

## Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat

Fernanda Cechetti<sup>a,\*</sup>, Paulo Valdeci Worm<sup>a</sup>, Viviane Rostirolla Elsner<sup>b</sup>, Karine Bertoldi<sup>b</sup>, Eduardo Sanches<sup>a</sup>, Juliana Ben<sup>c</sup>, Ionara Rodrigues Siqueira<sup>b</sup>, Carlos Alexandre Netto<sup>a,c</sup>

<sup>a</sup> Programa de Pós-graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil

<sup>b</sup> Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil

<sup>c</sup> Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Brazil

### ARTICLE INFO

#### Article history:

Received 10 February 2011

Revised 26 August 2011

Accepted 25 September 2011

Available online 7 October 2011

#### Keywords:

Chronic cerebral hypoperfusion

Oxidative state

Hippocampus

2VO

Water maze

### ABSTRACT

Physical activity impacts functional recovery following stroke in humans, however its effects in experimental animals submitted to chronic cerebral hypoperfusion have not been investigated. The aim of this study was to evaluate the therapeutic potential of exercise, as assessed by cognitive activity in the Morris water maze and the brain oxidative status, through measurement of macromolecules damage, TBARS levels and total cellular thiols, as well as antioxidant enzymes in hippocampus, striatum and cerebral cortex. Adult male Wistar rats were submitted to the modified permanent bilateral occlusion of the common carotid arteries (2VO) method, with right common carotid artery being first occluded, and tested 3 months after the ischemic event. The effects of three different exercise protocols were examined: pre-ischemia, post-ischemia and pre + post-ischemia. Physical exercise consisted of sessions of 20-min, 3 times per week during 12 weeks (moderate intensity). Rats were submitted to cognitive assessment, in both reference and working spatial memory and after the last testing session were sacrificed to have oxidative stress parameters determined. Hypoperfusion caused a significant cognitive deficit in both spatial water maze tasks and this effect was reversed in rats receiving exercise protocol post and pre + post the ischemic event. Moreover, forced regular treadmill exercise regulated oxidative damage and antioxidant enzyme activity in the hippocampus. These results suggest that physical exercise protects against cognitive and biochemical impairments caused by chronic cerebral hypoperfusion.

© 2011 Elsevier Inc. Open access under the [Elsevier OA license](http://www.elsevier.com/locate/ynlme).

### 1. Introduction

Chronic cerebral hypoperfusion is a pathological state that contributes to the establishment of neurodegenerative diseases (Hartman, Lee, Zipfel, & Wozniak, 2005; Masada et al., 1997; Pazos et al., 1999) and there is a correlation between the severity of memory dysfunction and the decline of cerebral blood flow in Alzheimer's disease, vascular dementia and post-stroke hypoperfusion (Komatani et al., 1988; Ohta, Nishikawa, Kimura, Anayama, & Miyamoto, 1997). In the laboratory rat, the permanent bilateral occlusion of both common carotid arteries in the rat (2-vessel occlusion, 2VO) causes an abrupt reduction of whole cerebral blood flow (CBF; Farkas, Berczi, Sule, & Bari, 2007; Farkas, Luiten, & Baric, 2007), decreasing to approximately 35–45% and 60% of control levels in cortical areas and hippocampus, respectively (Ohta et al., 1997; Otori et al., 2003; Tsuchiya, Sako, Yura, & Yonemasu, 1992; Ulrich, Kroppenstedt, Heimann, & Kempinski, 1998); this is widely accepted

as an adequate experimental model to cerebral hypoperfusion. The main clinical outcomes of chronic cerebral hypoperfusion include neural impairments and cognitive decline (Farkas, Berczi et al., 2007; Farkas, Luiten et al., 2007; Ni, Ohta, Matsumoto, & Watanabe, 1994; Otori et al., 2003; Pappas, De la Torre, Davidson, Keyes, & Fortin, 1996; Sarti, Pantoni, Bartolini, & Inzitari, 2002). Accordingly, rodent two-vessel occlusion provokes short and long term memory impairments using passive avoidance, Y-maze, eight-arm radial maze tasks (Zhao, Murakami, Tohda, Watanabe, & Matsumoto, 2005) and Morris Water Maze task (Ni et al., 1994; Pappas et al., 1996).

It is increasingly accepted that oxidative injury plays a key role on the pathogenesis of neurodegenerative diseases like stroke, Alzheimer's disease and vascular dementia (Chong, Li, & Maiese, 2005; Coyle & Puttfarcken, 1993). Oxygen free radicals and lipid peroxidation are important for the development of lesions caused by chronic cerebral hypoperfusion in the central nervous system (Markesbery, 1997). Free radicals produce oxidative damage to critical biological molecules and, in order to handle that, organisms utilize antioxidant defenses, including superoxide dismutase (SOD), catalase and glutathione peroxidase activities, as well as

\* Corresponding author. Address: Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600, 90050-170 Porto Alegre, RS, Brazil. Fax: +55 51 3308 3092.

E-mail address: [nandacechetti@gmail.com](mailto:nandacechetti@gmail.com) (F. Cechetti).

non-enzymatic antioxidants (as, for example, glutathione, ascorbate and tocopherol) (Halliwell, 1991). These endogenous molecules assist aerobic cells to maintain a reducing state, despite the oxidizing environment (Chung et al., 2005; Pogocki & Schöneich, 2001). On the other hand, thiols are extraordinarily efficient antioxidants in protecting cells against consequences of free radical damage due to their ability to react with the latter (Atmaca, 2004; Sen, Maiti, Puri, Andulov & Valdman, 1992). Both intracellular and extracellular redox states of thiols play a critical role in determining protein structure and function, as well as regulation of transcription factors activities (Wlodek, 2002).

Among new therapeutic strategies pursued to alleviate cognitive damage, physical activity has been shown to support brain health and function (Radák et al., 2001), with beneficial effects on learning, long-term potentiation and memory (Ogonovszky et al., 2005; Van Praag, Christie, Sejnowski, & Gage, 1999). Consistent to that regular physical activity is currently indicated as a preventive measure to age-related neurodegenerative diseases (Mattson, 2000).

Although the exact molecular mechanisms through which physical exercise affects brain function are unclear, it has been suggested to activate cellular and molecular pathways that contribute to neuroprotection. Some reports demonstrate a significant increase in antioxidant enzymes activities, what increases resistance against oxidative stress and therefore reduces cell damage (Leeuwenburgh et al., 1997; Powers et al., 1994; Servais et al., 2003), as well as demonstrate that regular exercise attenuates the protein oxidative damage in aged rats (Radák et al., 2001).

