RESEARCH PAPER

Acta Neurobiol Exp 2022, 82: 295–303
DOI: 10.55782/ane-2022-028

Effect of intranasal administration of caffeine on mPFC ischemia-induced cognitive impairment in BALB/c mice

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Caffeine is a psychoactive compound used widely to enhance cognitive functions in human or animal studies. The present study examined the effects of caffeine on cognitive performance and inflammatory factors in mice with medial prefrontal cortex (mPFC) ischemia. Mice underwent a photothrombotic mPFC ischemic stroke and were treated with normal saline or caffeine at different doses intranasally for 7 days. The sham surgery animals received normal saline intranasally. The Morris water maze test and social interaction test were performed to assess spatial and social memories, respectively. In addition, the levels of inflammatory proteins, including tumor necrosis factor-alpha, interleukin-6, and interleukin-10, were measured in the mPFC using immunoblotting. The results showed that mPFC ischemia impaired spatial memory and social behaviors, and caffeine at doses of 0.05 and 0.1 mg improved behavioral outcomes in the ischemic groups. Also, caffeine reversed ischemia-induced high levels of pro-inflammatory biomarkers and enhanced the expression of the anti-inflammatory mediator. Our findings indicate that caffeine alleviated mPFC ischemia-induced memory disturbances, probably through the modulation of the inflammatory mediators.

Key words: caffeine, ischemia, medial prefrontal cortex, memory, neuroinflammation

INTRODUCTION

Cerebral ischemia is caused by an insufficient supply of oxygen and nutrients to the brain due to interrupted blood flow, resulting in significant cognitive, motor, and sensory dysfunctions. Impaired memory is among the cognitive problems of ischemia, which is associated with dramatic impacts on patients' quality of life (Liu et al., 2017; Martins et al., 2017).

High-level cognitive abilities, including memory, emotion, executive function, motivation, and sociability, are mediated in part through the medial prefrontal cortex (mPFC) or its extensive connection with different brain regions; hence mPFC dysfunction disrupts normal cognition (Sadigh-Eteghad et al., 2018; Xu et al., 2019; Ji et al., 2020).

The rodent photothrombotic stroke model can be reproducibly used to study cellular and behavioral responses to neuroprotective regimens (Ahmed et al., 2016). Ischemia activates multiple pathological cascade, including excitotoxicity, neuroinflammatory responses, oxidative stress, and apoptosis (Liu et al., 2009). Evidence shows that ischemic stroke is associated with an increase in inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) and a decrease in anti-inflammatory products, which exacerbate brain injury. Anti-inflammatory mediators such as interleukin-10 (IL-10) neutralize the deleterious effects of pro-inflammatory cytokines by a feedback loop and exert a neuroprotective effect on brain cells (Kulesh et al., 2018; Li et al., 2018). Also, the systemic and local inflammatory responses are essen-

Received 18 August 2021, accepted 27 May 2022

tial in post-stroke cognitive impairments (Rothenburg et al., 2010; Kliper et al., 2013).

Caffeine, a central nervous system (CNS) stimulant, is present naturally in dietary sources such as coffee (Madeira et al., 2017; Sun et al., 2013; Waer et al., 2021). This bioactive compound is widely used worldwide and affects users' cognitive performance (Waer et al., 2021). Caffeine has anti-inflammatory, anti-apoptotic, and anti-oxidant properties that make it suitable for managing the pathologies associated with neurovascular and neurodegenerative diseases (Lee et al., 2017; Hosny et al., 2019). Though caffeine is a potential therapeutic agent for brain diseases, its systemic delivery may lead to gastrointestinal and hepatotoxic damage. Intranasal drug delivery can be a rapid, non-invasive, and efficient method for nervous system diseases approved in various experimental and clinical studies (Kolahdouzan et al., 2017; Inoue et al., 2020).

This study aimed to investigate the effects of different doses of intranasal caffeine on cognitive function and inflammatory markers in a mice model of photothrombotic mPFC ischemia.

METHODS

Animals

Adult male Balb/c mice (25-30 g) were purchased from the HTT Co, Tabriz, Iran. Animals were housed (5 mice per cage) under ambulatory conditions (22 ± 2°C and 12/12 h light/dark cycle) with free access to water and food. All animal procedures were performed according to the National Institute of Health (NIH; Publication No. 85-23, revised 1985) and approved by the regional Ethics Committee of Tabriz University of Medical Sciences (No: IR.TBZMED.VCR.REC.1398.468).

Experimental groups

Mice were randomly divided into five groups (n=10) of sham, NS, coff 0.025, coff 0.05, and coff 0.1. Animals in the sham group underwent sham surgery and received normal saline (as a caffeine vehicle). The remaining groups were submitted to mPFC ischemia and received normal saline (NS control) or caffeine at 0.25, 0.05, and 0.1 mg doses. All solutions were prepared before administration and given via intranasal route a day following surgery at a constant volume of 10 µl for 7 consecutive days.

