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Effects of dietary 5-methoxyindole-2-carboxylic acid on brain functional recovery after ischemic stroke

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Abstract

Stroke leads to devastating outcomes including impairments of sensorimotor and cognitive function that may be long lasting. New intervention strategies are needed to overcome the long-lasting effects of ischemic injury. Previous studies determined that treatment with 5-methoxyindole-2-carboxylic acid (MICA) conferred chemical preconditioning and neuroprotection against stroke. The purpose of the current study was to determine whether the preconditioning can lead to functional improvements after stroke (done by transient middle cerebral artery occlusion). After 4 weeks of MICA feeding, half the rats underwent ischemic injury, while the other half remained intact. After one week recovery, all the rats were tested for motor and cognitive function (rotorod and water maze). At the time of euthanasia, measurements of long-term potentiation (LTP) were performed. While stroke injury led to motor and cognitive dysfunction, MICA supplementation did not reverse these impairments. However, MICA supplementation did improve stroke-related impairments in hippocampal LTP. The dichotomy of the outcomes suggest that more studies are needed to determine optimum duration and dosage for MICA to lead to substantial motor and cognitive improvements, along with LTP change and neuroprotection.

Keywords

Stroke; MICA; preconditioning; cognition; DLDH

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1. Introduction

Stroke is the fifth leading cause of death and the leading cause of disability in the United States [1, 2]. It is associated with sensorimotor and cognitive impairments that may be long lasting. Therefore, it is critical to develop new strategies to alleviate the negative outcomes associated with stroke. Preconditioning, defined as an exposure to the non-injurious stimulus prior to the injurious stimulus, has been proposed as a promising strategy for ischemic stroke [3]. In particular, chemical preconditioning, via manipulations of oxidative phosphorylation or use of drugs, has gained support in the literature as an ethical and viable approach to alleviating the devastating outcomes of ischemic injury [4, 5]. Furthermore, chemical or pharmacological preconditioning has been shown to have promising prophylactic effects, an ideal characteristic for situations, such as surgeries, during which ischemic events are likely [6, 7].

During ischemia, oxygen and nutrient levels are depleted leading to a decrease in ATP production, suggesting mitochondria as a known target for injury [8, 9]. Because chemical or pharmacological conditioning targets a particular protein by affecting its function, any mitochondrial protein can be activated or inhibited under conditioning. So, proteins involved in oxygen consumption, nutrient electron extraction, or oxidative phosphorylation can be primary targets of chemical conditioning via a known activator/inhibitor or newly synthesized drugs [10]. One particular mitochondrial protein of interest is dihydrolipoamide dehydrogenase (DLDH).

DLDH, a flavin-dependent, pyridine dinucleotide oxidoreductase, is involved in complexes and pathways fundamental to mitochondrial function and ultimately cell survival. DLDH is part of the α -ketoglutarate dehydrogenase, pyruvate dehydrogenase, and branched amino acid dehydrogenase complexes, as well as the glycine cleavage system. Inhibition of DLDH does not affect survivorship and fertility of mice, and can therefore serve as a target of preconditioning. The compound 5-methoxyindole-2-carboxylic acid (MICA) is an indolecarboxylic acid acting as a hypoglycemic agent [11] and dihydrolipoamide dehydrogenase (DLDH) inhibitor. Supplementation for 4 weeks of MICA (0.33% dose) did not produce any adverse effects on body weights, glucose levels, or mitochondrial electron transport chain and oxidative phosphorylation [12]. Supplementation with MICA attenuated DLDH activity and activated NAD(P)H:ubiquinone oxidoreductase-1 (NQO1) via Nrf2 pathway. These observations were associated with decreased oxidative stress and cell death, and improved ATP output [12]. This study also determined that supplementation or injection of MICA resulted in a reduction of the infarct size following transient middle cerebral artery occlusion (tMCAO), and provided a delayed neuroprotection involving Nrf-2 signaling pathway [12]. In summary, our previous studies have determined that MICA supplementation or injection induced ischemic tolerance [12].

Based on these results, we proposed the following study to determine the functional outcomes of MICA supplementation and whether it could alleviate the effects of stroke on motor and cognitive function, as well as long-term potentiation (LTP). Accordingly, we supplemented young rats with MICA for one month. After reversing to the control diet, a subgroup of rats were subjected to tMCAO while the others remained intact. After a week

recovery, all the rats were tested for motor coordination (rotorod) and spatial learning and memory (water maze). Upon euthanization, brain slices were used for LTP assessments.