Interestingly, there are not many studies on the effects of exercise on the oxidative status of vulnerable brain regions (Candelario-Jalil, Mhadu, Al-Dalain, Martinez, & Leon, 2001) and after excitotoxic events, like brain ischemia. We have recently demonstrated that daily moderate intensity exercise [2 weeks of 20 min/day of training (Scopel et al., 2006) and a 12-week of three-times a week treadmill protocol (Cechetti et al., 2007)] reduces *in vitro* ischemia damage to the hippocampus of Wistar rats.

In present study, chronic cerebral hypoperfusion produced by permanent occlusion of bilateral common carotid arteries in the rat was used to evaluate the therapeutic potential of physical activity, as assessed by cognitive activity and brain oxidative status on the hippocampus, striatum and cerebral cortex. Spatial memory deficits in both reference and working memory tasks in the Morris water maze, and parameters of cellular oxidative status, namely free radicals content, index of macromolecules damage, were studied in adult rats receiving 2VO followed by three exercise protocols.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats were obtained from the Central Animal House of the Institute of Basic Health Sciences from the Universidade Federal do Rio Grande do Sul. They were maintained in a temperature-controlled room ( $21 \pm 2^\circ\text{C}$ ), on a 12/12 h light/dark cycle, with food and water available *ad libitum*.

### 2.2. Surgical procedure-two-vessel occlusion

Three months-old rats were anesthetized for surgery with halothane; a neck ventral midline incision was made and the common carotid arteries were then exposed and gently separated from the vagus nerve. Both carotids were occluded with 5–0 silk suture, with a one week interval in between; the right common carotid artery being the first to be occluded (Cechetti, Worm, Pereira,

Siqueira, & Netto, 2010). Sham-operated controls received the same surgical procedure with no artery ligation. Animals were randomly assigned to sham or 2VO groups, to avoid any litter effect, as following: (1) pre-surgery exercise group (from 20 days to 3 months of life) ( $n = 9$ ), (2) post-surgery exercise group (from 3 months until 6 months of life) ( $n = 9$ ) and (3) pre + post-surgery exercise group (from 20 days until 6 months of life) ( $n = 10$ ). All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by National Institute of Health (USA), with the Federation of Brazilian Societies for Experimental Biology (FESBE) and previously approved by the University's Committee of Ethics on Research.

### 2.3. Exercise training

Rats were habituated with the treadmill apparatus to minimize novelty stress and randomly assigned to different experimental groups. The exercise protocol chosen was of 20-min sessions run three times per week (Ben et al., 2010; Cechetti et al., 2007; Scopel et al., 2006); all procedures occurred between 17 and 19 pm. Animals in non-exercised (sedentary) groups were left on the treadmill for 5 min without any stimulus to run.

A moderate intensity exercise protocol (Ben et al., 2009; Ben et al., 2010; Cechetti et al., 2007; Scopel et al., 2006) was used, i.e., the exercise intensity was set at 60% of animal's maximal oxygen uptake (Brooks & White, 1978). Indirect assessment of oxygen uptake ( $\text{VO}_2$ ) peak was carried out for all rats before training, considering their exhaustion. Animals ran on the treadmill at a low initial speed followed by increases of 5 m/min every 3 min until the point of exhaustion (i.e., failure of rats to continue running); the time to fatigue (in min) and workload (in m/min) were taken as indexes of exercise capacity that were taken as  $\text{VO}_2$  max (Arida, Scorza, dos Santos, Peres, & Cavalheiro, 1999; Brooks & White, 1978; Cechetti et al., 2007; Scopel et al., 2006).

Animals were exercised to the treadmill by gradually increasing running speed and time, for 12 weeks, as follows: weeks 1 and 2, at 12 m/min for the first 3 min, 24 m/min for the next 4 min, 36 m/min for the following 6 min, 24 m/min for the following 4 min and 12 m/min for the last 3 min; weeks 3–6, at 24 m/min for the first 4 min, 36 m/min for the next 12 min, and 24 m/min for the last 4 min; weeks 7–10, at 24 m/min for the first 2 min, 36 m/min for the next 16 min, and 24 m/min for the last 2 min. By the end, rats were running at 48 m/min, with the first and the last 2-min run at 36 m/min (Cechetti et al., 2007; Scopel et al., 2006).

### 2.4. Morris water maze

Three months after surgery rats were submitted to behavioral testing for spatial memory in the Morris water maze. The maze consisted of a black circular pool with 200 cm in diameter filled with water (temperature around  $23^\circ\text{C}$ , depth 40 cm) situated in a room with visual cues on the walls. A black platform with 10 cm in diameter was submerged in the water (2 cm below the water surface) and the pool was conceptually divided into four quadrants and had four points designed as starting positions (N, S, W or E) (Morris, 1984; Pereira, Strapasson, Nabinger, Achaval, & Netto, 2007). Two behavioral protocols, for reference and working memory, were utilized.

#### 2.4.1. Reference memory protocol

In this task rats received five training days (sessions) and a probe trial in the 6th day. Each session consisted of four trials with a 15 min intertrial interval. A trial began when the rat was placed in the water at one of the four starting positions, chosen at random, facing the wall. The order of starting position varied in every trial and any given sequence was not repeated on acquisition phase

days. The rat was given 60 s to locate the platform; if the animal did not succeed it was gently guided to the platform and left on it for 10 s. Rats were dried and returned to their home cages after each trial. The latency to find the platform was measured in each trial and the mean latency for every training day was calculated. The probe consisted of a single trial, as described before, with the platform removed. Here, the latency to reach the original platform position, the number of crossings over that place and the time spent in the target, as well as in the opposite quadrants, were measured (Cechetti et al., 2010; Netto et al., 1993; Pereira et al., 2007). Videos were subsequently placed in randomized order in a separate ANY-maze protocol to be scored by a trained observer blind to the experimental condition using a keyboard-based behavioral tracking system.

#### 2.4.2. Working memory protocol

This protocol consisted of four trials/day, during four consecutive days, with the platform location daily changed. Each trial was conducted as described in the reference memory protocol, with a 5 min intertrial interval. Latency to find the platform was measured in each trial and the mean latency for every trial, along the 4 days, was calculated, allowing for the observation of the ability of animals in locating the novel platform position in the day (Cechetti et al., 2010; Netto et al., 1993; Pereira et al., 2007).

