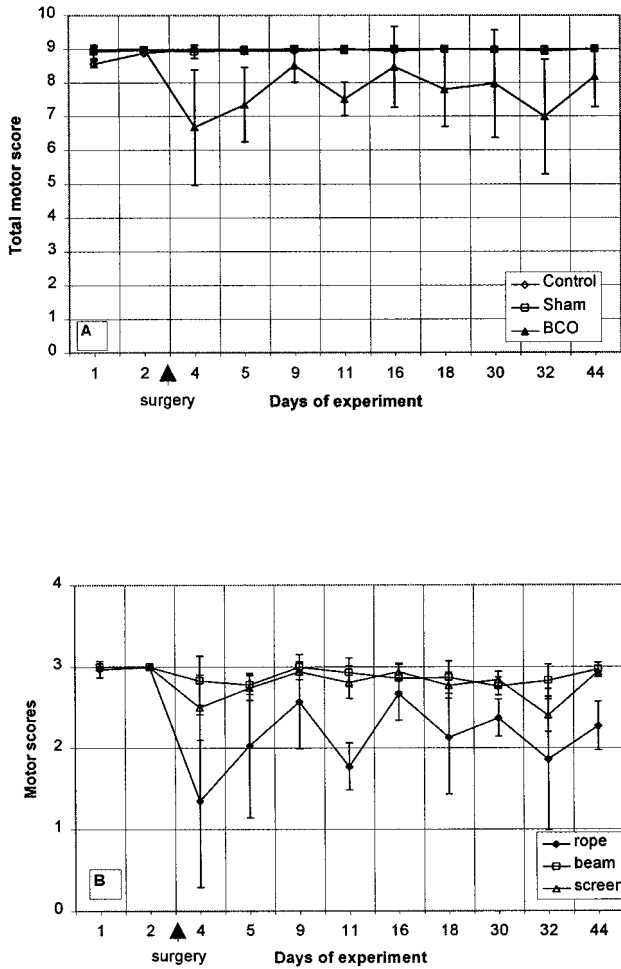


**Figure 4.** A, Labyrinth exploration time of the control, sham, and BCO groups in the training phase before surgery (trials 1 and 2), immediately thereafter, and before and after the follow-up CBF measurements. B, Changes of location in the maze, averaged for 1 trial (BCO group). Times of measurements are as in panel A.

compared with the baseline score before occlusion. In the BCO<sub>survival</sub> subgroup, the scores on day 5 ( $7.35 \pm 0.36$  points) and day 11 ( $7.51 \pm 0.39$  points) and the scores after 4 weeks (day 32:  $6.99 \pm 0.54$ ) were still significantly (*P*<0.05) diminished compared with the baseline score before BCO. During the entire follow-up period, scores never changed significantly in controls (*P*>0.999) and sham-operated rats (*P*=0.667).

Rats with reduced acetazolamide response after BCO showed a trend (*r*=−0.304) toward longer labyrinth exploration times than rats with normal or near normal acetazolamide responses (Figure 6B). A positive correlation (*r*=0.63; *P*<0.001) was found between the acetazolamide reactivity and prehensile traction test scores after the ICBF and reserve capacity measurements (Figure 7): rats with normal acetazolamide response could hold onto the rope longer than rats with a reduced response.

When the results of the 3 motor test scores are compared, the prehensile traction test reached the highest sensitivity, while most of the rats managed to complete the beam test. The time profile of the score distribution was similar in the prehensile