2. Materials and Methods

2.1 Animals

Young male Sprague-Dawley rats (2 months old) were obtained from Charles Rivers, and maintained in the UNT Health Science Center Vivarium. The studies were approved by the Institutional Care and Use Committee of UNT Health Science Center and were in accordance with NIH guidelines for the Care and Use of Laboratory Animals. Animals were group housed (2/cage). The rats were ID chipped and randomly assigned to one of four experimental groups. In summary and reflected in Figure 1, the rats were placed on either a control diet or a MICA supplemented diet for one month. The MICA diet was then discontinued, and those rats were placed on the control diet. After 3 days, a subgroup of rats were stroked while the others remained intact. After a one week recovery (Stroke rats) or rest period (No Stroke rats), all rats were tested for motor and cognitive function.

2.2 Chemicals and reagents

5-methoxyindole-2-carboxylic acid (MICA) was purchased from Sigma-Aldrich (M14951). Chemicals used in LTP recordings were purchased from Sigma-Aldrich or FisherBiotech.

2.3 Administration of MICA

Rats received either a control diet (TestDiet, Richmond, IN, 5LL2) or the control diet supplemented with 0.33% MICA (TestDiet, Richmond, IN, 5SVZ) which was previously used and was associated with an effect of the preconditioning [12]. After 1–2 weeks acclimation, the rats were randomly assigned to one of the diet (control or MICA). The rats had ad libitum access to food and water. Body weights were monitored throughout the study. After one month on their respective diets, all rats were placed back on the control diet for the remainder of the study. No death happened during the MICA feeding.

2.4 Transient Middle Cerebral Artery Occlusion (tMCAO)

Rats were randomly assigned to either a No Stroke or a Stroke group. Three days after the rats were all placed back on the control diet, the Stroke rats underwent surgery while the No Stroke group received no surgery and remained intact. The stroke group received tMCAO surgery as previously described [12, 13]. Under anesthesia (1–3% isoflurane in 30% oxygen using a vaporizer and flowmeter), the left MCA of the rats was occluded by a 4–0 monofilament suture (0.3–0.33mm coated with silicon) via internal carotid artery. The occlusion lasted 60 min, at which point the suture was removed and reperfusion took place [12, 13]. After one week recovery the rats were behaviorally characterized. We started with n=12 for the No Stroke rats, and n=24 for the Stroke rats. A total of 10 rats died from the stroke surgery (6 from the control group, and 4 from the MICA treated group) making the final Ns as follow: n=12 for No Stroke rats regardless of diet and n=18 for Stroke rats on control diet and n=20 for Stroke rats on MICA diet (Note: rotorod has n=21 for the Stroke rats on MICA diet as one rat was still alive during this test by died just before starting water maze).

2.5 Behavioral Tests

One week after stroke surgery (or at the same time point for the No Stroke group), the rats were tested for motor (rotarod) and cognitive (Morris water maze) function [13]. **Rotarod:** The rats were placed on a horizontal rod suspended 30-cm above a padded surface, which started rotating at a constant increasing speed. The rats received 4 trials/ session separated by 10 min, and received 2 sessions per day. The rats were trained for at least 7 sessions and until reached a criterion of maximal performance (no increase in performance of more than 15% over their last 3 sessions). **Morris water maze:** The rats received a pre-training phase during which they were allowed to swim from one end of straight-alley to the partially submerged platform at the other end to learn the components of swimming and climbing onto a platform. A black tent covered the tank to prevent exposure to spatial cues in the room. The pre-training phase was conducted during 2 days (Monday and Friday) consisting of 3 trials conducted at 10-min intervals. During the subsequent sessions, the tent and alley were removed and the rats were allowed to swim freely from one of 4 starting position to find the hidden platform using spatial cues around the room. Path length and speed were assessed using a tracking software (Any-Maze, Stoelting, Inc.). Each session consisted of 3 trials separated by 10 min. After 4 sessions of acquisition, the rats were not exposed to the maze over the week end and received a retention session on Monday. Then, they received 2 reversal sessions conducted the same way as the acquisition phase, but the platform was moved to a different and new location. Prior to the last session of acquisition and after the last reversal session, the rats received probe trials during which the platform is removed. The rats are given 30 s to swim and performance was measured the time spent in a 40-cm annulus around the platform location.

