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Repeated resveratrol administration confers lasting protection against neuronal damage but induces dose-related alterations of behavioral impairments after global ischemia

Catrinel Girbovan, Lucie Morin and Hélène Plamondon

Resveratrol, a naturally occurring polyphenol, has been shown to protect the heart and brain against ischemic injury. The current study investigated the effects of administration with either a 1 or 10-mg/kg dose of resveratrol on CA1 neuronal injury and behavioral/cognitive impairments after 10-min global ischemia in rats. The open-field, eight-arm radial maze and object recognition tests served to evaluate effects of resveratrol treatment on ischemia-induced locomotor activity, and spatial and recognition memory impairments, respectively. CA1 and CA3 neuronal injury was assessed upon completion of behavioral testing, 85 days postischemia. A separate series of groups served to assess neuronal injury at 7 days postischemia. Global ischemia (10 min) led to approximately 50% CA1 cell injury, which was prevented at both short (7 days) and long (85 days) postischemic intervals by resveratrol treatment. Importantly, despite comparable neuronal protection, the two resveratrol doses showed distinct behavioral effects. Thus, the 10-mg/kg resveratrol dose led to an enhanced locomotor activity in the open-field 4-days postischemia and an impaired spatial memory in the delayed nonmatching to sample and delayed matching to sample radial-maze tasks initiated on day 13 postischemia. These findings suggest independent actions of resveratrol on distinct physiological systems mediating cellular survival and functional recovery and dose-related actions of the polyphenol on behavioral and memory processes. *Behavioural Pharmacology* 00:000–000 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The naturally occurring polyphenol phytoalexin resveratrol has been shown to exert a number of biological benefits in various experimental paradigms including antioxidant, cardioprotective, antiaging, anti-inflammatory, and anti-carcinogenic actions (Fremont, 2000). In addition, in-vivo and in-vitro studies have demonstrated protective effects of resveratrol in multiple disease models, including stroke (Lu *et al.*, 2006; Tsai *et al.*, 2007; Dong *et al.*, 2008; Wang *et al.*, 2009; Simao *et al.*, 2011) epilepsy (Gupta *et al.*, 2002; Wu *et al.*, 2009), diabetes (Ates *et al.*, 2007), Alzheimer's (Sharma and Gupta, 2002; Wang *et al.*, 2006), and Parkinson's disease (Zhang *et al.*, 2010; Huber and Superti-Furga, 2011; Wang *et al.*, 2011). These effects have partly been attributed to the antioxidant and anti-inflammatory activities of resveratrol in the injured brain (Wang *et al.*, 2002). Similarly, inhibition of lipid peroxidation, upregulation of NO synthase, and increased vasorelaxation have been associated to resveratrol cardioprotective effects (Bradamante *et al.*, 2004; Miatello *et al.*, 2005).

Recently, different studies have demonstrated neuroprotective effects of resveratrol administration in a number of ischemic models. For example, resveratrol administration for 21 days before middle cerebral artery occlusion in rats was shown to significantly decrease infarct volume compared with controls (Sinha *et al.*, 2002), whereas a 7-day resveratrol pretreatment significantly attenuated CA1 neuronal injury and glial activation after global ischemia (Wang *et al.*, 2009; Simao *et al.*, 2011). These findings suggest that resveratrol represents a novel agent promoting neuronal survival poststroke (Bradamante *et al.*, 2004), which could have significant implications in cognitive recovery.

In rodents, global forebrain ischemia leads to selective and delayed degeneration of the CA1 pyramidal neurons of the hippocampus, which is initiated approximately 48 h postischemia (Kirino, 1982; Kirino *et al.*, 1984) and appears maximal at 7 days (Taoufik and Probert, 2008; Nikonenko *et al.*, 2009). CA1 neuronal loss leads to well-documented behavioral impairments, notably affecting spatial memory as assessed by the Morris water maze (Sandstrom and Rowan, 2007) as well as standard and alternate versions of radial arm maze tasks (Nelson *et al.*, 1997; Roberge *et al.*, 2008b).

To date, few studies have investigated the effect of resveratrol on behavioral recovery and it remains to be determined whether neuronal protection is paralleled by

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recovery of cognitive function after global ischemia. Furthermore, little is known about the effects of repeated administration of resveratrol in healthy subjects, with measures limited to metabolic and biochemical assessments (Simao *et al.*, 2011). Finally, neuronal assessments have been limited to short reperfusion intervals, ranging from a few hours to 7 days postischemia (Wang *et al.*, 2002; Simao *et al.*, 2011) and whether the protection can be maintained over weeks remains unknown.

Thus, the current study had two main goals. The first objective was to assess the effects of daily resveratrol administration, for 21 days preischemia at two doses (1 or 10 mg/kg; Mukherjee *et al.*, 2010), on neuronal injury in the CA1 and CA3 regions of the hippocampus observed at short-term and long-term reperfusion intervals (7 and 85 days, respectively), after 10-min global ischemia in rats. The second objective was to determine behavioral and cognitive effects of resveratrol treatment in controls, using the open-field, eight-arm radial maze and object recognition tests, as well as alterations of postischemic impairments. Administration of the 10 mg/kg resveratrol dose to a group of sham-treated rats allowed determining intrinsic actions of the drug on behavioral and memory performance.

Methods

Subjects

Male Wistar rats ($n = 35$ and 53 for the short and long reperfusion intervals, respectively) weighing between 100–125 g at time of arrival at the animal facility (325–375 g at time of surgery) were obtained from Charles River Laboratories (Rochefort, Quebec, Canada). Upon arrival, rats were individually housed and maintained on a 12-h light/dark cycle (lights on at 07 : 00 h), with free access to water and standard (Purina) rat chow. Room temperature was maintained at 21–23°C with 60% relative humidity. One week after arrival, subjects were randomly assigned to five experimental groups and chronic injections were initiated: ischemia + saline ($n = 10$), sham + saline ($n = 10$), ischemia + 1 mg/kg of resveratrol ($n = 12$), ischemia + 10 mg/kg of resveratrol ($n = 11$), and sham + 10 mg/kg of resveratrol ($n = 10$). Four weeks after arrival and upon completion of drug treatment, sham or ischemic surgeries were performed. An additional 35 rats were randomly assigned to the same five experimental groups but killed 7 days after sham or ischemic surgery (short-term interval): ischemia + saline ($n = 7$), sham + saline ($n = 6$), ischemia + 1 mg/kg of resveratrol ($n = 8$), ischemia + 10 mg/kg of resveratrol ($n = 8$), and sham + 10 mg/kg of resveratrol ($n = 6$). The surgery and injection schedule was identical to that of the long-term group with the exception of behavioral testing. Twenty-five of 81 rats operated on in the ischemic groups died during the ischemic surgery. The subject numbers indicated above represent rats remaining in each experimental group. All experimental procedures were in accordance with the guidelines set by the Canadian Council of Animal Care and approved by the University of Ottawa Animal Care and Ethics Committee. Efforts were made to minimize the number of subjects used.

Resveratrol administration

Resveratrol (Sigma, St. Louis, Missouri, USA) was dissolved in 50% ethanol and separated in 50 μg aliquots, which were lyophilized and then stored at −80°C until use. Aliquots were freshly dissolved daily in a vehicle consisting of 0.9% saline solution containing 20% hydroxypropyl h-cyclodextrin (Sigma). Twenty-one days before the surgery, sham and ischemic rats received daily, between 09 : 00 and 11 : 00 h, an intraperitoneal injection of either saline or 1 or 10 mg/kg of resveratrol (injection volume was 0.1 ml/100 g rat weight). The last saline or resveratrol injection was administered 1 h before ischemic or sham surgery. Rats were weighed daily and the injection volume adjusted to correspond to the subject's body weight. The resveratrol doses were selected on the basis of earlier reports showing beneficial effects in different experimental paradigms (Sinha *et al.*, 2002; Mukherjee *et al.*, 2010).

Transient forebrain ischemia

Forebrain ischemia was performed using the four-vessel occlusion model as previously described (Pulsinelli and Brierley, 1979). In brief, rats were deeply anesthetized by inhalation of 1.5% halothane in oxygen (1.5–2 l/min). Vertebral arteries were irreversibly occluded by electrocoagulation, and a small-diameter silk thread looped around the carotid arteries to facilitate subsequent occlusion. Twenty-four hours later, common carotid arteries were occluded with microaneurysm clamps for 10 min in spontaneously ventilating rats. Sham-operated rats underwent anesthesia and received the same dorsal and ventral surgical incisions as the ischemic groups, with the exception of electrocoagulation of the vertebral arteries. Twenty-four hours later, carotid arteries were exposed, but not clamped. Only rats that lost the righting reflex over the entire occlusion period were included in the study. Core temperature was kept at 37°C ± 0.5 throughout the surgery, using a feedback-regulated heating blanket connected to a rectal thermometer (Homeothermic Blanket Control Unit, Harvard Instruments, Natick, Massachusetts, USA). Body temperature was further supported with a heating pad in the hours after surgery and reperfusion. One day before behavioral testing, the pupillary reflex was assessed in all rats to determine visual system/retinal functioning possibly impacting vision during behavioral testing. Rats were transported to a dark room and left to habituate for a minimum of 15 min. The pupillary reflex was examined by illuminating the rats' dark-adapted eyes with a mini-flashlight producing a focused light beam. After examination of the first eye, chosen at random, an additional 60 s in the dark was imposed before examining the second eye. The reflex was considered intact

if constriction of both pupils in response to the light occurred under 10 s. All ischemic and sham-operated rats displayed a normal pupillary reflex, typically occurring within seconds of the light stimulus.