For intranasal administration, awake animals were placed supine, and drops were administered by pipette (5-6 µl) with alternation between the right and left nares every two min up to 10 µl. Fig. 1 shows the study design, including the experimental paradigm with the timeline.

Cerebral photothrombotic ischemia model

The photothrombotic method induced ischemic stroke in the mPFC region (Sadigh-Eteghad et al., 2018). For this purpose, mice were anesthetized by isoflurane in oxygen (3% for induction and 1.5% for maintenance) and mounted in a stereotaxic frame. After swabbing with 10% betadine, a midline skin incision was made on the skull, the periosteum was removed, and the skull was exposed. After that, a laser probe was fixed nearly 2 cm above the skin surface. Two minutes following intraperitoneal Rose Bengal (Sigma-Aldrich, St. Louis, Missouri) injection (150 µg/g), a 532 nm green laser beam was applied over the mPFC region (approximately 2.2 mm anterior to the bregma) for 10 min (Kucheryavykh et al., 2018). Then the surgical site was sutured by 6-0 silk suture. Mice in the sham group underwent the same surgical procedure and received the same amount of Rose Bengal but did not receive laser irradiation, and the incision was then closed.

Morris water maze (MWM)

A circular black water maze (75 cm in diameter, 35 cm in height, filled with 21 ± 1°C water) was conceptually divided into four equal quadrants. An escape platform (10 cm in diameter) was fixed inside the maze

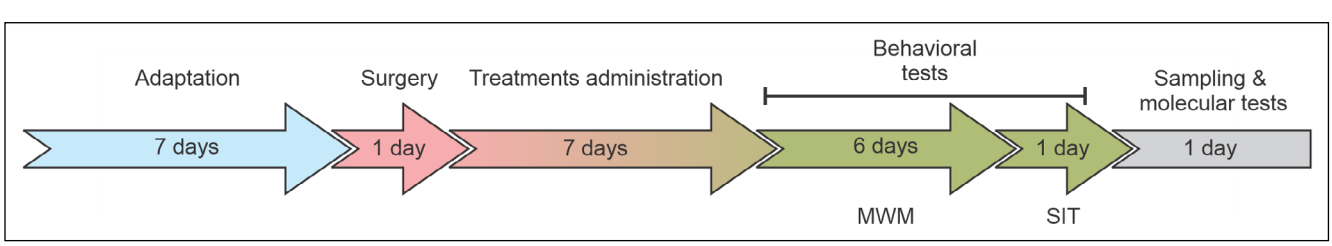

Fig. 1. The study timeline includes ischemia induction, treatment administration, behavioral tasks, sampling, and molecular tests.

in one of the four quadrants. The maze was located in a room with visual clues to allow orientation.

Each mouse completed three phases of testing (visible platform, hidden platform, and probe trial). The visible platform phase includes 4 trials in which the eyesight and swimming ability of the animals are screened. In this phase, the surface of the escape platform was raised 1 cm above the water surface and marked with a flag, and then each mouse was released in one of the quadrants facing the wall and allowed to find the platform over 60 s. If the mouse could not find the platform within 60 s, it was manually guided toward the platform and placed on it for 30 s.

After the visible platform, the hidden platform task was performed in four training trials per day for 5 consecutive days to evaluate spatial acquisition. In this phase, the escape platform was submerged 1.5 cm below the water surface in one of the quadrants, and its location remained unchanged during test days. Each trial was started by randomly releasing the mouse in one of the quadrants and allowing it to swim and find the hidden platform. The time spent to find the hidden platform was recorded as the escape latency. The probe test was performed 24 h following the last trial to assess spatial memory retention. The escape platform was removed in this test, and each mouse was released into the pool. The start point was the quadrant opposite the original position of the platform in the previous phase. The time spent in the target quadrant was recorded over the 60 s. The pool's water was changed with fresh water daily to prevent water pollution. A digital camera recorded all essions, and EthoVision™ (Noldus, The Netherlands) software was used to track the animals.

Social interaction test

A rectangular Plexiglas box (60 × 45 × 50 cm), separated into three equal chambers by transparent dividing walls with sliding doors (6 × 6 cm), was used. Two empty wire containment cups (11 × 10 cm) were placed on the side chambers. The task was done in three consecutive phases: adaptation, sociability, and social memory. For the adaptation phase, wire containment cups were both empty, and the mouse was placed in the center of the middle chamber with opened sliding doors. The mouse was allowed to explore all chambers for 10 min. Then, the mouse was guided toward the middle chamber, and the doors were closed. During this phase, the total distance traveled was considered the open field test activity.

During the second phase, an unfamiliar mouse (stranger 1) was located inside the wire containment cup of one of the side chambers. Then side doors were opened, and the test mouse was allowed to explore all chambers for 10 min. In this phase, the sociability index was calculated by dividing the time spent by the test mouse in direct contact with stranger 1 by the total time spent exploring both containment cups.