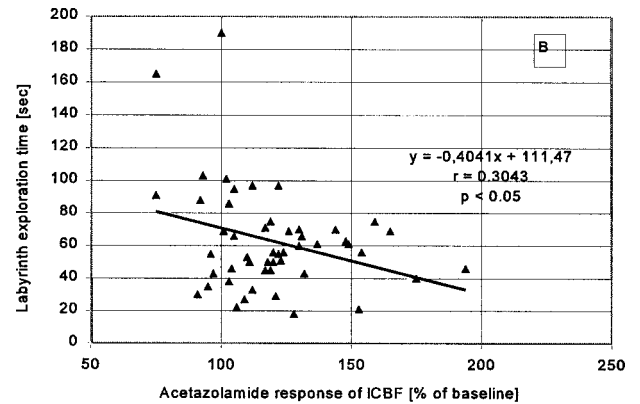
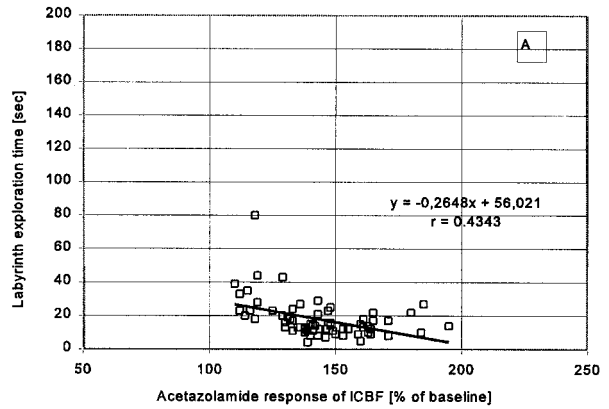
**Figure 5.** A, Total motor function score (maximum: 9 points) of the control, sham, and BCO groups in the training phase before surgery (trials 1 and 2), immediately thereafter, and before and after the follow-up CBF measurements. B, Prehensile traction (rope), beam, and screen test scores (maximum in each test: 3 points) of the BCO group. Time intervals are as in panel A.

traction test and the screen test, with minimum values on days 4 and 32. It is interesting to note that there were significant deteriorations of the prehensile traction scores at days 11, 18, and 32, ie, secondary to the ICBF measurements and acetazolamide tests performed with the animals under chloral hydrate anesthesia on days 10, 17, and 31.

There was no correlation between the maze exploration time and the scores of the prehensile traction test.

### Discussion

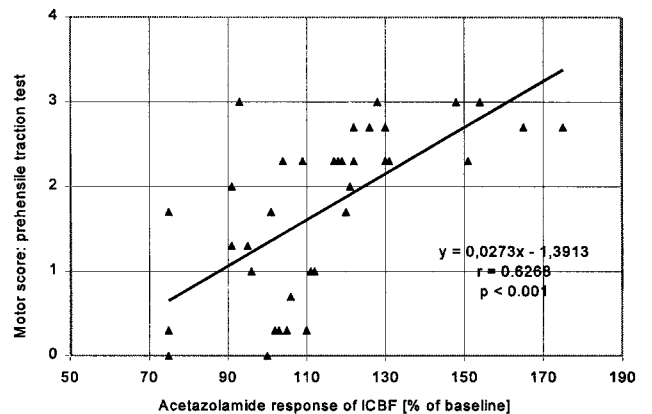
The 2-vessel occlusion model, proposed by Eklöf and Siesjö<sup>18</sup> in 1972 and modified by Smith et al<sup>19</sup> in 1984 without the induction of systemic hypotension, has been described as not leading to critical reductions of cortical CBF and not compromising the brain energy state<sup>20</sup> without induction of systemic hypotension. Therefore, the model seemed suitable for chronic survival studies, offering a low experimental failure rate from acute death of the animal. In modification of the original concept, we chose a permanent occlusion of the CCAs to create a chronic hemodynamic insufficiency com-



**Figure 6.** A, Correlation between ICBF responses to acetazolamide and labyrinth exploration times of control and sham-operated rats determined 1 day after ICBF measurements. B, Correlation between the ICBF responses to acetazolamide and labyrinth exploration times of BCO rats determined 1 day after ICBF measurements.

parable to human pathology and to investigate the spontaneous course of baseline CBF and perfusion reserve.

To compensate for the high variability of single LD flow readings found in cerebral cortex<sup>21</sup> and the absence of a calibration of LD flow data to absolute units, LD scanning



**Figure 7.** Correlation between ICBF responses to acetazolamide and prehensile traction test motor score of BCO rats determined 1 day after ICBF measurements.