### 2.5. Effect of exercise on several cellular on oxidative state

#### 2.5.1. Animals and tissue preparation

Rats were decapitated after the behavioral study, brain were removed and hippocampus striatum and cerebral cortex were quickly dissected out and instantaneously placed in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until biochemical assays. On the day of the experiments, brain tissue was homogenized in 10 volumes of ice-cold phosphate buffer (0.1 M, pH 7.4) containing containing KCl (140 mM), EDTA (1 mM) and phenylmethylsulfonyl fluoride (PMSF, 1 mM) in a Teflon-glass homogenizer. The procedures were performed at  $4^{\circ}\text{C}$ .

#### 2.5.2. Free radicals levels

To assess the free radicals content we used 2'-7'-dichlorofluorescein diacetate (DCFH-DA) as a probe (Lebel, Ali, McKee, & Bondy, 1990). An aliquot of the sample was incubated with DCFH-DA (100  $\mu\text{M}$ ) at  $37^{\circ}\text{C}$  for 30 min. The reaction was terminated by chilling the reaction mixture in ice. The formation of the oxidized fluorescent derivative (DCF) was monitored at excitation and emission wavelengths of 488 nm, 525 nm, respectively, using a fluorescence spectrophotometer (Hitachi F-2000). The free radicals content was quantified using a DCF standard curve and results were expressed as pmol of DCF formed/mg protein. All procedures were performed in the dark and blanks containing DCFH-DA (no homogenate) were processed for measurement of autofluorescence (Driver, Kodavanti, & Mundy, 2000; Sriram, Pai, Boyd, & Ravindranath, 1997).

#### 2.5.3. Thiobarbituric acid reactive substances (TBARS)

Lipoperoxidation (LPO) was evaluated by the thiobarbituric acid reactive substances (TBARS) test (Bromont, Marie, & Bralet, 1989). Aliquots of samples were incubated with 10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA). The mixture was heated (30 min) on a boiling water bath. Afterwards, *n*-butanol was added and the mixture was centrifuged (1000g for 10 min). The organic phase was collected to measure fluorescence at excitation and emission wavelengths of 515 and 553 nm, respectively. 1,1,3,3-tetramethoxypropane, which is converted to malondialdehyde (MDA), was used as standard. Results are expressed as pmol MDA/mg protein and reported as percentage of control.

#### 2.5.4. Total thiol content

Cellular thiols, as glutathione and protein thiols, were measured. Aliquots of samples were incubated with 100 mM DTNB (final concentration) for 15 min in darkness. Absorbance of the reaction mixture was measured at 412 nm (Khajuria, John, & Zutshi, 1999); results are expressed as nmoles SH per mg protein.

#### 2.5.5. Superoxide dismutase (SOD) activity

SOD activity was determined using a RANSOD kit (Randox Labs., USA). This method employs xanthine and xanthine oxidase to generate  $\text{O}_2^{\cdot-}$  that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye which is assayed spectrophotometrically at 505 nm at  $37^{\circ}\text{C}$ . The inhibition on production of the chromogen is proportional to the activity of SOD present in the sample.

#### 2.5.6. Protein determination

Protein was measured by the Coomassie blue method using bovine serum albumin as standard.

### 2.6. Statistical analysis

Training days behavioral performance was analyzed using two-way repeated-measures analysis of variance (ANOVA), with lesion and exercise as independent variables and session as the repeated measure. Reference memory probe trial, working memory in the MWM variables and oxidative status were analyzed by two-way ANOVA. All analyses were followed by *post hoc* Duncan's test for multiple comparisons, whenever indicated. Data are expressed as means  $\pm$  SEM. Probability values less than 5% were considered significant. All statistical analysis was performed using the Statistica® software package running on a compatible personal computer.

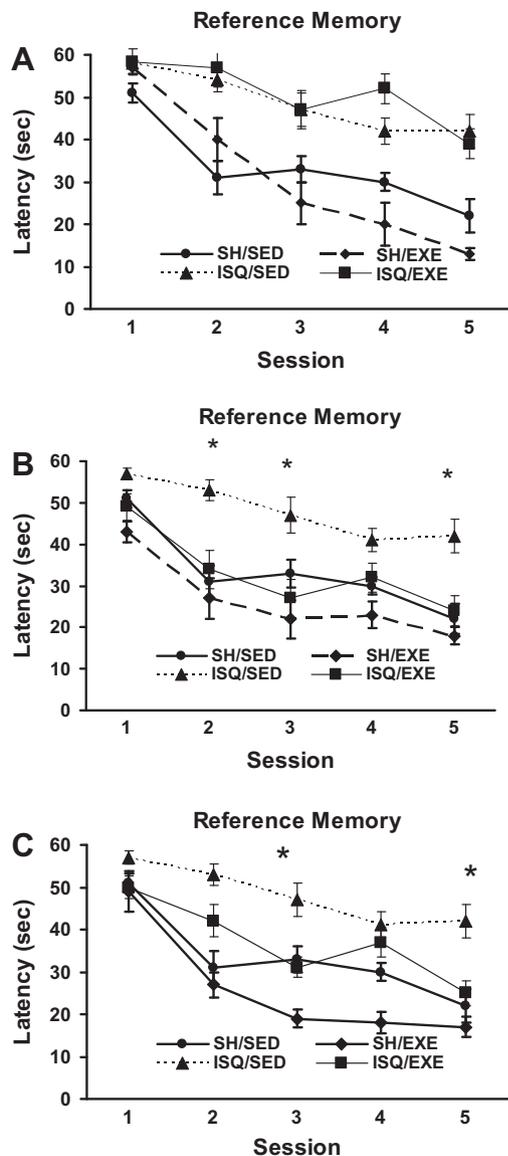
## 3. Results

### 3.1. Behavioral effects

Two-way repeated measures ANOVA of reference memory acquisition phase (Fig. 1A) revealed only significant main effects of *lesion* ( $F(5,25) = 11.80, p < .01$ ), with no significant *treatment* interaction ( $F(5,25) = 1.5, p > .05$ ) on the latency to find the platform, in pre-surgery exercise protocol. In post-surgery (Fig. 1B) and pre + post-surgery (Fig. 1C) protocols, ANOVA showed significant differences in factors *lesion* and *treatment*, where ischemic sedentary groups presented greater latencies than all other animals on sessions 2–5 ( $p < .05$ ).

Probe trial analysis showed effects of *lesion* ( $F(1,31) = 6.29, p < .01$ ) and *exercise* ( $F(1,31) = 4.17, p < .05$ ) on platform crossings in the post-surgery protocol. The *post hoc* test indicated that ischemic/sedentary rats had significantly greater latencies to reach the platform position and made fewer crossings than all other animals ( $p < .05$ , Table 1A). Pre + post-surgery protocol produced differences among ischemic/sedentary and other groups in time spent in the opposite and target quadrants, as well as crossings ( $p < .05$ , Table 1B). Two-way ANOVA did not reveal any significant difference in pre-surgery group (data not presented).