The last phase of the test was initiated by placing another unfamiliar mouse (stranger 2) inside the containment cup in the opposite side chamber that had been empty during the previous phase. In this phase, time spent by the test mouse for direct contact with both cages was recorded for over 10 min. The social memory index was calculated by dividing the time spent for direct contact with cup housing stranger 1 by the total time spent for direct contact with cups housing strangers 1 and 2. Sociability was defined as an inclination to spend time with the stranger 1 mouse compared to the time spent in the empty chamber. Social memory was defined as a propensity to spend more time with a new mouse than a familiar mouse (Moy et al., 2004).

Any olfactory cues and residues were cleaned with 70% ethanol after each phase to eliminate the odors. A digital camera recorded all sessions, and the EthoVision™ (Noldus, The Netherlands) software was used to obtain tracking data.

Western blot

One day after the behavioral tests, mice were anesthetized by intraperitoneal injection of ketamine and xylazine (90 and 10 mg/kg, respectively), and the whole brain was carefully removed. The mPFC was separated on a cold plate and homogenized in RIPA lysis buffer containing phosphatase and protease inhibitors. A BCA protein assay kit (BioRad Laboratories, CA, USA) was applied to measure the protein concentration. Equal amounts of protein from each group were separated by 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, USA). After blocking with 5% non-fat milk in Tris-buffered saline-Tween 20, the membranes were incubated with primary antibodies at 4°C overnight. The primary antibodies (1:500 dilution; Santa Cruz, CA) were used as follows: anti-IL-10 (sc-8438), TNF-α (sc-52746), and IL-6 (sc-28343); following this, the membranes were rinsed with TBST (5 min × 3) and incubated with the horseradish peroxidase-conjugated secondary antibody conjugated to HRP for 60 min at room temperature. The bound antibodies were visualized using an enhanced ECL system (Bio-Rad, USA) and analyzed using ImageJ software (National Institutes of Health, USA). We used β-actin (1:1000 dilution, Abcam)

as the internal loading control to normalize the data. The membrane was stripped by commercial stripping buffer in each step and then reprobed with the indicated marker antibody.

Statistical analysis

Data were reported as a mean ± SEM. Statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test using the GraphPad statistic software package. Group comparisons of the escape latency during the training phase of the MWM were performed by two-way ANOVA. Differences between the means were considered statistically significant if $p<0.05$.

RESULTS

Caffeine ameliorates mPFC ischemia-induced spatial learning and memory impairments

Escape latency platforming the MWM was markedly longer in the NS-treated ischemic group on day 3 ($p<0.05$), 4 ($p<0.001$), and 5 ($p<0.001$) of the training as compared to the NS-treated sham mice. By contrast, the escape latency reduced after administration of caffeine (0.05 mg on the day 5th, $p<0.05$; 0.1 mg on days 4 and 5, $p<0.05$) when compared to the NS-received ischemic group (Fig. 2A).

As Fig. 2B shows, in the probe test, the NS-treated ischemic mice spent less time in the target quadrant than the animals in the sham group ($p<0.001$). However, caffeine at 0.05 and 0.1 mg increased the time spent in the target quadrant compared to the NS-received ischemic group ($p<0.01$ for both doses).

Caffeine attenuates mPFC ischemia-induced social memory deficit

We first examined the locomotor activity of mice using the social interaction test during the habituation phase and found no significant differences among the experimental groups ($p>0.05$, Fig. 3A). Also, there was no significant difference in the sociability index among the experimental groups ($p>0.05$, Fig. 3B). Besides, animals in the NS-treated ischemic group showed a poorer social memory index than the NS-treated sham mice ($p<0.001$). Nevertheless, caffeine at the doses of 0.05 and 0.1 mg significantly ($p<0.05$ and $p<0.01$, respectively) improved the social memory index compared to the NS-treated ischemic group (Fig. 3C).

Caffeine modulates IL-10, IL-6, and TNF-α levels in the mPFC of the ischemic animals

The immunoblotting results revealed that protein expression of IL-10 was significantly decreased in the