Blood glucose measurements

Basal glycemic levels were assessed by the tail-bleeding method using Bayer's Contour meter (Bayer Canada, Montreal, Canada). Glucose levels (in mmol/l) were assessed 1 min before sham or ischemic occlusion and 3, 7, and 85 days after reperfusion. Assessments were performed between 9 : 00 and 10 : 00 h.

Behavioral tests

Behavioral testing was initiated 4 days postsurgery (see Fig. 1) and conducted in the light portion of the light/dark cycle.

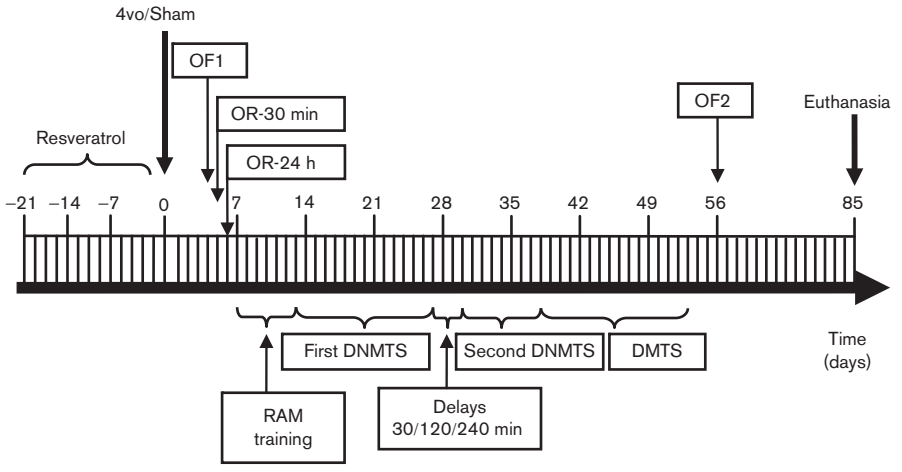
Open-field test

On days 4 and 56 after reperfusion, sham and ischemic rats were placed in the open field and behavior was monitored for a 15-min period. The observation arena was made of gray Plexiglas (LWH: $75 \times 75 \times 30$ cm) with a clear Plexiglas floor. A painted grid divided the Plexiglas floor into 25 identical squares each measuring 10×10 cm. The entire arena was kept on a table 90 cm above the floor in the same room as the rats' housing. Rats were monitored for frequency of line crossing, grooming, and rearing behavior by the experimenter using a portable PC computer (Toshiba 4250) and data logging software. To control for differences attributable to testing days, animals of all five groups were randomized so that rats of each group participated in each testing session.

Object-recognition test

On the fifth and sixth day after reperfusion, rats were tested in the object-recognition test, a memory test based on the natural propensity of rats to spend more time exploring a new rather than a formerly encountered object. Recognition memory was evaluated at two retention intervals (30 min and 24 h). Rats were transported from the vivarium to the testing room and allowed to rest for at least 30 min before behavioral testing began. Testing was monitored by an overhead camera, and behavioral measures quantified online using data logging software (ODlog 2.0, Macropod Software). The test was performed in the open-field arena as previously described (Plamondon *et al.*, 2006). In brief, each rat was exposed to three experimental conditions: in the initial trial (T1), one object stimulus (O1) was placed in one corner of the open field and the rat positioned in the opposite corner of the arena. The time spent exploring the object (i.e., touching the object with paws or exploring it by olfaction with direct contact of the snout) was measured. The session ended when the subject explored the object for 20 s or when 10 min had elapsed. During the second trial (T2), performed 30 min after T1, a second object (O2) was introduced in the adjacent corner to that of the reference object. The time spent exploring the familiar (O1) and the novel (O2) objects was measured for a period of 10 min. In the final trial (T3), performed 24 h after T1, O2 was replaced by a new object (O3) and the time the rat spent exploring the reference (O1) and novel (O3) objects was measured for 10 min. The object presentation order was randomly permuted from rat to rat. The arena and objects were extensively cleaned before each trial to eliminate any olfactory cues. The

Fig. 1



Experimental protocol. Schedule showing time intervals for all experimental procedures. 21-day presurgical period of resveratrol (1 mg/kg or 10 mg/kg;) or saline intraperitoneal injections; 4vo: four vessel occlusions; OF1 and OF2: open-field test 4 and 56 days after reperfusion; OR-30 and OR-24: object-recognition test with 30 min and 24 h retention intervals; RAM training: training in the radial arm maze; First DNMTS: first delayed nonmatching to sample task; delays 30, 120, 240 min: intertrial delays of 30, 120, and 240 min in the first DNMTS; Second DNMTS: second delayed nonmatching to sample task; DMTS: delayed matching to sample task. Day 0 refers to the day of surgery.

objects consisted of plastic toys heavy enough to prevent the rats from moving them. Objects were carefully selected to elicit similar levels of exploration.

Radial arm maze

Delayed nonmatching and matching to sample radial arm maze: apparatus: The radial maze consisted of eight arms (60×12 cm with a 5-cm lip around each arm) extending radially from a central octagonal area (32 cm in diameter with a 30-cm high-clear Plexiglas wall). Plexiglas sliding doors allowed entry into each arm. The floor of the arms and central area were covered with a black rubber lining. The apparatus was elevated 50 cm above the floor and surrounded by extra-maze cues such as posters or calendars along the sidewalls. The experimenter sat behind a panel where he could observe and record behavior unobtrusively and manipulate the overhead strings to open and close the maze doors. At the end of each arm, a food cup (1 cm deep into the floor) contained a reward (a piece of Fruit Loop).

Training procedures: From day 6 to day 12 postischemia, rats began training in the radial maze. Daily food ration of ad-lib rats was gradually reduced (over a 4-day period) and rats maintained at approximately 85% of their free-feeding rate during the testing period to ensure adequate motivation. Rats were familiarized with the radial arm maze during four daily sessions each lasting 10 min on successive days. Rewards were initially available throughout the maze to encourage exploration, but were gradually restricted to the food cups. Each subject was individually placed in the center of the maze with the doors to all arms closed. Upon opening the doors, the rat was permitted to enter any of the eight arms. Upon entry in any one arm, all other maze arms were closed. When the rat had consumed the reward at the end of one arm and returned to the center of the maze, all doors remained closed to confine the rat to the center zone of the maze for a 10-s delay. The doors were then reopened and the procedure repeated. The test continued until all baits were consumed or until 10 min had elapsed. An arm entry was counted when the rat had its four paws within an arm. The orientation of the subject's head when placed into the central area was randomly permuted from trial to trial to minimize the development of a response pattern on the basis of position. The floor of the eight arms and central area of the maze was cleaned with a 70% ethanol solution after each trial to reduce olfactory cues.

Delayed nonmatching to sample tasks: win shift: The delayed nonmatching to sample (DNMTS) task was initiated 13 days postischemia, and consisted in daily acquisition and retention trials separated by a 15-min delay. In the acquisition trials, four arms were blocked with transparent Plexiglas doors. Rats were free to navigate to the four available arms, which were baited with a small piece of Fruit Loop. After consuming all food rewards (or when 10 min had elapsed), rats were removed from the maze and returned to their home cage for 15 min. After this delay, rats were placed back in the clean maze with a different body orientation for the retention trial. At this stage, all eight arms were available for entry, but only those previously blocked contained a reward. At the completion of each day's retention trial, rats were given their daily food ration. The arms blocked in the acquisition trials were individually and randomly assigned to rats from a list of 30 possible patterns, and arm sequences were counterbalanced across groups. One sequence was assigned to each animal and maintained for the 15-day testing period. After this period, rats were tested for an additional 3 days in a DNMTS task using the same arm sequence but a different intertrial delay on each day (5, 30, or 240 min). In addition, to determine whether the rats had learned rules involved in the DNMTS task rather than simply memorized the arm sequences, we tested rats for an additional six days using the same methodology but novel arm sequences. Measures recorded in the acquisition trial (predelay) included total number of working memory errors and latencies to complete the task. In the retention trials, the error scores include retroactive errors (entry into a non-rewarded arm) and proactive errors (re-entry into an arm within the test trial).

Delayed matching to sample task: win stay: After the DNMTS tasks, rats underwent a delayed matching to sample (DMTS) task for an additional 8 days using a new arm sequence and a 15-min intertrial delay. In this procedure, rats were rewarded for selecting previously baited arms in the retention trials. Measures recorded in the acquisition and retention trials of the DMTS were identical as the DNMTS.