Analysis of working memory performance demonstrated significant differences in escape latency of ischemic sedentary in post-surgery protocol (Fig. 2B) on the 2nd, 3rd and 4th trials ( $p < .05$ ), as compared to other three groups. The same pattern of results appeared after the pre + post-surgery protocol (Fig. 2C) ( $p < .05$ ); however there was no differences after pre-surgery exercise protocol ( $p > .05$  in all trials) (Fig. 2A).



**Fig. 1.** Performance of rats receiving exercise protocol pre-surgery (A), post-surgery (B) and pre + post-surgery (C) in the water maze reference memory task. SH/SED – sham sedentary group, SH/EXE – sham group submitted to physical activity during 3 months, three times per week, ISQ/SED – ischemic sedentary group, ISQ/EXE – ischemic group submitted to physical activity during 3 months, three times per week. Lines represent mean  $\pm$  standard error of mean (SEM). \*Significant difference between sedentary ischemic group and all other groups (ANOVA followed by Duncan's test,  $p < .05$ ).

Permanent bilateral carotid occlusion did not cause any swimming deficit; general means of swimming speed were 26 cm/s for sham-control animals and 24.5 cm/s for ischemic rats.

### 3.2. Oxidative state

#### 3.2.1. Free radical levels

ANOVA revealed no changes on DCF measurements in the hippocampus, striatum and cerebral cortex after 2VO rats, irrespective of a physical exercise regimen (data not presented).

#### 3.2.2. Thiobarbituric acid reactive substances (TBARS) and total cellular thiols

There were changes on TBARS levels ( $F(3,53) = 4.1228$ ,  $p = .01$ ) in the hippocampus, as well as on total thiol levels

**Table 1**

Probe trial of the reference memory protocol: Number of platform crossings, latency to find the platform position, and time spent in target and opposite quadrants. Panel A: Post – surgery group and Panel B: Pre + post surgery group: SH/SED – control sedentary group, SH/EXE post – control submitted to exercise post-surgery group, ISQ/SED – ischemic sedentary group, ISQ/EXE post – ischemic group submitted to exercise post-surgery, SH/EXE pre-post – control submitted to exercise pre-post-surgery group, ISQ/EXE pre-post – ischemic group submitted to exercise pre-post-surgery. Data represent means  $\pm$  SEM.

	Crossings	Latency (s)	Target quadrant (s)
<i>Panel A</i>			
SH/SED	2 $\pm$ 0.4	10.1 $\pm$ 3.1	18 $\pm$ 1.8
SHAM/EXE post	2.5 $\pm$ 0.7	9.1 $\pm$ 4	23 $\pm$ 3.9
ISQ/SED	0.5 $\pm$ 0.2*	23.4 $\pm$ 6.5*	16.7 $\pm$ 2
ISQ/EXE post	1.8 $\pm$ 0.4	9.1 $\pm$ 2.8	18.2 $\pm$ 2.8
<i>Panel B</i>			
SH/SED	2 $\pm$ 0.4	12.11 $\pm$ 4.4	18 $\pm$ 1.9
SHAM/EXE pre + post	2.2 $\pm$ 0.4	16.6 $\pm$ 5.7	19.3 $\pm$ 2.5
ISQ/SED	0.7 $\pm$ 0.4*	17.9 $\pm$ 7.2	14.8 $\pm$ 2*
ISQ/EXE pre + post	2.3 $\pm$ 0.3	18 $\pm$ 7.1	24.9 $\pm$ 2.3

\* Significant difference between sedentary ischemic group and all other groups (two-way ANOVA,  $p < .05$ ).

( $F(3,52) = 4.6249$ ,  $p = .01$ ). Differences were associated to exercise factor in the hippocampus in both techniques utilized: TBARS ( $F(3,53) = 8.48$ ,  $p = .01$ ) and thiol levels ( $F(3,52) = 6.46$ ,  $p = .01$ ), where ischemic sedentary group was significantly different from all other groups analyzed (Fig. 3A and B).

ANOVA did not reveal any significant difference between groups in striatum and cerebral cortex (data not presented).

#### 3.2.3. Superoxide dismutase activity

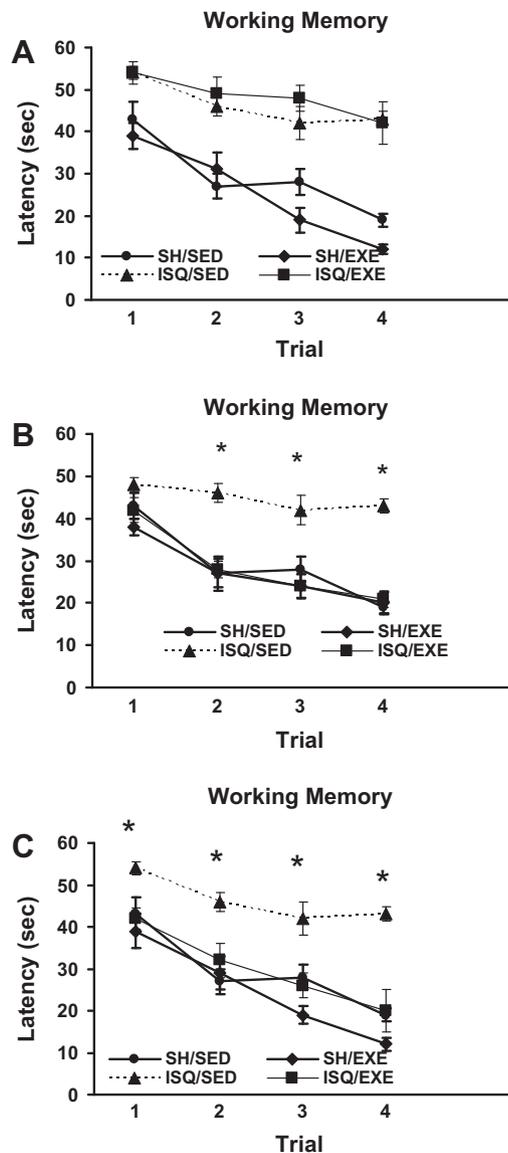
No changes appear on SOD activity in the hippocampus, striatum and cerebral cortex of 2VO rats, irrespective of exercise regimen (data not shown).

## 4. Discussion

Permanent bilateral occlusion of the common carotid arteries (2VO) in rats is an established procedure to investigate the effects of chronic cerebral hypoperfusion on cognitive dysfunction and neurodegenerative processes. (Sarti et al., 2002). We here report that forced treadmill running protects from spatial learning and memory deficits in 2VO rats when applied either post- and pre + post ischemia. To our knowledge, this is the first study demonstrating a positive effect of physical activity on the chronic cerebral hypoperfusion model.