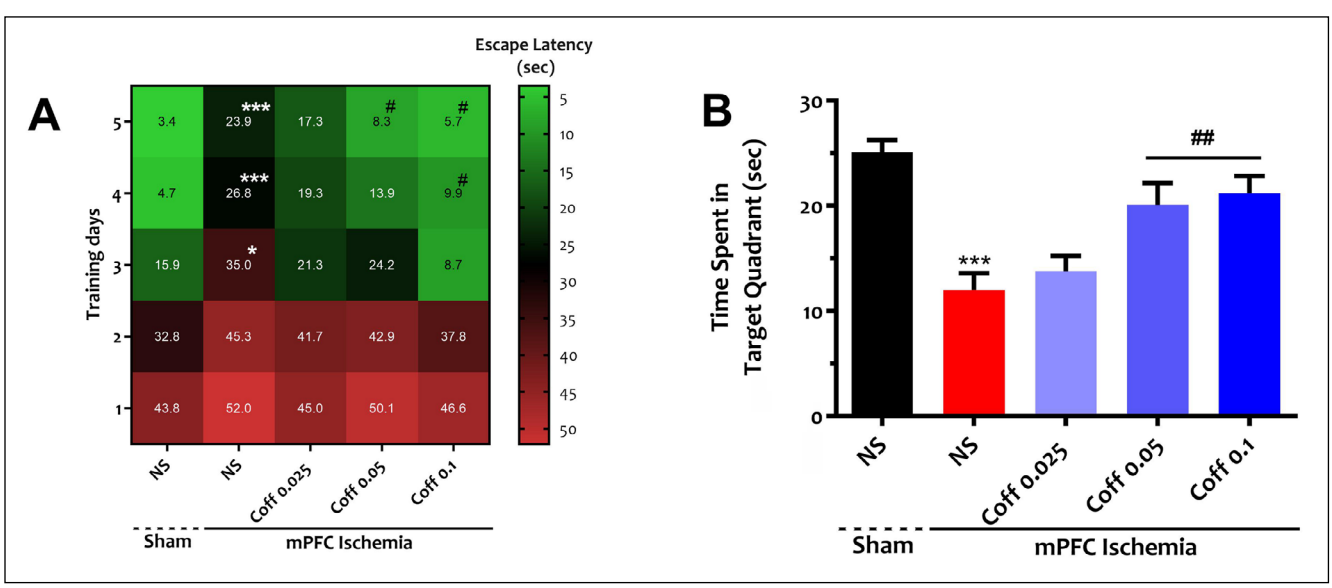

Fig. 2. Effect of different doses of intranasal caffeine (0.025, 0.05, and 0.1 mg) on MWM test outcomes. (A) The escape latency during the training trial and; (B) time spent in the target quadrant in the probe test. Data are shown as mean ± SEM (n=10). *$p<0.05$ and ***$p<0.001$ comparing the NS-treated and sham groups; $^{\#}p<0.05$ and $^{\#\#}p<0.01$ comparing the NS-treated and ischemic groups; (NS, normal saline; coff, caffeine; mPFC, medial prefrontal cortex; MWM, Morris water maze).

mPFC of the NS-treated ischemic mice compared to the NS-treated sham group (p<0.001, Fig. 4A). However, the expression levels of TNF-α (p<0.001, Fig. 4B) and IL-6 (p<0.01, Fig. 4C) were significantly increased in the ischemic group compared to the sham animals. Interestingly, intranasal caffeine at the doses of 0.05 mg (p<0.05) and 0.1 mg (p<0.05) significantly increased IL-10 levels, and at the dose of 0.1 mg (p<0.01), reduced the expression levels of TNF-α in the mPFC compared to the NS-treated ischemic group. Protein levels of IL-6 in the treatment groups showed no significant changes compared to the NS-treated ischemic group (p>0.05; Fig. 4A-D).

DISCUSSION

These findings demonstrate that intranasal delivery of caffeine at the doses of 0.05 and 0.1 mg for 7 consecutive days reduced inflammatory responses in the mPFC and improved spatial learning and memory impairments and social memory deficits in the animals with mPFC ischemia.

The mPFC, a crucial component of the forebrain with reciprocal connections with other cortical and subcortical regions, is essential for higher cognitive performances (Ji et al., 2020). Preclinical research demonstrated that ischemia in the mPFC region leads