Analysis of neuronal density on thionin-stained sections

Eighty-five days after reperfusion, rats were deeply anesthetized using sodium pentobarbital and perfused using 0.9% saline followed by 4% paraformaldehyde solution. Brains were removed and stored at -80°C. Serial coronal sections (14 μm) of the hippocampal regions were subsequently obtained using a cryostat and stained for Nissl bodies with thionin. Neuronal density of the hippocampal CA1 and CA3 subfields was determined using the method of Kirino *et al.* (1991) and performed on coronal sections located between 3.14 and 4.16 mm posterior to bregma (Paxinos and Watson, 1986). The total linear length of the CA1 and CA3 sectors [as defined by Paxinos and Watson (1986)] was measured by means of a digitizer. The number of intact neurons in the stratum pyramidale within CA1 and CA3 subfields was counted using a Leica DAS microscope attached to a Sony digital camera and computer-assisted cell counting was performed using Norton Eclipse (v 6.0; Empix Imaging, Missisauga, Ontario, Canada). Neurons that had shrunken cell bodies with surrounding empty spaces were excluded.

The neuronal density of the CA1 and CA3 sectors, that is, the number of intact pyramidal cells per 1-mm linear length of stratum pyramidale was quantified by a person that was blind to the treatment conditions. A mean value for each hippocampal substructure was obtained from six bilateral measurements per subject in each of the experimental groups. The neuronal density for a given subject represents the average of both the right and left hippocampal measures.

Data analysis

All statistical analyses were conducted using the PASW Statistics 18.0 software package. A difference was considered significant when the P value is less than or equal to 0.05. On rare occasions, rats showing two standard deviations from the group average performance were considered outliers and were excluded from the related statistical analysis. The assumptions of homogeneity of variance and of sphericity were verified. The Huynh-Feldt correction for violations to the assumption of sphericity was applied when appropriate and the degrees of freedom adjusted when the correction was used. Significant interactions were further analyzed using simple effect tests with Bonferroni modification of critical α level. Behavioral activity in the open field (calculated from frequencies of line crossing and the time spent rearing and grooming) was assessed in three blocks of 5 min each (total = 15 min) using a mixed analysis of variance (ANOVA) design, with two independent factors surgery and treatment and the repeated factor time. One-way ANOVAs followed by least significant difference post-hoc analyses were used to test for between group activity level differences for each time interval. In the object-recognition test, raw data obtained were transformed into a ratio, reflecting the preference of the animals for the novel versus the familiar object. The ratio formula was [tnovel/(tnovel + tfamiliar)], where tfamiliar is the time spent exploring the familiar object and tnovel is the time spent exploring the new object, in seconds (Gaskin *et al.*, 2010). The closer this ratio gets to 1, the more the animal spent time exploring the novel object. Two-factor (surgery and treatment) ANOVAs were performed on the dependent measure at each time interval to assess group differences. The data were analyzed as a partially crossed design taking into account the two factors being assessed but also the administration of the 1 mg/kg of resveratrol dose to a unique experimental group (i.e., the ischemic rats). Radial arm maze data were analyzed using mixed analysis of variance (ANOVA) designs with two independent factors surgery and treatment and various levels of the repeated factor time. Data obtained from neuronal densities in the CA1 and CA3 regions of the hippocampus were analyzed using two-factor (surgery and treatment) ANOVAs. Simple-effect tests were used for further analysis of significant interactions. In the radial arm maze, data are expressed as total errors (± standard error of the mean) for blocks of either 3 days (15-day DNMTS task) or 2 days (DNMTS; novel sequence and DMTS). Data from the different intertrial delays in the DNMTS tasks were analyzed separately. Values are expressed as mean ± standard error of the mean for all tests.

Results

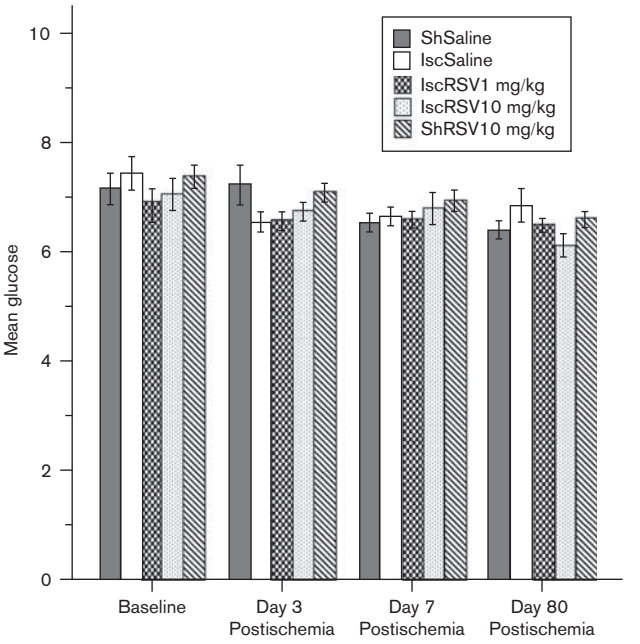
Body weight

Body weight values from both short-term and long-term studies were combined to assess possible effects of resveratrol treatments. Repeated-measures ANOVA revealed no significant effect of treatment [$F(2,80) = 1.48$, not significant] but a significant main effect of time [$F(3.53,\ 282.7) = 3535.57$, $P < 0.001$], attributable to increased body weight over time (data not shown).

Blood glucose level

Figure 2 presents the mean blood glucose level of each rat group collected at baseline and 3, 7, and 80 days post-surgery. Values from the short-term and long-term groups were combined for the initial three collection intervals. There was a significant main effect of time [$F(3,135) = 9.94$, $P < 0.001$], attributable to slightly higher glucose levels at baseline than at any other time point ($P < 0.05$) but baseline values remained within the normal glycemic range.

Fig. 2

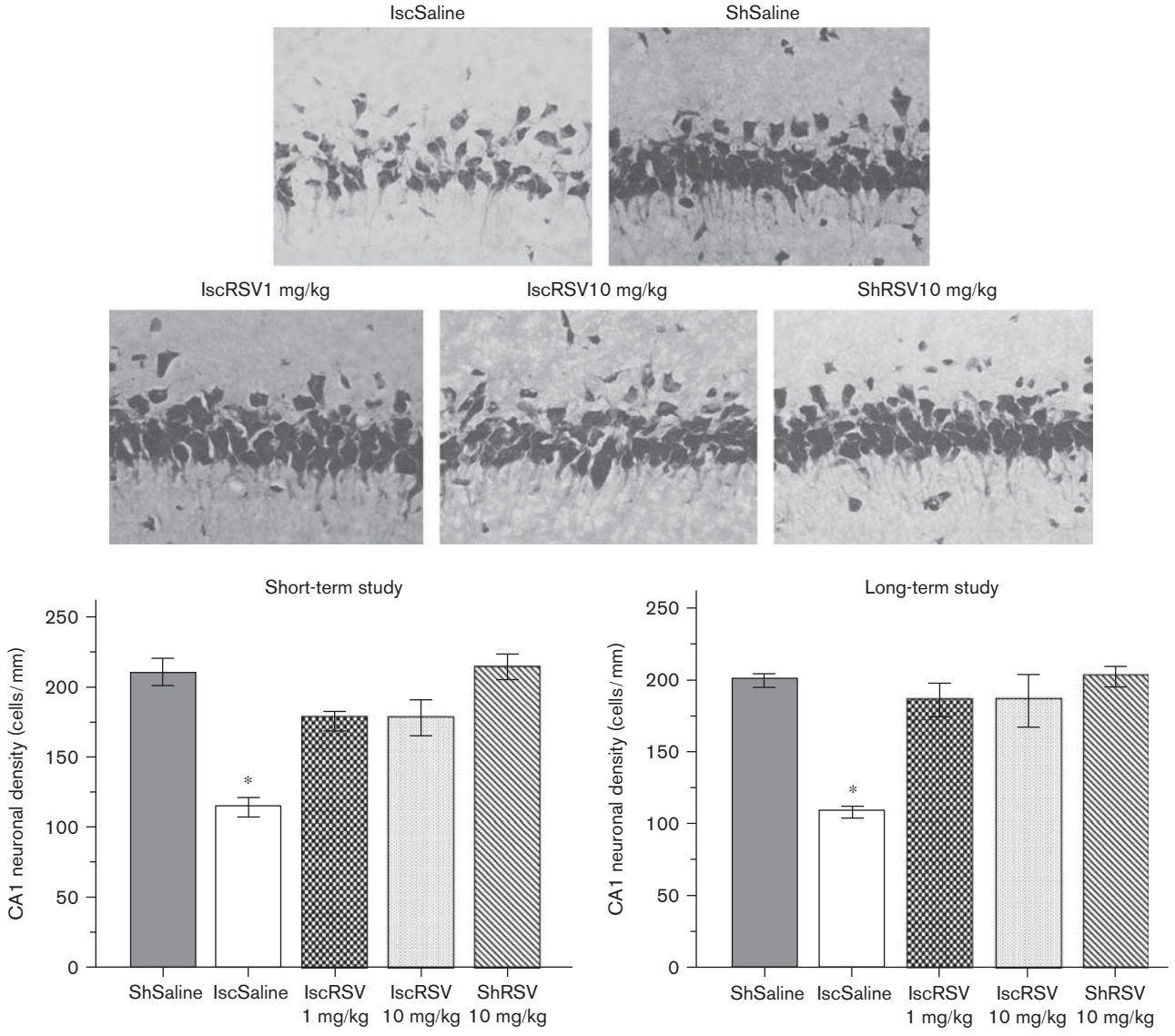


Mean blood glucose levels of saline and resveratrol treated ischemic and sham animals at baseline (presurgery) and 3, 7, and 85 days postischemia or sham surgery. No significant differences were observed between groups ($P > 0.05$).