It has been reported that learning and memory can be influenced by exercise. Rodent studies reported better cognitive performance as a result of increased physical activities (Anderson et al., 2000; Fordyce & Farrar, 1991a; Fordyce & Farrar, 1991b; Ji et al., 2010; Samorajski et al., 1985). Present results clearly demonstrate that forced running could significantly improve spatial learning and memory in rats after 2VO ischemia, especially when performed after the ischemic event.

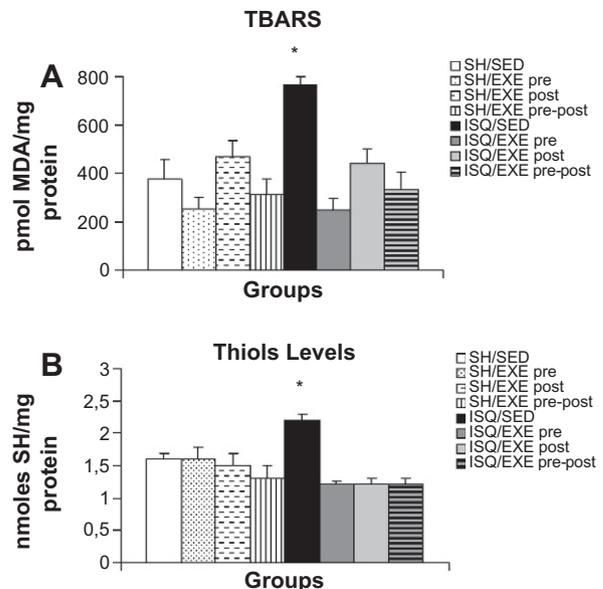
The majority of studies report positive effects when the exercise is administered after the lesion event (Jolitha, Subramanyam, & Asha Devi, 2006; Somani, Ravi, & Rybak, 1995). Possible mechanisms include exercise-induced neurogenesis (Van Praag et al., 1999), up-regulation of trophic factors expression (Ang, Wong, Mochhala, & Ng, 2003; Neeper, Gomez-Pinilla, Choi, & Cotman, 1996) and promotion of long-term potentiation (LTP) (Van Praag et al., 1999); the latter is particularly interesting since it is widely accepted that LTP may serve as a basis for some molecular and synaptic events underlying memory (Abraham, 2003), including



**Fig. 2.** Performance of rats receiving exercise protocol pre-surgery (A), post-surgery (B) and pre + post-surgery (C) in the water maze working memory task. SH/SED – sham sedentary group, SH/EXE – sham submitted to exercise pre-surgery group, SH/EXE post – sham submitted to exercise post-surgery group, SH/EXE pre + post – sham submitted to exercise pre + post-surgery group, ISQ/SED – ischemic sedentary group, ISQ/EXE pre – ischemic group submitted to exercise pre-surgery, ISQ/EXE post – ischemic group submitted to exercise post-surgery, ISQ/EXE pre + post – ischemic group submitted to exercise pre + post-surgery. Lines represent mean  $\pm$  standard error of mean (SEM). \*Significant difference between sedentary ischemic group and all other groups (ANOVA followed by Duncan's test,  $p < .05$ ).

the formation of dendritic spine (Toni, Buchs, Nikonenko, Bron, & Muller, 1999).

Brain tissue is sensitive to oxidative damage and it has been known that 2VO model induces oxidative events, however not many studies explored actions to reduce oxidative stress after cerebral hypoperfusion (Ghoneim, Abdel-Naim, Khalifa, & El-Denshary, 2002; Yanpallewar, Raib, Kumarb, & Chauhanc, 2005). Oxidative stress is defined as the imbalance between oxidants and antioxidants in favor of oxidants that potentially lead to tissue damage (Polidori, Stahl, Eichler, Niestroj, & Sies, 2001). Brain oxidative metabolism is high and large lipid contents of myelin sheaths are a target of reactive oxygen species (ROS) (Choi, 1993). Thiols levels, GSH and superoxide dismutase (SOD) are involved in the antioxidant system and are important for the protection of the brain tissue from oxidative damage.



**Fig. 3.** Thiobarbituric acid reactive substances (TBARS) (panel A) and total cellular thiols (panel B) levels in the hippocampus. SH/SED – sham sedentary group, SH/EXE pre – sham submitted to exercise pre-surgery group, SH/EXE post – sham submitted to exercise post-surgery group, SH/EXE pre + post – sham submitted to exercise pre + post-surgery group, ISQ/SED – ischemic sedentary group, ISQ/EXE pre – ischemic group submitted to exercise pre-surgery, ISQ/EXE post – ischemic group submitted to exercise post-surgery, ISQ/EXE pre + post – ischemic group submitted to exercise pre + post-surgery. Results are expressed as mean  $\pm$  standard error of mean (SEM). \*Significant difference between sedentary ischemic group and all other groups (two-way ANOVA;  $p < .05$ ).

Several studies show a direct relationship between impaired cognitive performance and dysfunction of neurochemical systems in specific brain regions (Brandeis, Brandys, & Yehuda, 1993; McNamara & Skelton, 1993; Raghavendra et al., 2007). However, the effects of exercise upon oxidative status in the hippocampus, striatum and cerebral cortex are conflicting, possibly because of biases due to the distinct experimental models and physical activities protocols employed (Cechetti et al., 2008; Ramsden, Berchtold, Patrick Kesslak, Cotman, & Pike, 2003; Risedal, Zeng, & Johansson, 1999). The protocol here used (three 20-min sessions per week, during 12 weeks) did not alter the basal free radical content in hippocampus, however it affected basal TBARS and thiols level.

It is shown that lipid peroxidation was significantly increased after chronic cerebral hypoperfusion, corroborating previous studies (Aytaca, Seymena, Uzunb, Dikmena, & Altugc, 2006; Ningaraj & Rao, 1998; Yanpallewar, Raib, Kumarb, & Acharya, 2004). Excessive generation of (ROS) results in lipid peroxidation of the cell membrane and subsequent damage is reflected by accumulation of MDA, a by-product of lipid peroxidation (Halliwell, 1991). Present study also showed that occlusion of both carotids caused an increase of thiols activity in hippocampus. We suggest this effect indicates that brain's antioxidant machinery is activated in response to excessive generation of free radicals, corroborating with numerous studies that address this issue both *in vivo* and *in vitro* (Bannister, Bannister, & Rotillio, 1987; Omar & McCord, 1990; Yanpallewar et al., 2004). It also demonstrated that forced regular physical activity was able to prevent the oxidative state and increased antioxidant capacity in the hippocampus after 2VO. Most studies on this model of hypoperfusion and oxidative stress demonstrate the neuroprotective effect of drugs and plant extracts (Yanpallewar et al., 2004; Yanpallewar et al., 2005); ours is the first to report exercise-induced neuroprotection.