The behavioral tests took 3 weeks to run after which the rats were anesthetized with isoflurane and decapitated for electrophysiological studies.

2.6 Slice preparation

Transverse hippocampal slices (~350 μ m) were prepared from the rats. After decapitation, the brain was quickly removed and placed into fresh ice-cold oxygenated (95% O₂/5% CO₂) artificial cerebrospinal fluid (ACSF) (composition in mM): 124 NaCl, 2 KCl, 1.2 MgSO₄, 26 NaHCO₃, 1.25 KH₂PO₄, 2.4 CaCl₂, 10 D-glucose, pH 7.4). The brain was first cut mid-sagittally at a 50–70° angle along the dorsal edge of each hemisphere and then mounted on the vibratome platform to be cut in ice-cold, oxygenated ACSF using a vibratome (VT1000S, Leica Microsystems). Slices were then incubated in oxygenated ACSF at 32°C for at least 1 hour before being transferred to the recording chamber.

2.7 Field excitatory postsynaptic potentials (fEPSPs) and long-term potentiation (LTP)

An individual slice was transferred to a submerged recording chamber, continuously superfused at 33–34 °C with oxygenated ACSF at a rate of 2 ml/min. A stimulating electrode (concentric bipolar microelectrode, FHC Inc, Bowdoin, ME) was positioned to stimulate the Schaffer collateral-commissural pathway fibers using a 150 μ s, 0.05 Hz protocol (Grass S44 stimulator and a PSIU6 isolation unit). fEPSPs were recorded extracellularly from the stratum radiatum of the CA1 region with a glass micropipette (4–8 M Ω , 2 M NaCl). Maximal fEPSP was determined initially by progressively increasing the

stimulus current. Once the maximal amplitude was determined, the current that elicits half-maximal amplitude of the response was utilized for the study. After evoked fEPSPs were stable, basal potentials were recorded at 0.05 Hz for 20 min. Theta burst stimulus (TBS) protocol was used to induce LTP (ten trains of 4 pulses at 100 Hz and 200 ms apart, and repeated three times with an interburst interval of 20 sec).

2.8 Data Analysis

The effects of diet and surgery on body weights, food intake, and behavioral performance were assessed using three-way analysis of variance (ANOVA) with weeks, days, or sessions as the repeated measure factor. Probe data were analyzed using two-way ANOVA with Diet and Surgery as between-group factors. Using the Kolmogorov-Smirnow test, datasets did not violate normality assumption. Planned individual comparisons between different diet groups and surgery groups were performed using a single degree-of-freedom F test involving the error term from the overall ANOVA. The alpha level was set at 0.05 for all analyses and Systat 13 statistical package was used to conduct the analyses. The fEPSP slopes (ratio of voltage and time (mV/ms) between time-point 10 and 90% of initial rising phase of EPSP) were calculated with Clampfit 10.2 (Molecular Devices, Sunnyvale, CA) and all the slope values were normalized against the average slope over the 10 min before the tetanus. Magnitude of LTP was expressed with average value of normalized fEPSP slopes from 50 to 60 min following the tetanus. Student's t-test (paired or unpaired) or one-way ANOVA with Student–Newman–Keuls multiple comparison test were used to determine statistical significance ($p < 0.05$).

3. Results

3.1 Influence of MICA diet and stroke on body weights

Body weights were recorded weekly throughout the study and are presented in Figure 1. All rats gained weight throughout the study, with the stroke rats having their growth delayed due to the stroke surgery. There was no effect of the MICA diet on body weights from either No Stroke or Stroke rats. These observations were supported by a significant effect of Weeks [$F(9,522)=720.2$, $p < 0.001$], Surgery [$F(1,58)=17.2$, $p < 0.001$] and a significant Weeks x Surgery interaction [$F(9,522)=11.2$, $p < 0.001$], and lack of main effect of Diet [$F(1,58)=0.04$, $p=0.847$] or Weeks x Diet interaction [$F(9,522)=0.54$, $p=0.85$].