Neuronal density assessment

Figure 3 shows the effect of resveratrol treatment and 10-min global ischemia on hippocampal CA1 and CA3 neuronal densities, 7 and 85 days postischemia. In the short-term groups, there were significant main effects of surgery [$F(1,30) = 49.59$, $P < 0.001$] and treatment

Fig. 3



		ShSaline	IscSaline	IscRSV 1 mg/kg	IscRSV 10 mg/kg	ShRSV 10 mg/kg
Long-term study	CA3	176.58±7.08	160.53±8.45	163.68±6.75	169.95±5.27	171.13±3.4
Short-term study	CA3	193.25±17.98	178.75±6.15	180.19±5.34	172.97±5.75	186.38±8.62

Upper panels show representative photomicrographs of thionin-stained neurons in the CA1 layer of sham and ischemic animals treated with either saline or resveratrol at 85 days postischemia. * indicates a significant reduction in the number of surviving CA1 neurons (approxiamtely 50%) by saline-treated ischemic rats compared with all other groups at both short-term and long-term intervals ($P < 0.001$). 1 and 10 mg/kg of resveratrol administration led to significant reduction in CA1 neuronal damage compared with saline-treated ischemic rats at both time intervals ($P < 0.001$) (lower panels - left and right figures, respectively). Bottom table presents the cell density (cells/mm) in the hippocampal CA3 region of sham and ischemic rats treated with either saline or resveratrol. There were no significant differences in CA3 cell density among the experimental groups ($P > 0.05$). Values represent mean ± standard error of the mean.

[$F(2,30) = 9.35$, $P < 0.005$] and a significant surgery X treatment interaction [$F(1,30) = 10.32$, $P < 0.005$]. Simple effect tests indicated that saline-treated ischemic rats had significantly fewer CA1 hippocampal neurons than their sham-counterparts, irrespective of treatment ($P < 0.001$) and that resveratrol-treated ischemic groups had significantly more CA1 neurons than ischemic rats treated with saline ($P < 0.005$, for both the short-term and long-term experiments). In the long-term groups, there were significant main effects of surgery [$F(1,46) = 24.83$, $P < 0.001$] and treatment [$F(2,46) = 11.37$, $P < 0.001$] and a significant surgery X treatment interaction [$F(1,46) = 10.67$, $P < 0.005$]. Simple effect tests indicated that saline-treated ischemic rats had significantly fewer CA1 hippocampal neurons than saline-treated sham counterparts ($P < 0.001$), ischemic rats treated with 1 mg/kg ($P = 0.001$) and 10 mg/kg ($P < 0.001$) of resveratrol and shams treated with 10 mg/kg of resveratrol ($P < 0.001$). Simple effect tests also indicated that both resveratrol dosages significantly attenuated ischemia-induced CA1 neuronal damage ($P < 0.005$) compared with saline-treated rats. No significant differences in the number of CA3 neurons were observed among the groups.

Behavioral tests

Open-field exploration (4 days postischemia)

Figure 4 (top row) highlights the total number of squares crossed by each experimental group during each of the three 5-min intervals. With regard to locomotion, there were significant main effects of time [$F(1.8, 81.16) = 41.99$, $P < 0.001$] and treatment [$F(2,45) = 5.75$, $P < 0.01$] and a significant time X treatment interaction [$F(3.21, 81.16) = 3.18$, $P < 0.05$]. Simple effects tests revealed that during the initial 5-min interval, rats treated with 10 mg/kg of resveratrol explored significantly more than saline and 1 mg/kg-treated rats ($P < 0.001$), irrespective of surgical treatment. Exploration also diminished with time, with all rats showing significantly decreased locomotion in the last 5 min of the test than at any other time interval ($P < 0.01$). With regard to rearing (Fig. 4, second row), mixed ANOVA revealed significant main effects of time [$F(1.59, 3796.13) = 59.9$, $P < 0.001$], surgery [$F(1,45) = 4.24$, $P < 0.05$] and treatment [$F(2,45) = 12.86$, $P < 0.05$]. This was attributable to ischemic rats spending significantly more time rearing than sham rats. Post-hoc analyses also revealed that ischemic rats treated with 1 mg/kg of resveratrol spent significantly less time rearing than saline-treated and 10-mg/kg resveratrol-treated rats ($P < 0.01$ and < 0.001, respectively). With regard to grooming, there was a significant main effect of time [$F(1.6, 72.04) = 21.78$, $P < 0.001$]. Post-hoc analyses revealed that, in an inverse relationship with the rearing and locomotion data, all rats gradually increased the time spent grooming from one interval to the next, with the most time spent grooming during the last 5 min of the test ($P < 0.05$).

Second open-field test (56 days postischemia)

Table 1 shows the locomotion, rearing, and grooming values for the three time intervals. Analysis of locomotion (total crossed squares) revealed no significant differences in exploratory activity among the groups but a significant main effect of time [$F(2,90) = 98.33$, $P < 0.001$] attributable to heightened exploration of the arena in the first 5-min interval compared with the subsequent two ($P = 0.001$). Analyses of rearing time also revealed a significant main effect of time [$F(2,90) = 38.05$, $P < 0.001$] with an enhanced rearing behavior present for all groups in the initial 5 min of the test ($P = 0.001$). Finally, analysis of grooming activity revealed significant main effects of time [$F(1.31, 58.93) = 17.15$, $P < 0.001$] and surgery [$F(1,45) = 4.42$, $P < 0.05$] and a significant time X surgery interaction [$F(1.31, 58.93) = 3.97$, $P < 0.05$]. Simple effect tests revealed that all rats spent the least amount of time grooming during the first 5 min of the test ($P < 0.05$) and that ischemic subjects spent significantly less time grooming than sham rats at every time interval ($P = 0.05$).

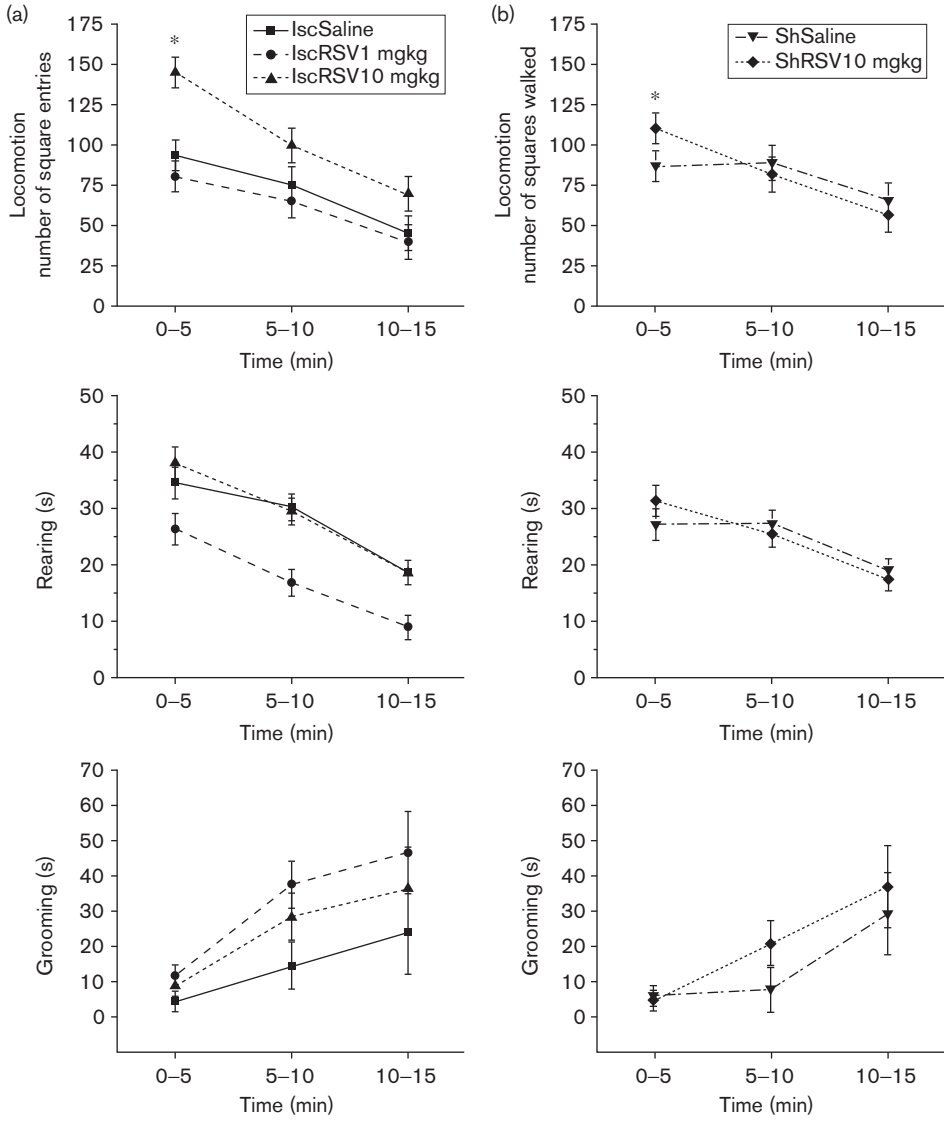
Object-recognition test

Figure 5 shows the investigation ratios for all groups of subjects for the two retention intervals (30 min and 24 h). At the 30-min retention interval, statistical analysis revealed a significant main effect of surgery [$F(1,48) = 4.95$, $P < 0.05$] and a significant surgery X treatment interaction [$F(1,48) = 5.84$, $P < 0.05$]. Simple effect tests indicated that this difference was attributable to heightened exploration of the novel object in saline-treated sham compared with ischemic rats ($P < 0.01$). In addition, a significant reduction of the exploration of the novel object was observed in resveratrol-treated sham rats compared with saline-treated rats ($P < 0.05$). There were no significant differences between ischemic groups ($P > 0.05$). At the 24 h retention interval all groups showed comparable exploration ratios ($P > 0.05$). There were no significant between-group differences in exploration time of O1 during familiarization, with all rats reaching the 20-s exploration criteria within the allocated 10-min session.