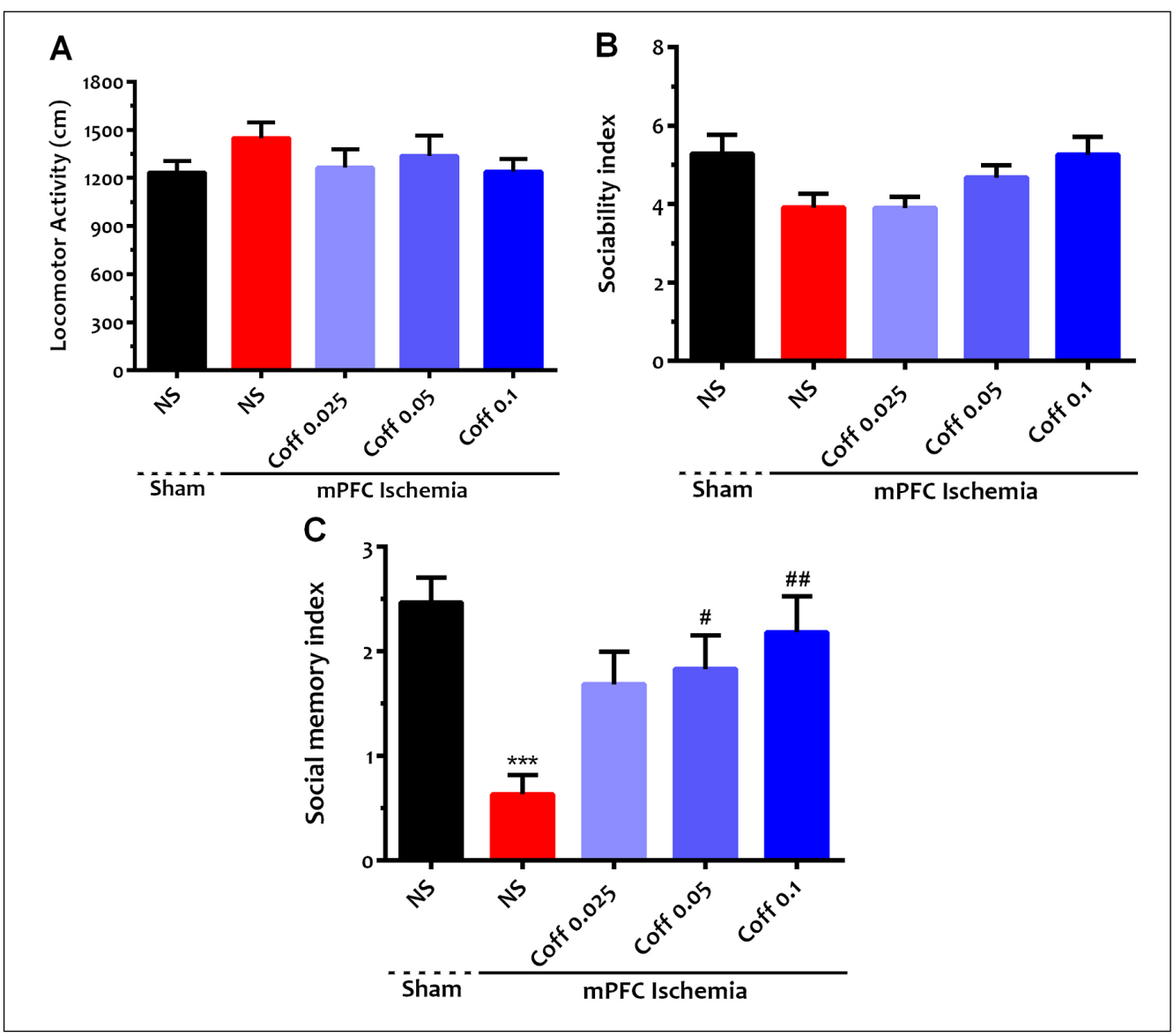

Fig. 3. Effect of different doses of intranasal caffeine (0.025, 0.05, and 0.1 mg) on the performance of study groups in the social interaction test. (A) Locomotor activity, (B) sociability index and (C) social memory index. Data are shown as mean ± SEM (n=10). ***p<0.001 comparing the NS-treated and sham groups; $^{\#}p$<0.05 and $^{\#\#}p$<0.01 comparing the NS-treated and ischemic groups; (NS, normal saline; coff, caffeine; mPFC, medial prefrontal cortex).