3.2 Influence of MICA diet and stroke on coordinated running

Coordinated running performance across training sessions and at criterion (Last) are presented in Figure 2. All rats learned to balance on the rotating rod and their latency to fall increased across sessions until the rats reached a plateau of performance. While the stroke rats fell faster than the No Stroke rats in the earliest training sessions, they reached the same level of performance by the end of training and at criterion. There was no effect of the MICA diet on rotorod performance from either No Stroke or Stroke rats. These observations were supported by a significant effect of Session [$F(6,354)=23.7$, $p < 0.001$] and a significant Session x Surgery interaction [$F(6,354)=3.2$, $p=0.004$], and a lack of main effect of Diet [$F(1,59)=1.5$, $p=0.22$] or interaction with any factors (in-between subjects [$F(1,59)=0.07$, $p=0.8$; within-subjects all $F(6,354) < 0.5$, all $ps > 0.7$).

3.3 Influence of MICA diet and stroke on spatial learning and memory

Spatial learning and memory was tested across 3 phases: Acquisition (Session 1–4), Retention (Session 5), and Reversal (Session 6–7), and the data are presented in Figure 3. All rats regardless of diet and surgery learned to locate the platform efficiently during the acquisition, retained the information during retention, and learned a new platform location during reversal (Figure 3A), supported by significant main effect of Sessions during acquisition and reversal [all $F_s(3,174) > 43.5$, all $p_s < 0.001$]. The Stroke rats took longer path length to find the platform than the No Stroke ones during the acquisition and retention phases but performed similarly to the No Stroke rats during reversal. The MICA diet had no effect on the performance of the rats. ANOVAs revealed significant main effects of Surgery during acquisition and retention [all $F_s(1,58) > 5.0$, all $p_s < 0.04$] but not during reversal [$F(1,58) = 1.96$, $p = 0.167$]. During acquisition, the MICA-treated rats seem to perform worse than the control rats in the stroke group, while they seem to be doing slightly better in the No Stroke groups. However, this observation did not reach a significant interaction between Diet and Surgery [$F(1,58) = 3.9$, $p = 0.054$].

Speed of all rats decreased during acquisition and leveled out during retention and reversal (Figure 3B). Overall, the Stroke rats had higher speeds than the No Stroke rats, most notably during acquisition and reversal. These observations were supported by significant main effects of Surgery during both phases [all $F_s(1,58) > 6$, all $p_s < 0.02$] and of Sessions [all $F_s(3,174) > 10$, all $p_s < 0.01$]. Overall, there was no effect of diet in any of the phases, with the exception of small effect during acquisition's last session during which the MICA-treated rats had slower speeds than the Control-treated rats. This observation did not reach a significant interaction between Sessions and Diet [$F(3,174) = 2.6$, $p = 0.054$].

Spatial bias was assessed during 2 probe trials (Probe 1 done prior to Session 4 (end of acquisition), and probe 2 done after Session 7 (end of reversal) and is presented in Figure 4. During Probe 1, MICA-treated rats spent less time in the area of the platform than the controls when they were not stroked and more time when they were stroked. This differential effect was supported by a significant interaction between Surgery and Diet [$F(1,57) = 4.2$, $p = 0.046$]. During Probe 2, the No Stroke rats spent more time in the annulus 40 cm than the Stroke rats, regardless of which diet they were fed, which was supported by a significant main effect of Surgery [$F(1,58) = 13.7$, $p < 0.001$].

3.4 Influence of MICA diet on hippocampal CA1 synaptic plasticity

Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1 areas of two bisected hippocampal slices obtained from control or MICA diet rats. Long-term potential (LTP) was induced by theta burst stimulation (TBS) protocol. As shown in Figure 5, input/output curves and paired-pulse facilitation were comparable for the two groups, suggesting that basal excitatory synaptic function was not altered by MICA diet.

In addition, LTP recorded from MICA animals was not significantly different from those recorded from control diet animals (Figure 6 A–B). The average of LTP amplitude is $146 \pm 8.2\%$ ($n=9$) and $142 \pm 6.6\%$ ($n=10$) for control and MICA diet animals, respectively ($t=0.327$, $p=0.75$, unpaired t-test, Figure 6C).

3.5 Influence of MICA diet on hippocampal CA1 synaptic plasticity following ischemia stroke

The fEPSPs at CA1 were recorded from hippocampus prepared from tMCAO rats with control diet and with MICA diet, respectively. Similar to the observation on non-stroke animals, basal excitatory neurotransmission at CA3-CA1 synapse was not altered by MICA diet (Figure 7). As shown in Figure 8, MICA diet significantly attenuated post-stroke LTP impairment. For the animals receiving control diet, the LTP amplitude was reduced from $146 \pm 8.2\%$ ($n=14$) in sham-control rats to $124 \pm 4.5\%$ ($n=14$) following stroke ($t=57.5$, $p=0.015$, unpaired t-test).