Radial arm maze

Figures 6 and 7 show the number of working memory errors during retention trials for the DNMTS and DMTS tasks, respectively. For the first DNMTS task, analyses of the performance in the acquisition trials revealed a significant main effect of time on the number of working memory errors [$F(3.4, 146.04) = 30.03$, $P < 0.001$] but no significant group differences, indicating that all groups learned to visit the four baited arms at the same rate (data not shown). However, analysis of the retention trial revealed significant main effects of time [$F(3.57, 153.62) = 77.92$, $P < 0.001$], surgery [$F(1,43) = 16.65$, $P < 0.001$], and treatment [$F(2,43) = 4.92$, $P < 0.05$], and a significant time X surgery interaction [$F(3.57, 153.62) = 3.5$, $P < 0.05$].

Fig. 4



The effect of 10-min global ischemia and resveratrol treatment on locomotion, rearing and grooming activity during the 15-min open-field test period on day 4 postischemia in ischemic (a) and sham (b) rats. During the first 5 min of the test, 10 mg/kg of resveratrol-treated rats explored the arena significantly more than all other groups. * indicates a significant difference between saline, 1 mg/kg and 10 mg/kg of resveratrol-treated groups ($P<0.001$). Values represent mean score ± standard error of the mean.

Simple effect analyses revealed that ischemic rats made significantly more errors than sham-operated rats on days 4–6 and 13–15. A trend was present on days 1–3, although it did not reach significance ($P = 0.062$). The significant main effect of time was due to significant improvement of all subject groups over time ($P < 0.05$).

When presented with a second DNMTS using different sequences of baited arms, analysis revealed no significant differences among the groups during both acquisition and retention trials. This suggests that once the animals have learned the win-shift rule from initial exposure to the DNMTS task, performance when presented with the same paradigm but using a new arm sequence is maintained, reducing between group differences.

Analysis of the number or working memory errors when intertrial intervals of 30, 120, and 240 min were applied revealed a significant main effect of time interval [$F(2,90) = 9.49$, $P < 0.001$] and a significant time X surgery interaction [$F(2,90) = 3.24$, $P < 0.05$]. Simple effect tests revealed that ischemic rats made significantly more errors during the 240-min delay than sham-operated rats ($P = 0.025$). Furthermore, ischemic rats gradually made more errors as the intertrial

Table 1 Effect of 10-min global ischemia and resveratrol treatment on locomotion, rearing, and grooming activities during the 15-min open-field test performed on day 56 postischemia

	Time (min)		
	0–5	5–10	10–15
Locomotion			
ShSaline	116.8 ± 11.02	91.5 ± 10.26	68.5 ± 11.06
IscSaline	117.5 ± 9.06	83.5 ± 8.93	50.6 ± 7.10
IscRSV1 mg/kg	132.0 ± 7.91	95.6 ± 10.49	54.5 ± 7.64
IscRSV10 mg/kg	127.7 ± 9.31	104.6 ± 9.68	75.6 ± 6.25
ShRSV10 mg/kg	118.4 ± 10.22	85.2 ± 9.01	56.9 ± 8.02
Rearing (s)			
ShSaline	29.2 ± 1.69	24.9 ± 1.38	18.3 ± 1.51
IscSaline	38.4 ± 3.28	31.1 ± 3.18	22.5 ± 2.74
IscRSV1 mg/kg	29.2 ± 1.93	28.1 ± 1.89	24.2 ± 3.64
IscRSV10 mg/kg	32.3 ± 2.01	25.9 ± 1.80	23.1 ± 2.00
ShRSV10 mg/kg	33.0 ± 3.12	27.2 ± 2.55	18.9 ± 2.37
Grooming (s)			
ShSaline	2.05 ± 0.85	8.32 ± 2.98	21.97 ± 10.13
IscSaline	0.36 ± 0.21	9.13 ± 3.08	8.19 ± 3.48
IscRSV1 mg/kg	2.33 ± 0.99	5.28 ± 1.85	12.99 ± 5.07
IscRSV10 mg/kg	4.40 ± 1.59	8.50 ± 3.27	12.90 ± 3.81
ShRSV10 mg/kg	7.34 ± 4.76	18.24 ± 5.88	34.53 ± 12.32

Locomotion values represent number of square entries. Values represent mean score ± standard error of the mean.

interval increased ($P < 0.05$), whereas shams were making just as many errors with delays of 120 and 240 min.

In the DMTS (win-stay) task, analysis of the number of errors during the acquisition trial revealed no significant differences between groups. However, analysis of errors during the retention trial, revealed significant main effects of time [$F(2.9, 130.57) = 43.3$, $P < 0.001$], associated with improved performance of all rats with increased trials, and of treatment [$F(2,45) = 10.46$, $P < 0.001$], attributable to increased errors in rats treated with 10 mg/kg of resveratrol compared with 1 mg/kg of resveratrol ($P < 0.001$) and saline-treated rats ($P < 0.05$).

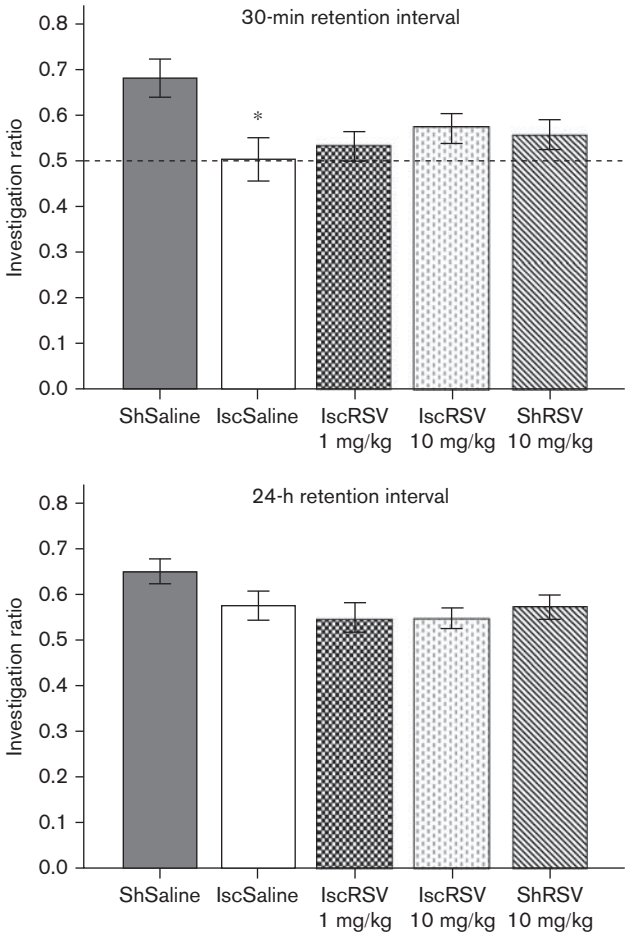
Discussion

The current study had two main goals: to determine the effects of repeated administration of two resveratrol doses on cognitive and behavioral impairments after global ischemia and to evaluate its effects on hippocampal neuronal injury at a remote postischemic interval.