was introduced.<sup>13,22,23</sup> The analysis of flow observation frequency histograms allows discrimination of the different effects of acetazolamide on microcirculation and on larger cortical vessels. A detailed correlation of anatomic structures and ICBF findings (S. Kroppenstedt, MD, unpublished data, 1997) shows that the most pronounced flow increase occurs in regions with a flow <60 LDU (microcirculation), while the flow in the vicinity of larger vessels remains largely unaffected. The flow histograms reveal that flow readings possibly influenced by large pial vessels (>90 LDU) were obtained from <15% of all locations. A distinct evaluation of flow from locations with baseline ICBF values <60 LDU, ie, from the microcirculation (S. Kroppenstedt, MD, unpublished data, 1997), yielded a reduction of flow in acute ischemia from a median 38 to 20 LDU, ie, a 47.4% reduction, which is somewhat less pronounced than the 62% seen if all ICBF values are considered. Interestingly the occlusion of carotid arteries appears to rather homogeneously reduce LD flow, since median flow at all measured locations was 21 LDU. Acetazolamide increased flow from 20 to 21 LDU in the <60 LDU subgroup, which is a 5% increase comparable to the 4.8% in the total population. During the later course of the experiment, changes observed are comparable to those seen in the total population. A similar analysis during the chronic stage is hampered by the fact that locations of measurement cannot be identified again after the animal has been removed from the stereotaxic frame.

Analysis of the ICBF data of the sham group reveals a higher variability than expected. The ICBF means after preparation of CCAs were significantly ( $P<0.05$ ) lower than those of the control rats, which had no manipulation of the vessels. Nevertheless, the acetazolamide response in this phase was not impaired compared with the control group. As a possible cause of the lowered and unstable perfusion in the sham group, the mechanical irritation of sympathetic nerves on the vessel walls must be considered. An impairment of the neurogenic component of cerebral autoregulation<sup>24,25</sup> may contribute to a transient perfusion deficit. The ICBF measurement 1 week after the vessel manipulation yielded a complete recovery. To detect such effects, the control group without any manipulation of neck or intracranial vessels was useful. Six weeks after surgery, baseline ICBF in all groups declined. This phenomenon might be due to the thickening of the remaining bony layer by healing processes. To avoid leakage of cerebrospinal fluid, we did not drill down to the dura after removal of some superficial scar tissue. Adaptation of the animals to the procedures and decreasing stress while anesthesia doses remained constant may also have contributed to the decline of ICBF values.

BCO led to an immediate drop of cortical perfusion in both hemispheres by 62% (by 69% in the rats that did not survive). Eklöf and Siesjö,<sup>18</sup> using a similar model, estimated a perfusion decrease of 50% calculated from the cerebral arteriovenous oxygen difference. They observed an inhomogeneous flow distribution but no influence on the energy state of the brain unless the flow reduction exceeded 45% of normal values. Choki et al<sup>26</sup> describe in their model of permanent BCO in Wistar rats a reduction of flow between 38% and 9%, depending on the brain structure measured. Similar to our

model, Tsuchiya et al<sup>27</sup> reported a mortality of 21% in their spontaneously breathing Wistar rats after BCO and a reduction of cortical CBF to 25% to 39%. Lower  $PO_2$  and no tendency to hyperventilation in our Wistar-Kyoto rats may be due to the deep anesthesia that we had to maintain to avoid head movements in the stereotaxic frame and dislocations of the LD flow probe.

Because a calibration of LD flowmetry data with absolute perfusion values (milliliters per 100 g per minute) is not possible, data expressed in LD units should be interpreted with caution. A detailed analysis, however, has shown that the biological zero in our system is very low, ie, 0 to 2 LDU, and that repeated measurements in many control cases yield similar median ICBF readings, which represent regional CBF.<sup>13,23</sup> Therefore, it may be concluded that BCO may well produce critical flow levels that can be detected by LD. When the 23% mortality rate in the BCO group is considered, median cortical flow values <20 LDU are likely to be in a critical range.

Even more pronounced than the depression of baseline flow values is the reduction of the acetazolamide response in the BCO group still seen 4 and 6 weeks after BCO. The acetazolamide response was severely diminished initially in the BCO<sub>survival</sub> animals and was inverse in the animals with lethal ischemia. Only in the early postocclusion phase did a temporary improvement of the acetazolamide reaction occur in BCO<sub>survival</sub> animals. This phenomenon may be partly explained by a more homogeneous perfusion of the available capillary bed, sufficient for a temporary compensation of an acute state of incomplete ischemia and a transient recovery of the reserve capacity. This has been described as the mechanism of flow increase in hypercapnia by Goebel and coworkers,<sup>28</sup> since a recruitment of nonperfused capillaries could be excluded. An exhaustion of this reserve can be suspected because the acetazolamide response was again markedly reduced after 2 weeks. Studies of Coyle and Panzenbeck<sup>11</sup> show that basilar carotid anastomoses widen within 6 weeks after unilateral permanent CCA occlusion and a temporary ligation of the contralateral CCA.