4. Discussion

The major findings of the study are that (1) stroke impaired motor and cognitive function, and LTP, (2) MICA supplementation did not reverse stroke-associated behavioral impairments, and (3) MICA supplementation improved stroke-related impairments in hippocampal LTP.

Sensorimotor and cognitive impairments have been observed and studied extensively in rats after tMCAO. It is noteworthy that there is a partial or complete recovery of sensorimotor function developed over time [14, 15], while cognitive impairments seem to increasingly get worse with time [13, 16]. Deficits in spatial learning and memory, a hippocampal-dependent task [17], have been consistently observed in studies of stroke [13–15, 18], while other indices of cognitive function have wavered depending on experimental protocols and tasks used [18]. Our current data confirm motor and cognitive declines 7–14 days post-surgery. The behavioral deficits observed in the Morris water maze test might be in part due to impaired hippocampal LTP [19, 20]. Our data replicated a previous finding that tMCAO impairs hippocampal CA1 LTP [13]. GABAergic inhibitor neurons have been implicated in the induction of LTP [21]. Our previous studies have indicated that enhanced GABAergic neurotransmission after stroke might play a role in cognitive deficits and anxiolytic response [13, 22, 23]. The study also implicated a potential role of the ERK/MAPK signaling pathway in the hippocampal LTP induction and spatial learning and memory impairments [13], which has also been reported by other laboratories [24–26].

While any mitochondrial proteins can be evaluated as preconditioning targets for neuroprotection, two major ones have been well studied: succinate dehydrogenase and KATP channels [5, 27]. However, the caveats with the inhibitors of these proteins, 3-nitropropionic acid and diazoxide respectively, are that they can be highly toxic and target only one protein [5]. The multitude of events involved in the preconditioning effects for ischemic events such as oxidative stress increase, membrane depolarization affecting membrane potential make the DLDH inhibition via MICA intake a unique and attractive concept, as it affects multiple targets [10], though which specific pathway is more affected by MICA remains unknown. Furthermore, our previous and current studies have shown that MICA supplementation does not result in any toxicity [12]. Our previous studies have supported the role of MICA, as a reversible DLDH inhibitor in an *in vivo* setting and provided evidence that it reduced oxidative stress, cell death, and improved mitochondrial functioning. Furthermore, we have shown that this preconditioning effect of MICA resulted

in a significant reduction in the infarct size following tMCAO, regardless of the route of administration used for MICA. This preconditioning effect was also shown to be long lasting as it was present 4 weeks after a 7 day injection period of MICA. However, in the current study these preconditioning effects did not translate into improved functional outcomes. Motor and cognitive performance were impacted by the ischemic injury, however it was not prevented by MICA supplementation. This suggests that the neuroprotective effect of chemical preconditioning by MICA are not enough to lead to functional recovery. A caveat of this study, however, is that we did not do TTC staining to assess the impact of the surgery on these rats, which could in turn impact the functional outcome assessments. Furthermore, while MICA conferred protection in the cortical areas, the impact on the hippocampus remains undetermined. Perhaps a cortical-based test would have provided a more positive outcomes, and correlated with reduced infarct size. Also not in line with the behavioral outcomes, the LTP impairment was prevented by MICA supplementation, suggesting another beneficial effect of chemical preconditioning. One possible explanation is that water maze testing reflects the coordinated actions of multiple areas of the brain and neurotransmitter systems constituting a fully integrated neural network [28]. Therefore, it is likely that the effects of MICA are enough to protect LTP in the hippocampus but the dose or duration was not enough to provide more protection to other pathways and brain regions involved in learning and memory.

5. Conclusion

MICA has shown some promising preconditioning effects as a neuroprotectant of ischemic injury by reducing infarct size, improving oxidative stress status, and improving LTP. However, these molecular events have not translated to improved motor and cognitive performance. These data suggest that more studies are needed to determine the optimum concentration and length of treatment that could turn these molecular improvements into functional benefits.