Resveratrol leads to robust and long-lasting neuronal protection postischemia

To our knowledge findings from this study represent the first demonstration that pretreatment with resveratrol confers lasting neuronal protection against ischemia-induced hippocampal damage (40% greater neuronal survival than observed in vehicle-treated ischemic animals), maintained up to 85 days postischemia. To date, preservation of CA1 hippocampal neurons in experimental models has consistently been reported at reperfusion intervals ranging from 6 h to 7 days after ischemic injury (Wang *et al.*, 2002; Lu *et al.*, 2006; Della-Morte *et al.*, 2009; Simao *et al.*, 2011). The robust and persistent neuronal preservation suggests that resveratrol treatment

Fig. 5

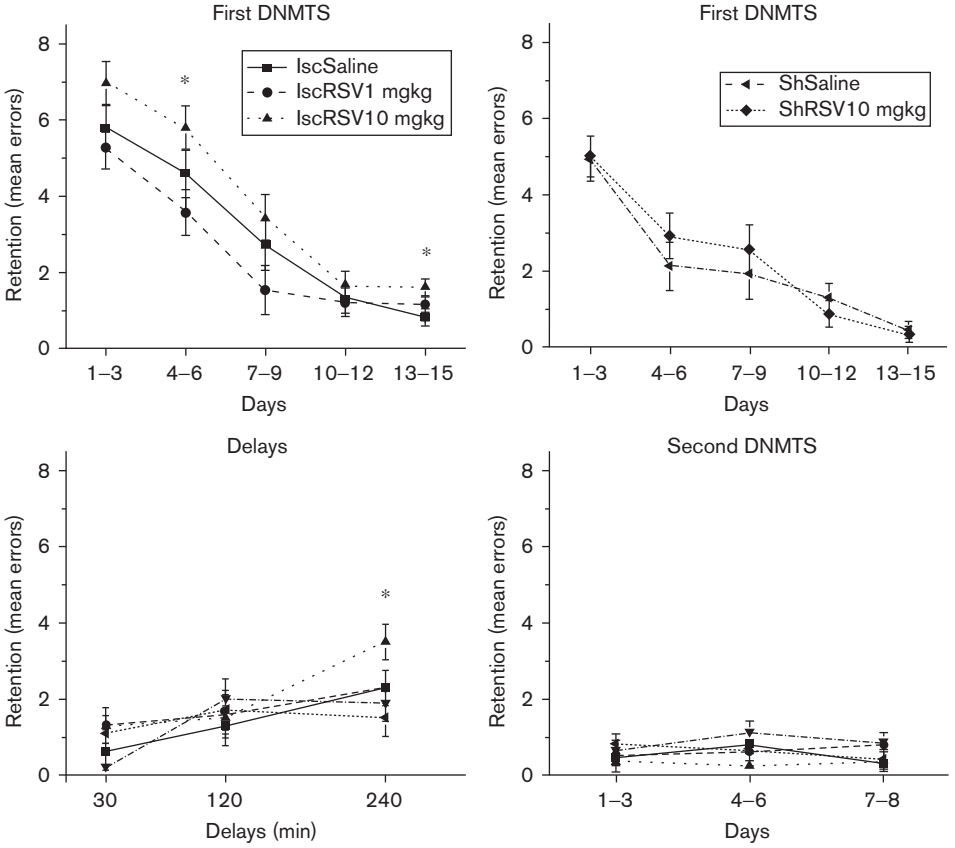


Mean (± standard error of the mean) investigation ratio of saline and resveratrol-treated ischemic and sham rats in the object-recognition test at the 30 min and 24 h retention intervals. * indicates a significant reduction of the exploration ratio after 10 min of global ischemia in saline-treated rats ($P < 0.05$).

can induce long-term cellular effects. This is important as many therapeutic approaches have been shown to lose their beneficial impact as time following reperfusion is increased [see Corbett and Nurse (1998) for a review]. Of note, repeated 21-day resveratrol administration before ischemic and sham surgery did not affect body weight and blood glucose measures at 3, 7, and 85 days postsurgery. This is consistent with a study in which 10 mg/kg of resveratrol was administered to controls by gavage chronically for 45 days and noted no significant differences in metabolic measures of body weight, glycemia, and high-density lipoprotein-cholesterol levels compared with vehicle-treated rats (Miatello *et al.*, 2005).

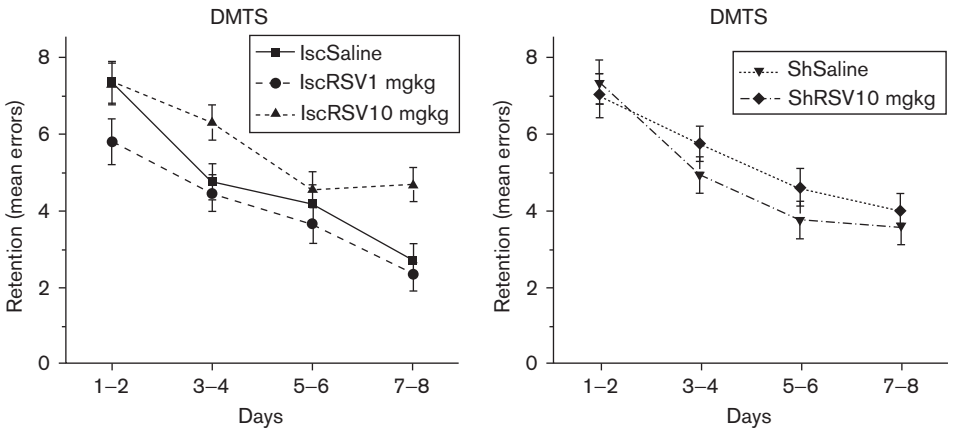
Current hypotheses underlying the neuroprotective actions of resveratrol include its effect on reducing oxidative stress through scavenging of reactive oxygen species, releasing trophic factors important for angiogenesis (Fukuda *et al.*, 2006; Dong *et al.*, 2008; Wang *et al.*,

Fig. 6



Effect of ischemia and resveratrol treatment on the total number of errors during retention trials in the first delayed nonmatching to sample task (DNMTS) (15 days), DNMTS (variable delays) and second DNMTS (6 days). * During the first DNMTS, ischemic rats made significantly more errors during days 3–5 and again on days 12–15, than sham-operated controls ($P<0.001$). Upon acquisition of the first DNMTS task, extending retention delays did not have an impact on performance except for the 240-min intertrial delay; * 10 mg/kg of resveratrol-treated ischemic rats made significantly more errors than either 1 mg/kg of resveratrol and saline-treated rats ($P<0.05$). Values represent mean error ± standard error of the mean.

Fig. 7



Effect of ischemia and resveratrol treatment on the total number of errors during retention trials in the delayed matching to sample task (DMTS) task in the radial arm maze. Throughout the 8-day testing period, rats treated with 10 mg/kg of resveratrol made significantly more errors than saline and 1 mg/kg of resveratrol-treated rats ($P<0.05$). Values represent mean error ± standard error of the mean.

2010) and reducing inflammation (Manna *et al.*, 2000; Simao *et al.*, 2011). In particular, resveratrol has been shown to enhance free radical scavenging and cerebral blood flow via upregulation of endothelial nitiric oxide synthase release (Das *et al.*, 2005; Lu *et al.*, 2006; Tsai *et al.*, 2007) and downregulation of inducible nitiric oxide synthase (Bi *et al.*, 2005). For instance, 21-day resveratrol pretreatment at a dose of 20 mg/kg led to significant inhibition of infarct volume after MCA occlusion in rats, a phenomenon associated with alterations of discrete oxidative stress signals, including malondialdehyde and glutathione levels (Sinha *et al.*, 2002). The presence of similar physiological effects in our study remains to be determined but appears possible.

Resveratrol effects on behavioral performance are dose related

At present, very few studies have characterized the impact of daily resveratrol administration on behavioral performance in normal animals or following global cerebral ischemia (Miatello *et al.*, 2005; Wang *et al.*, 2009). We have used the open-field, object-recognition, and radial arm maze tests to determine resveratrol effects on locomotor activity, visual and spatial memory. Our findings indicated dose-related effects of resveratrol on behavior in ischemic animals and altered performance of sham animals treated with 10 mg/kg of resveratrol in discrete behavioral tests, the latter suggesting long-lasting intrinsic actions of resveratrol to regulate locomotion and memory. Thus, in the open field, pretreatment with 10 mg/kg of resveratrol led to enhanced locomotor activity in ischemic rats 4 days postsurgery compared with 1 mg/kg-treated and saline-treated controls. Our study further indicated that locomotor activity was enhanced in resveratrol-treated sham rats at the same time interval. At present, no study has examined the impact of repeated resveratrol administration on locomotor activity in control rats or animals exposed to global ischemia. In a recent study, Wang *et al.* (2009) reported reduced open-field activity in gerbils associated with oral administration of grape polyphenol extract (resveratrol content was estimated to be 0.7 µmol/l) 4 days before and in the 48 h preceding postischemic open-field testing. Together, these observations suggest dose-related effects, although direct comparison of findings remains difficult considering a much shorter administration protocol and oral administration of a polyphenol extract. Of interest, saline-treated ischemic animals, whose global activity is commonly enhanced postischemia (Green *et al.*, 1995; Plamondon and Khan, 2005; Yan *et al.*, 2007; Milot and Plamondon, 2008, 2009), showed locomotion comparable with sham rats. These observations further suggest that increased locomotion observed in 10 mg/kg-treated ischemic rats is linked to intrinsic effects of higher resveratrol dosages on neurochemical pathways mediating this response (Poignet *et al.*, 1989; Araki *et al.*, 1999). The reason for the absence of hyperactivity in saline-treated ischemic rats at this time interval is not fully understood but has been previously reported. Change in emotional reactivity postischemia has been proposed to play a role in novelty-induced hyperactivity in ischemic animals (Plamondon and Khan, 2005; Walsh *et al.*, 2008). In the current study, rats were handled daily for 21 days before ischemia, while being injected with resveratrol or saline, a procedure which could have effects on emotional reactivity. This is supported by similar observations in cohorts of ischemic rats that received extensive handling associated to discrete preischemic feeding or injection protocols (Plamondon and Roberge, 2008; Roberge *et al.*, 2008a; De Butte-Smith *et al.*, 2009). Furthermore, healthy animals receiving daily handling prior to open-field exposure have shown reduced open-field activity (Schmitt and Hiemke, 1998; Izidio *et al.*, 2005). Upon re-exposure to the open-field 56 days postischemia, all groups showed comparable open-field activity. Other researchers have reported a similar decline in hyperactivity upon habituation to the testing environment (Wang and Corbett, 1990; Dowden and Corbett, 1999; Plamondon and Khan, 2005).