Hippocampus plays an important role on learning and memory processing and is highly sensitive to ischemic insults (Ni et al., 1994; Pappas et al., 1996). Ischemia-induced neuronal degeneration is also observed in other structures, such as striatum, cerebral cortex and thalamus (Freund, Buzsaki, Leon, Baimbridge, & Somogyi, 1990; Pereira et al., 2009), but the favored brain region for the study of 2VO-induced neurodegeneration is the hippocampus. The main reasons are that the hippocampus: (a) displays the most characteristic neuropathological damage in Alzheimer's disease; (b) is highly implicated in spatial learning and memory as assessed by the Morris water maze and the 8-arm radial maze; (c) is one of the brain regions most sensitive to ischemia, particularly its CA1 subfield; (d) bears a distinct laminar structure that allows to exact cell-type (e.g. pyramidal cells) or layer specific measurements (Farkas, Berczi et al., 2007; Farkas, Luiten et al., 2007).

The beneficial effects of endurance training on the redox status of the nervous system of rodents have been reported after swimming (Jolitha et al., 2006), treadmill training (Cosk, Gonul, Guzel, et al., 2005; Somani et al., 1995), and running wheel training (Suzuki, Katamine, & Tatsumi, 1993). On the other hand, there are also studies where swimming (Radák et al., 2006) and treadmill (Ozkaya, Agar, Yargicoglu, et al., 2002) training did not affect brain antioxidant defenses. It is proposed that exercise, through its continuous free radical generating effect, thus induce the adaptation of the cellular antioxidant system (Leeuwenburgh et al., 1997; Powers et al., 1994; Servais et al., 2003).

Summarizing, present results demonstrate that chronic cerebral hypoperfusion causes cognitive damage associated to increased lipoperoxidation in the hippocampus, and that post- and pre + post-ischemia moderate treadmill running exercise prevents both effects. This implies a neuroprotective action of running exercise over cognitive and biochemical impairments caused by cerebral hypoperfusion; further studies are needed to investigate the mechanisms involved.