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References

1. Kochanek KD, et al., Mortality in the United States, 2013. NCHS Data Brief, 2014(178): p. 1–8.
2. Mozaffarian D, et al., Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation*, 2015 131(4): p. e29–322. [PubMed: 25520374]
3. Gidday JM, Cerebral preconditioning and ischaemic tolerance. *Nat Rev Neurosci*, 2006 7(6): p. 437–48. [PubMed: 16715053]
4. Riepe MW, et al., Increased hypoxic tolerance by chemical inhibition of oxidative phosphorylation: “chemical preconditioning”. *J Cereb Blood Flow Metab*, 1997 17(3): p. 257–64. [PubMed: 9119898]
5. Wiegand F, et al., Respiratory chain inhibition induces tolerance to focal cerebral ischemia. *J Cereb Blood Flow Metab*, 1999 19(11): p. 1229–37. [PubMed: 10566969]
6. Goulton CS, et al., Pharmacological Preconditioning with GYKI 52466: A Prophylactic Approach to Neuroprotection. *Front Neurosci*, 2010 4.

7. Bell KF, et al., Activation of Nrf2-regulated glutathione pathway genes by ischemic preconditioning. *Oxid Med Cell Longev*, 2011 2011: p. 689524. [PubMed: 21904646]
8. Woodruff TM, et al., Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. *Mol Neurodegener*, 2011 6(1): p. 11. [PubMed: 21266064]
9. Sims NR and Anderson MF, Mitochondrial contributions to tissue damage in stroke. *Neurochem Int*, 2002 40(6): p. 511–26. [PubMed: 11850108]
10. Jin Z, Wu J, and Yan LJ, Chemical Conditioning as an Approach to Ischemic Stroke Tolerance: Mitochondria as the Target. *Int J Mol Sci*, 2016 17(3): p. 351. [PubMed: 27005615]
11. Bauman N and Hill CJ, Inhibition of gluconeogenesis and alpha-keto oxidation by 5-methoxyindole-2-carboxylic acid. *Biochemistry*, 1968 7(4): p. 1322–7. [PubMed: 4878041]
12. Wu J, et al., Administration of 5-methoxyindole-2-carboxylic acid that potentially targets mitochondrial dihydrolipoamide dehydrogenase confers cerebral preconditioning against ischemic stroke injury. *Free Radic Biol Med*, 2017 113: p. 244–254. [PubMed: 29017857]
13. Li W, et al., Transient focal cerebral ischemia induces long-term cognitive function deficit in an experimental ischemic stroke model. *Neurobiol Dis*, 2013 59: p. 18–25. [PubMed: 23845275]
14. DeVries AC, et al., Cognitive and behavioral assessment in experimental stroke research: will it prove useful? *Neurosci Biobehav Rev*, 2001 25(4): p. 325–42. [PubMed: 11445138]
15. Karhunen H, et al., Long-term functional consequences of transient occlusion of the middle cerebral artery in rats: a 1-year follow-up of the development of epileptogenesis and memory impairment in relation to sensorimotor deficits. *Epilepsy Res*, 2003 54(1): p. 1–10. [PubMed: 12742590]
16. Yang SH, et al., Endovascular middle cerebral artery occlusion in rats as a model for studying vascular dementia. *Age (Dordr)*, 2006 28(3): p. 297–307. [PubMed: 22253496]
17. Winocur G and Moscovitch M, Hippocampal and prefrontal cortex contributions to learning and memory: Analysis of lesion and aging effects on maze learning in rats. *Behavioral Neuroscience*, 1990 97: p. 13–27.
18. Bingham D, et al., Watermaze performance after middle cerebral artery occlusion in the rat: the role of sensorimotor versus memory impairments. *J Cereb Blood Flow Metab*, 2012 32(6): p. 989–99. [PubMed: 22373646]
19. Grant SG, et al., Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science*, 1992 258(5090): p. 1903–10. [PubMed: 1361685]
20. Tsien JZ, Huerta PT, and Tonegawa S, The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell*, 1996 87(7): p. 1327–38. [PubMed: 8980238]
21. Kullmann DM and Lamsa KP, LTP and LTD in cortical GABAergic interneurons: emerging rules and roles. *Neuropharmacology*, 2011 60(5): p. 712–9. [PubMed: 21185319]
22. Clarkson AN, et al., Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature*, 2010 468(7321): p. 305–9. [PubMed: 21048709]
23. Gonzalez LE, Ouagazzal AM, and File SE, Stimulation of benzodiazepine receptors in the dorsal hippocampus and median raphe reveals differential GABAergic control in two animal tests of anxiety. *Eur J Neurosci*, 1998 10(12): p. 3673–80. [PubMed: 9875346]
24. Irving EA and Bamford M, Role of mitogen- and stress-activated kinases in ischemic injury. *J Cereb Blood Flow Metab*, 2002 22(6): p. 631–47. [PubMed: 12045661]
25. Lynch MA, Long-term potentiation and memory. *Physiol Rev*, 2004 84(1): p. 87–136. [PubMed: 14715912]
26. Davis S, et al., The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. *J Neurosci*, 2000 20(12): p. 4563–72. [PubMed: 10844026]
27. Blanco M, et al., Ischemic preconditioning: a novel target for neuroprotective therapy. *Cerebrovasc Dis*, 2006 21 Suppl 2: p. 38–47.
28. D'Hooge R and De Deyn PP, Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev*, 2001 36(1): p. 60–90. [PubMed: 11516773]