The presence of intrinsic effects of resveratrol on behavior was also apparent from findings in the object-recognition test, where sham rats treated with 10 mg/kg of resveratrol showed recognition memory impairments comparable with that of ischemic rats at the 30-min retention interval. In addition, the 1 and 10 mg/kg of resveratrol doses did not prevent ischemia-induced memory impairments. The presence of recognition impairments in resveratrol-treated sham as well as CA1-protected ischemic rats further supports the dissociation of performance in this test from CA1 neuronal injury (Mumby, 2001; De Butte-Smith *et al.*, 2009; Aggleton *et al.*, 2010).

The participation of CA1 neurons in spatial memory is acknowledged and the radial maze is a reliable tool to measure spatial memory impairments. We selected DNMTS and DMTS to assess spatial memory impairments, the latter paradigm (Win-stay) being considered more challenging for rats due to their natural propensity to select alternate arms than those previously explored in retention trials. In the DNMTS radial maze task, spatial memory impairments were apparent in both saline-treated and resveratrol-treated ischemic animals, with a tendency for the 10mg/kg dose to worsen the rats' performance, whereas ischemic rats treated with the lower 1mg/kg dose showed a trend toward improved performance. Ischemic rats displayed an increased number of errors in the DNMTS task when a 240-min intertrial interval was used, which was particularly evident in ischemic rats treated with 10 mg/kg of resveratrol. The DMTS task led to increased errors for all groups, illustrating increased difficulty of the animals to apply a win-stay rule. Interestingly, yet consistent with observations

in the DNMTS task, 10-mg/kg resveratrol treatment accentuated memory impairments in ischemic and sham rats, a difference maintained over days.

A number of studies have examined the relationship between hippocampal density and performance in various spatial memory tasks and consistently associated moderate-to-severe CA1 neuronal loss with significant spatial memory impairments (Hodges *et al.*, 1996; Nelson *et al.*, 1997; Block, 1999; Briones and Therrien, 2000; Hartman *et al.*, 2005). Among the CA1 protected resveratrol ischemic rats, rats treated with the 10-mg/kg dose showed increased and long-lasting spatial memory impairments, suggesting that factors other than CA1 injury contribute to the memory deficits observed. Dissociations between CA1 neuronal injury and behavioral recovery postischemia have been reported by earlier studies (Lyeth *et al.*, 1990; Roberge *et al.*, 2008a; De Butte-Smith *et al.*, 2009). De Butte-Smith *et al.* (2009) recently demonstrated that increased CA1 cell survival associated with chronic estradiol treatment in female rats failed to improve visual or spatial recognition memory impairments after global ischemia (De Butte-Smith *et al.*, 2009). Conversely, recovery of functional impairments post stroke has been demonstrated in the absence of CA1 neuronal survival (Lyeth *et al.*, 1990; Olsen *et al.*, 1994; Ma *et al.*, 2008). In this context, it has recently been suggested that pharmacologically manipulating norepinephrine release after ischemia, by administering the α2-adrenoceptor agonist clonidine, could attenuate working memory deficits in the radial arm maze, despite significant CA1 cell loss (Milot and Plamondon, 2011). It is thus possible that higher resveratrol doses could be associated with physiological changes affecting attentional and/or emotional processes interfering with memory performance.

Conclusion

Our findings demonstrate that CA1 neuronal protection in ischemic rats treated with either resveratrol doses is not predictive of improved behavioral recovery. Rather, resveratrol effects appeared dose-related and independent of CA1 injury in discrete behavioral tests. Our findings are consistent with those of different studies suggesting a dose-dependent profile of resveratrol actions under basal or experimental conditions (Harper *et al.*, 2007; Dudley *et al.*, 2009; Mukherjee *et al.*, 2010) and with recent findings highlighting disease specific physiological effects (Calabrese *et al.*, 2010). Although definite dosage-related effects remain difficult to confirm, due to combined in-vitro and in-vivo evaluations and the range of resveratrol doses investigated, these observations do emphasize a continuum of physiological resveratrol actions dependent on dosage. Growing evidence also supports dose-dependent resveratrol effects in other pathological models, including osteoporosis (Dai *et al.*, 2007) and Alzheimer's disease (Conte *et al.*, 2003).

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Conflicts of interest

There are no conflicts of interests.

References

Aggleton JP, Albasser MM, Aggleton DJ, Poirier GL, Pearce JM (2010). Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. *Behav Neurosci* **124**: 55–68.

Araki H, Hino N, Karasawa Y, Kawasaki H, Gomita Y (1999). Effect of dopamine blockers on cerebral ischemia-induced hyperactivity in gerbils. *Physiol Behav* **66**:263–268.

Ates O, Cayli SR, Yucel N, Altinoz E, Kocak A, Durak MA, *et al.* (2007). Central nervous system protection by resveratrol in streptozotocin-induced diabetic rats. *J Clin Neurosci* **14**:256–260.

Bi XL, Yang JY, Dong YX, Wang JM, Cui YH, Ikeshima T, *et al.* (2005). Resveratrol inhibits nitric oxide and TNF-alpha production by lipopolysaccharide-activated microglia. *Int Immunopharmacol* **5**:185–193.

Block F (1999). Global ischemia and behavioural deficits. *Prog Neurobiol* **58**:279–295.

Bradamante S, Barenghi L, Villa A (2004). Cardiovascular protective effects of resveratrol. *Cardiovasc Drug Rev* **22**:169–188.

Briones TL, Therrien B (2000). Behavioral effects of transient cerebral ischemia. *Biol Res Nurs* **1**:276–286.

Calabrese EJ, Mattson MP, Calabrese V (2010). Resveratrol commonly displays hormesis: occurrence and biomedical significance. *Hum Exp Toxicol* **29**:980–1015.

Conte A, Pellegrini S, Tagliazucchi D (2003). Synergistic protection of PC12 cells from [beta]-amyloid toxicity by resveratrol and catechin. *Brain Res Bull* **62**:29–38.

Corbett D, Nurse S (1998). The problem of assessing effective neuroprotection in experimental cerebral ischemia. *Prog Neurobiol* **54**:531–548.

Dai Z, Li Y, Quarles LD, Song T, Pan W, Zhou H, Xiao Z (2007). Resveratrol enhances proliferation and osteoblastic differentiation in human mesenchymal stem cells via ER-dependent ERK1/2 activation. *Phytomedicine* **14**: 806–814.

Das S, Alagappan VK, Bagchi D, Sharma HS, Maulik N, Das DK (2005). Coordinated induction of iNOS-VEGF-KDR-eNOS after resveratrol consumption: a potential mechanism for resveratrol preconditioning of the heart. *Vascul Pharmacol* **42**:281–289.

De Butte-Smith M, Gulinello M, Zukin RS, Etgen AM (2009). Chronic estradiol treatment increases CA1 cell survival but does not improve visual or spatial recognition memory after global ischemia in middle-aged female rats. *Horm Behav* **55**:442–453.

Della-Morte D, Dave KR, DeFazio RA, Bao YC, Raval AP, Perez-Pinzon MA (2009). Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. *Neuroscience* **159**:993–1002.

Dong W, Li N, Gao D, Zhen H, Zhang X, Li F (2008). Resveratrol attenuates ischemic brain damage in the delayed phase after stroke and induces messenger RNA and protein express for angiogenic factors. *J Vasc Surg* **48**:709–714.

Dowden J, Corbett D (1999). Ischemic preconditioning in 18- to 20-month-old gerbils: long-term survival with functional outcome measures. *Stroke* **30**:1240–1246.

Dudley J, Das S, Mukherjee S, Das DK (2009). Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium depending on dose. *J Nutr Biochem* **20**:443–452.

Fremont L (2000). Biological effects of resveratrol. *Life Sci* **66**:663–673.

Fukuda S, Kaga S, Zhan L, Bagchi D, Das DK, Bertelli A, Maulik N (2006). Resveratrol ameliorates myocardial damage by inducing vascular endothelial growth factor-angiogenesis and tyrosine kinase receptor Flk-1. *Cell Biochem Biophys* **44**:43–49.

Gaskin S, Tardif M, Cole E, Piterkin P, Kayello L, Mumby DG (2010). Object familiarization and novel-object preference in rats. *Behav Processes* **83**: 61–71.

Green EJ, Pazos AJ, Dietrich WD, McCabe PM, Schneiderman N, Lin B, *et al.* (1995). Combined postischemic hypothermia and delayed MK-801 treatment attenuates neurobehavioral deficits associated with transient global ischemia in rats. *Brain Res* **702**:145–152.

Gupta YK, Briyal S, Chaudhary G (2002). Protective effect of trans-resveratrol against kainic acid-induced seizures and oxidative stress in rats. *Pharmacol Biochem Behav* **71**:245–249.