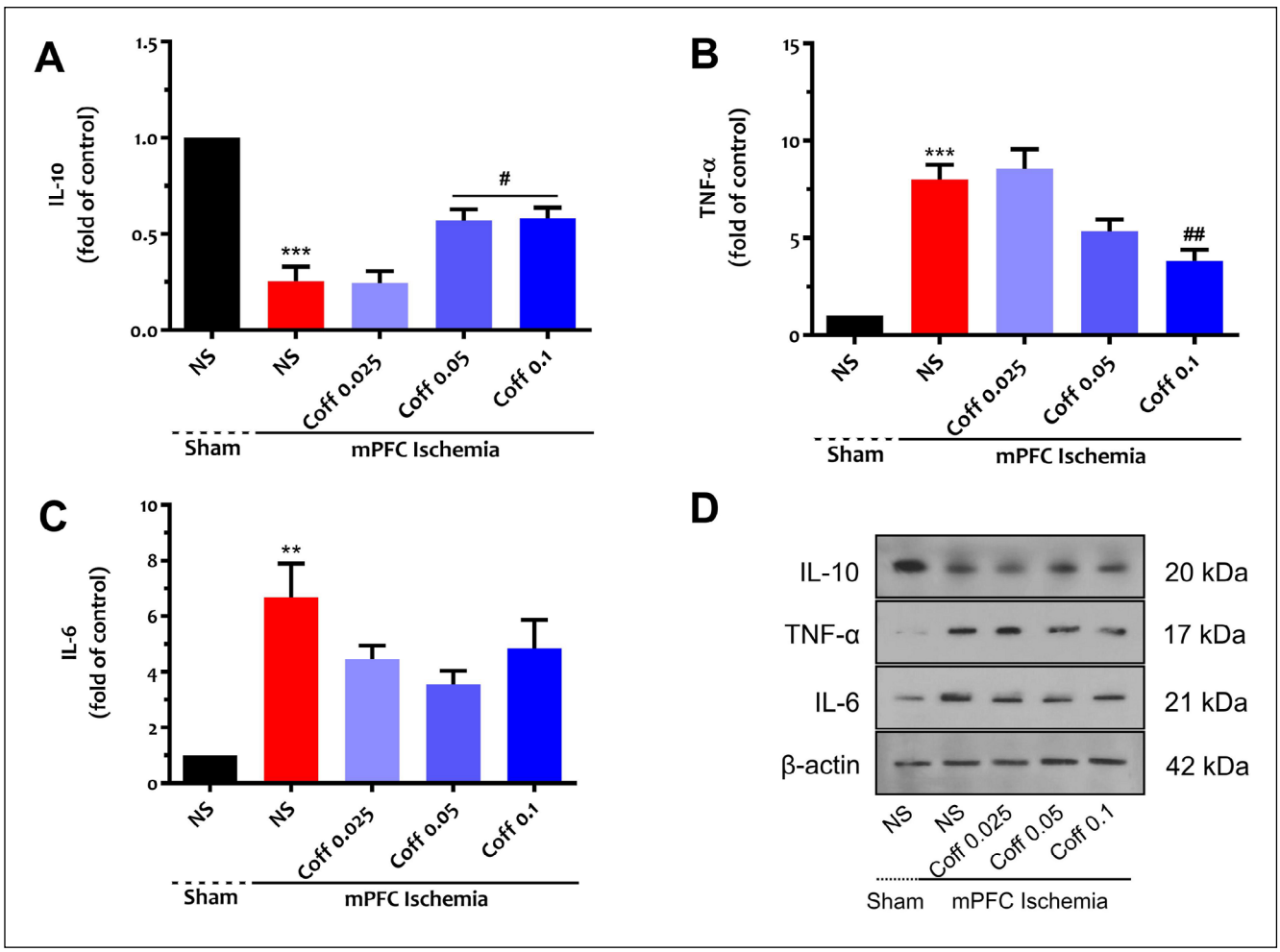

Fig. 4. Effect of different doses of intranasal caffeine (0.025, 0.05, and 0.1 mg) on the protein expressions of (A) IL-10, (B) TNF-α, and (C) IL-6 levels in the mPFC of the study groups. (D) Representative images obtained by Western blotting. Data are shown as mean ± SEM (n=3). $^{**}p<0.01$ and $^{***}p<0.001$ comparing the NS-treated and sham groups; $^{\#}p<0.05$ and $^{\#\#}p<0.01$ comparing the NS-treated and ischemic groups; (NS, normal saline; coff, caffeine; mPFC, medial prefrontal cortex; IL-10, Interleukin-10; TNF-α, tumor necrosis factor-alpha; IL-6, Interleukin-6).

to dysfunction in various cognitive processes (Déziel et al., 2015; Zhou et al., 2016; Park et al., 2019).

In this study, the mPFC ischemic stroke caused spatial learning and memory impairments, as indicated by longer escape latency in the MWM. Additionally, the ischemic group exhibited less time spent in the target quadrant in the probe test, suggesting that mice with mPFC ischemia could not remember the original platform position. However, intranasal caffeine shortened the escape latency in the last two days of the training trials. Furthermore, caffeine increased the time spent in the target quadrant in the probe test, suggesting an improvement in spatial learning and memory. The results of the social interaction test also showed that mPFC ischemia leads to social memory deficits that was improved by caffeine administration.

Previous studies demonstrated the effectiveness of caffeinol, a mixture of low doses of ethanol plus caffeine, in reducing cortical infarct volume and improving motor coordination and memory function following ischemic stroke and traumatic brain injury (Belayev et al., 2004; Dash et al., 2004; Piriyawat et al., 2003). Additionally, Strong et al. (2000) reported that acute administration of a combination of caffeine (10 mg/kg) and ethanol 2-3 h after the onset of the unilateral common carotid artery (CCA) and middle cerebral artery (MCA) occlusion markedly decreased infarct size up to 80%. In contrast, caffeine or ethanol alone did not affect infarct volume in rats. Aronowski et al. (2003) also showed that the doses of 6 mg/kg and 10 mg/kg of caffeine in the caffeinol mixture reduced cortical infarct volume and sensorimotor dysfunction induced by transient CCA/MCA occlusion in rats. Moreover, Belayev et al. (2004) found that caffeinol therapy decreased infarct volumes and brain swelling produced by MCA occlusion in rats. In

support of the results of Strong et al. (2000), Sun et al. (2013) also reported that a single treatment with caffeine (10 mg/kg) had no significant effect on the brain infarct volume and brain edema induced by MCA occlusion in rats. Thus, it seems that caffeine attenuated memory impairment provoked by mPFC ischemia through mechanisms other than reducing the infarct size, such as, e.g., inhibition of the inflammatory responses.