Figure 1.

Effects of stroke and MICA supplementation on body weights of young rats. Data presented are means $\pm$ SEM of $n=12$ (No Stroke) or $n=18-20$ (Stroke) from rats surviving at the end of behavioral tests.

Figure 2.

Effect of stroke and MICA supplementation on rotorod performance in young rats.

Performance was measured as latency to fall from the rod across sessions and as the latency on the session at which they reached criterion (Last). Data is presented as mean $\pm$ SEM (n=12 for No Stroke, n= 18–21 for Stroke).

Figure 3.

Effect of stroke and MICA supplementation on spatial learning and memory in young rats.

Performance was determined by path length taken to reach the platform (A) and speed (B).

Data presented are means $\pm$ SEM of $n=12$ (No Stroke) or $n=18-20$ (Stroke).

Figure 4.

Effect of stroke and MICA supplementation on spatial bias in young rats. Performance was determined by the percent time spent in an annulus 40cm around the platform location during probe trials. Data presented are means $\pm$ SEM of $n=11-12$ (No Stroke) or $n=18-20$ (Stroke). # $p < 0.05$ vs. Diet-matched No Stroke group.

Figure 5.

Input and output relationship (A) and paired-pulse facilitation (B) in control and MICA-treated animals. **A**, Input (stimulus)/output (fEPSP amplitude) curves, where fEPSPs were evoked by stimulating Schaffer collateral-CA1 pathway at a current intensity ranging from 30 to 100 μ A. Note that there was no significant difference between control and MICA animals. **B**, EPSPs were induced by a paired-pulse protocol with varying interpulse intervals from 25 to 250 ms. The ratios of the EPSPs amplitude evoked by second stimulus to that by the first stimulus (EPSP2/EPSP1) are plotted against interpulse intervals. Note that there was no significant difference in PPF between the two groups.

Figure 6.

Hippocampal LTP in control and MICA-treated animals. **A**, Representative traces of fEPSPs recorded before (a) and after (b) theta-burst stimulation (TBS) in hippocampal slices from control or MICA rats. **B**, Time course of the fEPSP slopes in control and MICA animals. fEPSPs slope are normalized to 10 min of baseline before theta-burst stimulation (TBS). **C**, The LTP magnitude is expressed as the mean values of the potentiation of fEPSPs averaged from 50 to 60 min following TBS. Note that no significant difference in LTP is found between control and MICA animals.

Figure 7.

Effects of MICA diet on input/output (A) and paired-pulse facilitation following ischemia stroke. **A**, Input (stimulus)/output (fEPSP amplitude) curves, where fEPSPs were evoked by stimulating Schaffer collateral-CA1 pathway at a current intensity ranging from 30 to 120 μ A. Note that there was no significant difference between stroke and stroke + MICA animals. **B**, EPSPs were induced by a paired-pulse protocol with varying interpulse intervals from 25 to 250 ms. The ratios of the EPSPs amplitude evoked by second stimulus to that by the first stimulus (EPSP2/EPSP1) are plotted against interpulse intervals. Note that there was no significant difference in PPF between the two groups.

Figure 8.

Effect of MICA diet on hippocampal CA1 LTP following ischemia stroke. **A**, Representative traces of fEPSPs recorded before (a) and after (b) TBS in hippocampal slices from animals receiving control diet or MICA diet. **B**, Time course of the fEPSP slopes control (n=14) and MICA diet rats (n=17). **C**, LTP amplitude was significantly impaired in stroke animals and protected against stroke by MICA diet. The LTP magnitude is expressed as the mean values of the potentiation of fEPSPs averaged from 50 to 60 min following TBS. $p<0.05$, compared to control or stroke animals, unpaired t-test.