## References

- Abraham, W. C. (2003). How long will long-term potentiation last? *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, 358, 735–744.
- Anderson, B. J., Rapp, D. N., Baek, D. H., McCloskey, D. P., Coburn-Litvak, P. S., & Robinson, J. K. (2000). Exercise influences spatial learning in the radial arm maze. *Physiology and Behavior*, 70, 425–429.
- Ang, E. T., Wong, P. T., Moochhal, S., & Ng, Y. K. (2003). Neuroprotection associated with running: is it a result of increased endogenous neurotrophic factors? *Neuroscience*, 118, 335–345.
- Arida, R. M., Scorza, F. A., dos Santos, N. F., Peres, C. A., & Cavalheiro, E. A. (1999). Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. *Epilepsy Research*, 37, 45–52.
- Atmaca, G. (2004). Antioxidant effects of sulfur-containing amino acids. *Yonsei Medical Journal*, 45, 776–788.
- Aytaca, E., Seymen, H. O., Uzun, H., Dikmen, G., & Altug, T. (2006). Effects of iloprost on visual evoked potentials and brain tissue oxidative stress after bilateral common carotid artery occlusion. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 74, 373–378.
- Bannister, J. V., Bannister, W. H., & Rotillio, G. (1987). Aspects of structure, function and application of superoxide dismutase. *CRC Critical Reviews in Biochemistry*, 22, 111–180.
- Ben, J., Soares, F. M., Cechetti, F., Vuaden, F. C., Bonan, C. D., Netto, C. A., et al. (2009). Exercise effects on activities of Na(+), K(+)-ATPase, acetylcholinesterase and adenine nucleotides hydrolysis in ovariectomized rats. *Brain Research*, 1302, 248–255.
- Ben, J., Soares, F. M., Scherer, E. B., Cechetti, F., Netto, C. A., & Wyse, A. T. (2010). Running exercise effects on spatial and avoidance tasks in ovariectomized rats. *Neurobiology of Learning and Memory*, 2010 (Epub ahead of print).
- Brandeis, R., Brandys, Y., & Yehuda, S. (1993). The use of the Morris water maze in the study of memory and learning. *International Journal of Neuroscience*, 48, 29–69.
- Bromont, C., Marie, C., & Bralet, J. (1989). Increased lipid peroxidation in vulnerable brain regions after transient forebrain ischemia in rats. *Stroke*, 20, 918–924.
- Brooks, G. A., & White, T. P. (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. *Journal of Applied Physiology*, 45, 1009–1015.
- Candelario-Jalil, E., Mhadu, N. H., Al-Dalain, S. M., Martinez, G., & Leon, O. S. (2001). Time course of oxidative damage in different brain regions following transient cerebral ischemia in gerbils. *Neuroscience Research*, 41, 233–241.
- Cechetti, F., Worm, P. V., Pereira, L. O., Siqueira, I. R., Netto, C. A. (2010). The modified 2VO ischemia protocol causes similar cognitive impairment with better survival rate, as compared to standard method. *Brazilian Journal of Medical and Biological Research*.
- Cechetti, F., Fochesatto, C., Scopel, D., Nardin, P., Gonçalves, C. A., Netto, C. A., et al. (2008). Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. *Brain Research*, 1188(January 10), 182–188.
- Cechetti, F., Rhod, A., Simão, F., Santin, K., Salbego, C., Netto, C. A., et al. (2007). Effect of treadmill exercise on cell damage in rat hippocampal slices submitted to oxygen and glucose deprivation. *Brain Research*, 1157, 121–125.
- Choi, B. H. (1993). Oxygen, antioxidants and brain dysfunction. *Yonsei Medical Journal*, 34, 1–10.
- Chong, Z. Z., Li, F., & Maiese, K. (2005). Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. *Progress in Neurobiology*, 75, 207–246.
- Chung, K. et al. (2005). Generation of free radical by interaction of iron with thiols in human plasma and its possible significance. *Thrombosis Research*, 116, 157–164.
- Cosk, S., Gonul, B., Guzel, N. A., et al. (2005). The effects of vitamin C supplementation on oxidative stress and antioxidant content in the brains of chronically exercised rats. *Molecular and Cellular Biochemistry*, 1–2, 135–138.
- Coyle, J. T., & Puttfarcken, P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science*, 262, 689–695.
- Driver, A. S., Kodavanti, P. R. S., & Mundy, W. R. (2000). Age-related in reactive oxygen species production in rat brain homogenates. *Neurotoxicology and Teratology*, 22, 175–181.
- Farkas, E., Berczi, S., Sule, Z., & Bari, F. (2007). Effects of cyclooxygenase (COX) inhibition on memory impairment and hippocampal damage in the early period of cerebral hypoperfusion in rats. *European Journal of Pharmacology*, 574, 29–38.
- Farkas, E., Luiten, P. G. M., & Baric, F. (2007). Permanent, bilateral common carotid artery occlusion in the rat: A model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Research Reviews*, 54, 162–180.
- Fordyce, D. E., & Farrar, R. P. (1991a). Physical activity effects on hippocampal and parietal cortical cholinergic function and spatial learning in F344 rats. *Behavioral Brain Research*, 43, 115–123.
- Fordyce, D. E., & Farrar, R. P. (1991b). Enhancement of spatial learning in F344 rats by physical activity and related learning-associated alterations in hippocampal and cortical cholinergic functioning. *Behavioural Brain Research*, 46, 123–133.
- Freund, T. F., Buzsaki, G., Leon, A., Baimbridge, K. G., & Somogyi, P. (1990). Relationship of neuronal vulnerability and calcium binding protein immunoreactivity in ischemia. *Experimental Brain Research*, 83, 55–66.
- Ghoneim, A. I., Abdel-Naim, A. B., Khalifa, A. E., & El-Denshary, E. S. (2002). Protective effects of curcumin against ischaemia/reperfusion insult in rat forebrain. *Pharmacological Research*, 46, 273.
- Halliwell, B. (1991). Reactive oxygen species in living system: Source, biochemistry and role in human disease. *American Journal of Medicine*, 99, 145–225.
- Hartman, R. E., Lee, J. M., Zipfel, G. J., & Wozniak, D. F. (2005). Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. *Brain Research*, 1043, 48–56.
- Ji, H., Hu, J. F., Wang, Y. H., Chen, X. Y., Zhou, R., & Chen, N. H. (2010). Osthole improves chronic cerebral hypoperfusion induced cognitive deficits and neuronal damage in hippocampus. *European Journal of Pharmacology*, 636, 96–101.
- Jolitha, A. B., Subramanyam, M. V. V., & Asha Devi, S. (2006). Modification by vitamin E and exercise of oxidative stress in regions of aging rat brain: Studies on superoxide dismutase isoenzymes and protein oxidation status. *Experimental Gerontology*, 41, 753–763.
- Khajuria, A., John, R. K., & Zutshi, U. (1999). Piperine mediated alterations in lipid peroxidation and cellular thiol status of rat intestinal mucosa and epithelial cells. *Phytomedicine*, 6, 351–355.
- Komatani, A., Yamaguchi, K., Sugai, Y., Takanashi, T., Kera, M., Shinohara, M., et al. (1988). Assessment of demented patients by dynamic SPECT of inhaled xenon-133. *Journal of Nuclear Medicine*, 29, 1621–1626.
- Lebel, C. P., Ali, S. F., McKee, M., & Bondy, S. C. (1990). Organometal induced increases in oxygen reactive species: the potential of 20,70-dichloro-fluorescein diacetate as an index of neurotoxic damage. *Toxicology and Applied Pharmacology*, 104, 17–24.
- Leeuwenburgh, C., Hollander, J., Leichtweis, S., Griffiths, M., Gore, M., & Ji, L. L. (1997). Adaptation of glutathione antioxidant system 523 to endurance training are tissue and muscle fiber specific. *American Journal of Physiology*, 272, 363–369.
- Markesbery, W. R. (1997). Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology and Medicine*, 23, 134–147.
- Masada, T., Itano, T., Fujisawa, M., Miyamoto, O., Tokuda, M., Matsui, H., et al. (1997). Embryonic transplantation and ischemic memory deficit. *Neuroscience Research*, 27, 249–255.
- Mattson, M. P. (2000). Neuroprotective signaling and the aging brain: Take away my food and let me run. *Brain Research*, 886, 47–53.
- McNamara, R. K., & Skelton, R. W. (1993). The neuropharmacological and neurochemical basis of place learning in the Morris water maze. *Brain Research Reviews*, 18, 33–49.
- Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, 11, 47–60.