Harper CE, Patel BB, Wang J, Arabshahi A, Eltoum IA, Lamartiniere CA (2007). Resveratrol suppresses prostate cancer progression in transgenic mice. *Carcinogenesis* **28**:1946–1953.

Hartman RE, Lee JM, Zipfel GJ, Wozniak DF (2005). Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. *Brain Res* **1043**:48–56.

Hodges H, Sowinski P, Fleming P, Kershaw TR, Sinden JD, Meldrum BS, Gray JA (1996). Contrasting effects of fetal CA1 and CA3 hippocampal grafts on deficits in spatial learning and working memory induced by global cerebral ischaemia in rats. *Neuroscience* **72**:959–988.

Huber K, Superti-Furga G (2011). After the grape rush: sirtuins as epigenetic drug targets in neurodegenerative disorders. *Bioorg Med Chem* **19**:3616–24.

Izidio GS, Lopes DM, Spricigo L Jr, Ramos A (2005). Common variations in the pretest environment influence genotypic comparisons in models of anxiety. *Genes Brain Behav* **4**:412–419.

Kirino T (1982). Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* **239**:57–69.

Kirino T, Tamura A, Sano K (1984). Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. *Acta Neuropathol (Berl)* **64**:139–147.

Kirino T, Tsujita Y, Tamura A (1991). Induced tolerance to ischemia in gerbil hippocampal neurons. *J Cereb Blood Flow Metab* **11**:18.

Lu KT, Chiou RY, Chen LG, Chen MH, Tseng WT, Hsieh HT, Yang YL (2006). Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation. *J Agric Food Chem* **54**:3126–3131.

Lyeth BG, Jenkins LW, Hamm RJ, Dixon CE, Phillips LL, Clifton GL, *et al.* (1990). Prolonged memory impairment in the absence of hippocampal cell death following traumatic brain injury in the rat. *Brain Res* **526**:249–258.

Ma B, Li M, Nong H, Shi J, Liu G, Zhang J (2008). Protective effects of extract of Coeloglossum viride var. bracteatum on ischemia-induced neuronal death and cognitive impairment in rats. *Behav Pharmacol* **19**:325–333.

Manna SK, Mukhopadhyay A, Aggarwal BB (2000). Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol* **164**:6509–6519.

Miatello R, Vazquez M, Renna N, Cruzado M, Zumino AP, Risler N (2005). Chronic administration of resveratrol prevents biochemical cardiovascular changes in fructose-fed rats. *Am J Hypertens* **18**:864–870.

Milot M, Plamondon H (2008). Ischemia-induced hyperactivity: effects of dim versus bright illumination on open-field exploration and habituation following global ischemia in rats. *Behav Brain Res* **192**:166–172.

Milot MR, Plamondon H (2009). Time-dependent effects of global cerebral ischemia on anxiety, locomotion, and habituation in rats. *Behav Brain Res* **200**:173–180.

Milot MR, Plamondon H (2011). Changes in HPA reactivity and noradrenergic functions regulate spatial memory impairments at delayed time intervals following cerebral ischemia. *Horm Behav* **59**:594–604.

Mukherjee S, Dudley JI, Das DK (2010). Dose-dependency of resveratrol in providing health benefits. *Dose Response* **8**:478–500.

Mumby DG (2001). Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behav Brain Res* **127**:159–181.

Nelson A, Lebessi A, Sowinski P, Hodges H (1997). Comparison of effects of global cerebral ischaemia on spatial learning in the standard and radial water maze: relationship of hippocampal damage to performance. *Behav Brain Res* **85**:93–115.

Nikonenko AG, Radenovic L, Andjus PR, Skibo GG (2009). Structural features of ischemic damage in the hippocampus. *Anat Rec (Hoboken)* **292**:1914–1921.

Olsen GM, Scheel-Kruger J, Moller A, Jensen LH (1994). Relation of spatial learning of rats in the Morris water maze task to the number of viable CA1 neurons following four-vessel occlusion. *Behav Neurosci* **108**:681–690.

Paxinos G, Watson C (1986). *The rat brain in stereotaxic coordinates*. San Diego: Academic Press.

Plamondon H, Khan S (2005). Characterization of anxiety and habituation profile following global ischemia in rats. *Physiol Behav* **84**:543–552.

Plamondon H, Roberge MC (2008). Dietary PUFA supplements reduce memory deficits but not CA1 ischemic injury in rats. *Physiol Behav* **95**:492–500.

Plamondon H, Morin A, Charron C (2006). Chronic 17beta-estradiol pretreatment and ischemia-induced hippocampal degeneration and memory impairments: a 6-month survival study. *Horm Behav* **50**:361–369.

Poignet H, Beaughard M, Lecoin G, Massingham R (1989). Functional, behavioral, and histological changes induced by transient global cerebral ischemia in rats: effects of cinnarizine and flunarizine. *J Cereb Blood Flow Metab* **9**:646–654.

Pulsinelli WA, Brierley JB (1979). A new model of bilateral hemispheric ischemia in the unanesthetized rat. *Stroke* **10**:267–272.

Roberge MC, Hotte-Bernard J, Messier C, Plamondon H (2008a). Food restriction attenuates ischemia-induced spatial learning and memory deficits despite extensive CA1 ischemic injury. *Behav Brain Res* **187**: 123–132.

Roberge MC, Messier C, Staines WA, Plamondon H (2008b). Food restriction induces long-lasting recovery of spatial memory deficits following global ischemia in delayed matching and non-matching-to-sample radial arm maze tasks. *Neuroscience* **156**:11–29.

Sandstrom NJ, Rowan MH (2007). Acute pretreatment with estradiol protects against CA1 cell loss and spatial learning impairments resulting from transient global ischemia. *Horm Behav* **51**:335–345.

Schmitt U, Hiemke C (1998). Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling. *Pharmacol Biochem Behav* **59**:807–811.

Sharma M, Gupta YK (2002). Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. *Life Sci* **71**:2489–2498.

Simao F, Matte A, Matte C, Soares FM, Wyse AT, Netto CA, Salbego CG (2011). Resveratrol prevents oxidative stress and inhibition of Na(+)K(+)-ATPase activity induced by transient global cerebral ischemia in rats. *J Nutr Biochem* **22**:921–928.

Sinha K, Chaudhary G, Gupta YK (2002). Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci* **71**:655–665.

Taoufik E, Probert L (2008). Ischemic neuronal damage. *Curr Pharm Des* **14**:3565–3573.

Tsai SK, Hung LM, Fu YT, Cheng H, Nien MW, Liu HY, *et al.* (2007). Resveratrol neuroprotective effects during focal cerebral ischemia injury via nitric oxide mechanism in rats. *J Vasc Surg* **46**:346–353.

Walsh SJ, Harley CW, Corbett D, Skinner DM, Martin GM (2008). CA1 ischemic injury does not affect the ability of Mongolian gerbils to solve response, direction, or place problems. *Brain Res* **1187**:194–200.

Wang D, Corbett D (1990). Cerebral ischemia, locomotor activity and spatial mapping. *Brain Res**5*** **33**:78–82.

Wang H, Zhou H, Zou Y, Liu Q, Guo C, Gao G, *et al.* (2010). Resveratrol modulates angiogenesis through the GSK3beta/beta-catenin/TCF-dependent pathway in human endothelial cells. *Biochem Pharmacol* **80**: 1386–1395.

Wang J, Ho L, Zhao Z, Seror I, Humala N, Dickstein DL, *et al.* (2006). Moderate consumption of Cabernet Sauvignon attenuates Abeta neuropathology in a mouse model of Alzheimer's disease. *FASEB J* **20**:2313–2320.

Wang Q, Xu J, Rottinghaus GE, Simonyi A, Lubahn D, Sun GY, Sun AY (2002). Resveratrol protects against global cerebral ischemic injury in gerbils. *Brain Res* **958**:439–447.

Wang Q, Sun AY, Simonyi A, Miller DK, Smith RE, Luchtefeld RG, *et al.* (2009). Oral administration of grape polyphenol extract ameliorates cerebral ischemia/reperfusion-induced neuronal damage and behavioral deficits in gerbils: comparison of pre- and post-ischemic administration. *J Nutr Biochem* **20**:369–377.

Wang Y, Xu H, Fu Q, Ma R, Xiang J (2011). Protective effect of resveratrol derived from Polygonum cuspidatum and its liposomal form on nigral cells in Parkinsonian rats. *J Neurol Sci* **304**:29–34.

Wu Z, Xu Q, Zhang L, Kong D, Ma R, Wang L (2009). Protective effect of resveratrol against kainate-induced temporal lobe epilepsy in rats. *Neurochem Res* **34**:1393–1400.

Yan X-B, Wang S-S, Hou H-L, Ji R, Zhou J-N (2007). Lithium improves the behavioral disorder in rats subjected to transient global cerebral ischemia. *Behav Brain Res**1*** **77**:282–289.

Zhang F, Shi JS, Zhou H, Wilson B, Hong JS, Gao HM (2010). Resveratrol protects dopamine neurons against lipopolysaccharide-induced neurotoxicity through its anti-inflammatory actions. *Mol Pharmacol* **78**:466–477.