Neuroinflammation is one of the mechanisms underlying cognitive impairment in neuronal disorders (Chin et al., 2013). Numerous studies highlighted the role of inflammatory and anti-inflammatory cytokines such as TNF-α, IL-6, and IL-10 in the modulation of ischemia-induced cerebral inflammation (Ma and Yin, 2016; Kulesh et al., 2018; He et al., 2020). TNF-α is released by the activated microglia and macrophages and acts as an inflammatory molecule in cerebrovascular events. It triggers the migration of neutrophils into the brain parenchyma, resulting in exacerbation of brain injury while blockade of TNF-α activity diminishes infarct size following focal ischemia (Barone et al., 1997). IL-6 is another prominent mediator of the inflammatory process involved in the pathogenesis of ischemia. In brain damage, IL-6 is released by different cells, including microglial cells, astrocytes, leukocytes, and endothelial cells. (He et al., 2020; Pawluk et al., 2020). Clinical studies indicated an increase in serum IL-6 levels within a few hours after stroke, which is associated with patients' functional state (Waje-Andreassen et al., 2005). Conversely, IL-10 has an anti-inflammatory property and counteracts neuroinflammatory responses by inhibiting the production of inflammatory cytokines (Ewen et al., 2013).

In the present study, photothrombotic mPFC ischemic stroke increased the expressions of TNF-α and IL-6 while attenuated IL-10 levels in the mPFC. We also found that intranasal caffeine for 7 days down-regulated TNF-α levels at the dose of 0.1 mg and up-regulated IL-10 levels at the doses of 0.05 mg and 0.1 mg, suggesting its protective effect against ischemia-induced neuroinflammation in a dose-dependent manner.

Previous animal studies have shown the cognitive enhancing effect of caffeine in AD, aging, and ischemia models in different behavioral tasks, including the novel object recognition, social preference memory, Y-maze, and MWM (Alexander et al., 2013; Costa et al., 2008; Leite et al., 2011; Prediger et al., 2005). Also, it has been indicated that caffeine could prevent the production of pro-inflammatory markers and attenuates inflammation-related cognitive impairments (Gonçalves et al., 2020; Wadhwa et al., 2018).

According to previous studies, caffeine has a dose-dependent action on cognitive function (Hwang et al., 2016; Mahdi et al., 2019). However, in this study, the cognitive enhancer effects of caffeine were observed at the doses of 0.05 and 0.1 mg, while 0.025 mg did not affect cognitive function.

The ability of caffeine to antagonize adenosine receptors plays a central role in its impact on behavior and cognitive performance (McLellan et al., 2016; Reyhani-Rad and Mahmoudi, 2016). Besides, animal studies have demonstrated that the neuroprotective effects of caffeine against cognitive impairment are mainly mediated through the blockade of A2A receptors rather than A1 receptors (Abreu et al., 2011; Canas et al., 2009). Furthermore, evidence also shows that activation of A2A receptors augments inflammatory responses by microglial activation resulting in cognitive impairment in animal models of ischemia (Ran et al., 2020). Therefore, caffeine's blockade of A2A receptors may explain its protective effects against neuroinflammation and cognitive impairment (Muhammad et al., 2018).

Caffeine at low to moderate doses (0.001 to 0.5 nM) blocks A1 and A2A adenosine receptorsbut has no considerable effect on cAMP breakdown (Fredholm 1979; 1995). Besides, blockade of G protein-associated A2A receptors decreases the production of cyclic adenosine monophosphate (cAMP) (Pleli et al., 2018). However, at high doses (plasma concentration >250 mM), caffeine is an inhibitor of phosphodiesterase, leading to an increase in the intracellular concentration of cAMP (Institute of Medicine, 2001). Recently, Xie et al. (2021) reported that oral administration of a low dose of caffeine (36 mg/kg/day) for 12 days improved spatial learning by increasing intracellular cAMP and inducing phosphorylation of protein kinase A (PKA) and Ca^{2+}/cAMP response element-binding protein (CREB) in the hippocampus, while administration of a high dose (144 mg/kg/day) of caffeine produced an opposite effect on this pathway. It has been confirmed that phosphorylated CREB up-regulates the expression of neurotrophic factors such as BDNF, promoting neuronal growth and survival, synaptic plasticity, and learning and memory (Lonze et al., 2002). Another study also demonstrated that caffeine stimulated CREB activity and increased the expression of CREB-dependent genes, including c-Fos and BDNF, in the cortical neurons (Connolly and Kingsbury, 2010).

The present study suggests that caffeine at 0.025 mg had no effect on memory function in the MWM, possibly owing to the blocking of adenosine receptors, resulting in decreased intracellular cAMP. However, caffeine at higher doses (0.05 and 0.1 mg) improved memory performance in the MWM, possibly by inhibiting phosphodiesterase and enhancing intracellular cAMP levels. Therefore, these pathways are highly

relevant to future studies in understanding the effect of caffeine on cognitive improvements in the cerebral ischemia models.

In conclusion, we showed the protective effects of intranasal caffeine against mPFC ischemia-induced cognitive impairments in a mice model, at least in part, through reduction of TNF-α and augmentation of IL-10 levels in the mPFC.

ACKNOWLEDGMENTS

The current research was supported by a grant (No: 65042) from the Tabriz University of Medical Sciences, Tabriz, Iran.