- Neeper, S., Gomez-Pinilla, F., Choi, J., & Cotman, C. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Research*, 726, 49–56.
- Netto, C. A., Hodges, H., Sinden, J. D., Le Peillet, E., Kershaw, T., Sowinski, P., et al. (1993). Effects of fetal hippocampal Weld grafts on ischaemic-induced deWcits in spatial navigation in the water maze. *Neuroscience*, 54, 69–92.
- Ni, J. W., Ohta, H., Matsumoto, K., & Watanabe, H. (1994). Progressive cognitive impairment following chronic cerebral hypoperfusion induced by permanent occlusion of bilateral carotid arteries in rats. *Brain Research*, 653, 231–236.
- Ningaraj, N. S., & Rao, M. K. (1998). Disulfiram augments oxidative stress in rat brain following bilateral carotid artery occlusion. *Journal of Biomedical Science*, 5, 226–230.
- Ogonovszky, H., Berkes, I., Kumagai, S., Kaneko, T., Tahara, S., Goto, S., et al. (2005). The effects of moderate-strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochemistry International*, 46, 635–640.
- Ohta, H., Nishikawa, H., Kimura, H., Anayama, H., & Miyamoto, M. (1997). Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. *Neuroscience*, 79, 1039–1050.
- Omar, B. A., & McCord, J. M. (1990). The cardioprotective effect of Mn-superoxide dismutase is lost at high doses in the postschemic rabbit heart. *Free Radical Biology and Medicine*, 9, 473–478.
- Otori, T., Katsumata, T., Muramatsu, H., Kashiwagi, F., Katayama, Y., & Terashi, A. (2003). Long-term measurements of cerebral blood flow and metabolism in a rat chronic hypoperfusion model. *Clinical and Experimental Pharmacology and Physiology*, 30, 266–272.
- Ozkaya, Y. G., Agar, A., Yargicoglu, P., et al. (2002). The effect of exercise on brain antioxidant status of diabetic rats. *Diabetes and Metabolism*, 5, 377–384.
- Pappas, B. A., De la Torre, J. C., Davidson, C. M., Keyes, M. T., & Fortin, T. (1996). Chronic reduction of cerebral blood flow in the adult rat: Late-emerging CA1 cell loss and memory dysfunction. *Brain Research*, 708, 50–58.
- Pazos, A. J., Green, E. J., Busto, R., McCabe, P. M., Baena, R. C., Ginsberg, M. D., et al. (1999). Effects of combined postschemic hypothermia and delayed N-tert-butyl-alpha-phenyltrone (PBN) administration on histopathological and behavioral deficits associated with transient global ischemia in rats. *Brain Research*, 846, 186–195.
- Pereira, L. O., Nabinger, P. M., Strapasson, A. C. P., Nardin, P., Gonçalves, C. A., Siqueira, I. R., et al. (2009). Long-term effects of environmental stimulation following hypoxia-ischemia on the oxidative state and BDNF levels in rat hippocampus and frontal cortex. *Brain Research*, 1247, 188–195.
- Pereira, L. O., Strapasson, A. C., Nabinger, P. M., Achaval, M., & Netto, C. A. (2007). Early enriched housing results in partial recovery of memory deficits in female, but not in male, rats after neonatal hypoxia-ischemia. *Brain Research*, 1218, 257–266.
- Pogocki, D., & Schöneich, C. (2001). Thiyl radicals abstract hydrogen atoms from carbohydrates: Reactivity and selectivity. *Free Radical Biology and Medicine*, 31, 98–107.
- Polidori, M. C., Stahl, W., Eichler, O., Niestroj, I., & Sies, H. (2001). Profiles of antioxidants in human plasma. *Free Radical Biology and Medicine*, 30, 456–462.
- Powers, S. K., Criswell, D., Lawler, J., Ji, L. L., Martin, D., Herb, R. A., et al. (1994). Influence of exercise and fiber type on 561 antioxidant enzyme activity in rat skeletal muscle. *American Journal of Physiology*, 266, 376–380.
- Radák, Z., Kaneko, T., Tahara, S., Nakato, H., Pucso, J., Sasvári, M., et al. (2001). Regular exercise improves cognitive function and decreases oxidative damage in rat brain. *Neurochemistry International*, 38, 17–23.
- Radák, Z., Toldy, A., Szabo, Z., Siamilis, S., Nyakas, C., Silye, G., et al. (2006). The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. *Neurochemistry International*, 49, 387–392.
- Raghavendra, M., Trigunayat, A., Singh, R. K., Mitra, S., Goel, R. K., & Acharya, S. B. (2007). Effect of ethanolic extract of root of *Pongamia pinnata* (L.) pierre on oxidative stress, behavioral and histopathological alterations induced by cerebral ischemia – Reperfusion and long-term hypoperfusion in rats. *Indian Journal of Experimental Biology*, 45(10), 868–876.
- Ramsden, M., Berchtold, N. C., Patrick Kesslak, J., Cotman, C. W., & Pike, C. J. (2003). Exercise increases the vulnerability of rat hippocampal neurons to kainate lesion. *Brain Research*, 971, 239–244.
- Risedal, A., Zeng, J., & Johansson, B. B. (1999). Early training may exacerbate brain damage after focal brain ischemia in the rat. *Journal of Cerebral Blood Flow and Metabolism*, 19, 997–1003.
- Samorajski, T., Delaney, C., Durham, L., Ordy, J. M., Johnson, J. A., & Dunlap, W. P. (1985). Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. *Neurobiology of Aging*, 6, 17–24.
- Sarti, C., Pantoni, L., Bartolini, L., & Inzitari, D. (2002). Persistent impairment of gait performances and working memory after bilateral common carotid artery occlusion in the adult Wistar rat. *Behavioural Brain Research*, 136, 13–20.
- Scopel, D., Fochesatto, C., Cimarosti, H., Rabbo, M., Belló-Klein, A., Salbego, C., et al. (2006). Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. *Brain Research Bulletin*, 71, 155–159.
- Sen, P., Maiti, P. C., Puri, S., Andulov, N. A., & Valdman, A. V. (1992). Mechanism of antistress activity of *Ocimum sanctum* Linn, eugenol, *Tinospora malabarica* in experimental animals. *Indian Journal of Experimental Biology*, 30, 592–596.
- Servais, S., Couturier, K., Koubi, H., Rouanet, J. L., Desplanches, D., Sornay-Mayet, M. H., et al. (2003). Effect of voluntary exercise on H2O2 release by subsarcolemmal, and intermyofibrillar mitochondria. *Free Radical Biology and Medicine*, 35, 24–32.
- Somani, S. M., Ravi, R., & Rybak, L. P. (1995). Effect of exercise training on antioxidant system in brain regions of rat. *Pharmacology, Biochemistry and Behavior*, 4, 635–639.
- Sriram, K., Pai, K. S., Boyd, M. R., & Ravindranath, V. (1997). Evidence for generation of oxidative stress in brain by MPTP: In vitro and in vivo studies in mice. *Brain Research*, 749, 44–52.
- Suzuki, M., Katamine, S., & Tatsumi, S. (1993). Exercise-induced enhancement of lipid peroxid metabolism in tissues and their transference into brain in rat. *Journal of Nutritional Science and Vitaminology*, 29, 141–151.
- Toni, N., Buchs, P. A., Nikonenko, I., Bron, C. R., & Muller, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature*, 402, 421–425.
- Tsuchiya, M., Sako, K., Yura, S., & Yonemasu, Y. (1992). Cerebral blood flow and histopathological changes following permanent bilateral carotid artery ligation in Wistar rats. *Experimental Brain Research*, 89, 87–92.
- Ulrich, P. T., Kroppenstedt, S., Heimann, A., & Kempfski, O. (1998). 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. *Stroke*, 29, 2412–2420.
- Van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 13427–13431.
- Wlodek, L. (2002). Beneficial and harmful effects of thiols. *Polish Journal of Pharmacology*, 54, 215–223.
- Yanpallewar, S., Raib, S., Kumar, M., & Acharya, S. B. (2004). Evaluation of antioxidant and neuroprotective effect of *Ocimum sanctum* on transient cerebral ischemia and long-term cerebral hypoperfusion. *Pharmacology, Biochemistry and Behavior*, 79, 155–164.
- Yanpallewar, S., Raib, S., Kumar, M., & Chauhanc, S. (2005). Neuroprotective effect of *Azadirachta indica* on cerebral post-ischemic reperfusion and hypoperfusion in rats. *Life Sciences*, 76, 1325–1338.
- Zhao, Q., Murakami, Y., Tohda, M., Watanabe, H., & Matsumoto, K. (2005). Preventive effect of chotosan, a Kampo medicine, on transient ischemia-induced learning deficit is mediated by stimulation of muscarinic M1 but not nicotinic receptor. *Biological and Pharmaceutical Bulletin*, 28, 1873–1878.