Possible negative effects of acetazolamide on ischemic tissue must be considered. The substance that inhibits the enzyme carbonic anhydrase causes hypercapnia and decreases the pH of nonischemic areas, leading to vasodilatation, increases of intracranial pressure, and decreases of CBF in ischemic regions.<sup>29</sup> This steal phenomenon supposedly occurs more often in the first minutes after acetazolamide application.<sup>30</sup> Acetazolamide may also disturb  $O_2$  delivery to the tissue by blocking the Bohr effect. These effects may contribute to the observed worsening of the prehensile traction test and, in the BCO group, after acetazolamide application during the follow-up period.

Memory functions (determined by the labyrinth test) and total motor score were most severely impaired 1 day after carotid occlusion. For 1 week there was a clear recovery trend of both parameters (Figures 4 and 5). This development, first described by Combs and D'Alecy,<sup>17</sup> was interpreted by these authors as a reemergence of functionally depressed neurons. Nevertheless, both functions appear significantly impaired during the complete follow-up, even though the motor scores

tend to deteriorate after the CBF and acetazolamide tests. The decline in maze performance is accompanied by more location changes in the maze in futile attempts to find the dark compartment. This proves that prolonged labyrinth exploration times are not due to motor or psychomotor deficits but rather originate from impaired memory retention function. Observation of rats in the maze shows that their behavior exhibits anxious excitement. In addition, the lack of a correlation between labyrinth times and motor scores makes a direct influence of motor deficits on the maze exploration behavior unlikely. However, as a result of the wide scattering of the labyrinth exploration times in chronic forebrain ischemia, they correlate at most loosely with the acetazolamide responses. Prehensile traction test proved to be the most sensitive motor test, which correlated best with reserve capacity (Figure 7).

Imamura et al<sup>31</sup> describe a partial recovery of learning and working memory of gerbils 4 weeks after a 5-minute BCO. In our experiment the possible negative effects of acetazolamide application on functional test scores have to be considered since significant deteriorations, particularly of forelimb grasping strength, were observed after anesthesia for ICBF measurement and acetazolamide test compared with the scores 1 day before those measurements. Another possible explanation for the late worsening of the functional parameters could be the delayed progress of neuronal damage.<sup>32</sup>

A negative correlation between the ICBF response to acetazolamide and the labyrinth time, which shortens with intact memory retention function, and a positive correlation between the acetazolamide response and the motor score could be expected from theoretical considerations and clinical experience.<sup>12</sup> However, our results could only confirm a loose correlation between hemodynamic responses and the data of the memory retention test. Nevertheless, the severity of ischemic motor deficits seemed to be predictable by reserve capacity testing. This offers perspectives for further investigations on the impact of reduced reserve capacity on brain function and histopathology with this easily performed and inexpensive 2-vessel occlusion rat model, which reproduces the clinical presentation of chronic hemodynamic insufficiency.

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## Editorial Comment

Ulrich and colleagues have provided an interesting characterization of incomplete cerebral ischemia as an animal model of chronic cerebral hemodynamic insufficiency. Within the limitations and restrictions of the LD technique, they have provided evidence of continued reduction of cerebral circulation lasting  $\geq 6$  weeks after initiation of BCO. The use of the hypercapnia challenge with systemic administration of acetazolamide provides a functional indicator of the chronic vascular impairment in this model. The authors also included behavioral outcome measures. However, the vascular insult had no effect on 2 of the 3 motor tasks and produced only modest deleterious effects on the prehensile traction test. In contrast, the more complex behavior demands of the labyrinth test demonstrated more robust and reliable behavioral conse-

quences of BCO. The lack of convincing data for the acetazolamide challenge to predict memory performance may provide a clue as to the nature of this behavioral deficit. The data suggest that the complex behavior deficits may be more a function of ischemia-related tissue damage rather than a function of the ability of the vasculature to respond normally to the demands of a hypercapnic challenge. One might further explore the contribution of impaired reserve capacity to behavioral performance deficits by testing control and BCO rats while perturbed by the acetazolamide challenge.

**Bruce G. Lyeth, PhD**, *Guest Editor*  
*Department of Neurological Surgery*  
*University of California, Davis*  
*Davis, California*