REFERENCES

Abreu RV, Silva-Oliveira EM, Moraes MF, Pereira GS, Moraes-Santos T (2011) Chronic coffee and caffeine ingestion effects on the cognitive function and antioxidant system of rat brains. Pharmacol Biochem Behav 99: 659–664.

Ahmed ME, Tucker D, Dong Y, Lu Y, Zhao N, Wang R, et al. (2016) Methylene Blue promotes cortical neurogenesis and ameliorates behavioral deficit after photothrombotic stroke in rats. Neuroscience 336: 39–48.

Alexander M, Smith AL, Rosenkrantz TS, Fitch RH (2013) Therapeutic effect of caffeine treatment immediately following neonatal hypoxic-ischemic injury on spatial memory in male rats. Brain Sci 3: 177–190.

Aronowski J, Strong R, Shirzadi A, Grotta JC (2003) Ethanol plus caffeine (caffeinol) for treatment of ischemic stroke: preclinical experience. Stroke 34: 1246–1251.

Barone F, Arvin B, White R, Miller A, Webb C, Willette R, et al. (1997) Tumor necrosis factor-α: a mediator of focal ischemic brain injury. Stroke 28: 1233–1244.

Belayev L, Khoutorova L, Zhang Y, Belayev A, Zhao W, Busto R, et al. (2004) Caffeinol confers cortical but not subcortical neuroprotection after transient focal cerebral ischemia in rats. Brain Res 1008: 278–283.

Canas PM, Porciúncula LO, Cunha GM, Silva CG, Machado NJ, Oliveira JM, et al. (2009) Adenosine A2A receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. J Neurosci 29: 14741–14751.

Chin Y, Kishi M, Sekino M, Nakajo F, Abe Y, Terazono Y, et al. (2013) Involvement of glial $P2Y_1$ receptors in cognitive deficit after focal cerebral stroke in a rodent model. J Neuroinflammation 10: 95.

Connolly S, Kingsbury TJ (2010) Caffeine modulates CREB-dependent gene expression in developing cortical neurons. Biochem Biophys Res Commun 397: 152–156.

Costa MS, Botton PH, Mioranzza S, Souza DO, Porciúncula LO (2008) Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tyrosine kinase receptor (TrkB) content in mice. Neuroscience 153: 1071–1078.

Dash PK, Moore AN, Moody MR, Treadwell R, Felix JL, Clifton GL (2004) Post-trauma administration of caffeine plus ethanol reduces contusion volume and improves working memory in rats. J Neurotrauma 21: 1573–1583.

Déziel RA, Ryan CL, Tasker RA (2015) Ischemic lesions localized to the medial prefrontal cortex produce selective deficits in measures of executive function in rats. Behav Brain Res 293: 54–61.

Ewen T, Qiuting L, Chaogang T, Tao T, Jun W, Liming T, et al. (2013) Neuroprotective effect of atorvastatin involves suppression of TNF-α and upregulation of IL-10 in a rat model of intracerebral hemorrhage. Cell Biochem Biophys 66: 337–346.

Fredholm BB (1979) Are methylxanthine effects due to antagonism of endogenous adenosine? Trends in Pharmacological Sciences 1: 129–132.

Fredholm BB (1995) Adenosine, adenosine receptors and the actions of caffeine. Pharmacol Toxicol 76: 93–101.

Gonçalves DF, Tassi CC, Amaral GP, Stefanello ST, Dalla Corte CL, Soares FA, et al. (2020) Effects of caffeine on brain antioxidant status and mitochondrial respiration in acetaminophen-intoxicated mice. Toxicol Res 9: 726–734.

He J, Zhou D, Yan B (2020) Eriocitrin alleviates oxidative stress and inflammatory response in cerebral ischemia reperfusion rats by regulating phosphorylation levels of Nrf2/NQO-1/HO-1/NF-κB p65 proteins. Ann Transl Med 8: 757.

Hosny EN, Sawie HG, Elhadidy ME, Khadrawy YA (2019) Evaluation of antioxidant and anti-inflammatory efficacy of caffeine in rat model of neurotoxicity. Nutr Neurosci 22: 789–796.

Hwang JH, Kim KJ, Ryu SJ, Lee BY (2016) Caffeine prevents LPS-induced inflammatory responses in RAW264.7 cells and zebrafish. Chem Biol Interact 248: 1–7.

Inoue D, Furubayashi T, Tanaka A, Sakane T, Sugano K (2020) Effect of cerebrospinal fluid circulation on nose-to-brain direct delivery and distribution of caffeine in rats. Mol Pharm 17: 4067–4076.

Institute of Medicine (US) Committee on Military Nutrition Research (2001) Caffeine for the Sustainment of Mental Task Performance: Formulations for Military Operations. Washington (DC), National Academies Press: 1–172.

Ji MH, Lei L, Gao DP, Tong JH, Wang Y, Yang JJ (2020) Neural network disturbance in the medial prefrontal cortex might contribute to cognitive impairments induced by neuroinflammation. Brain Behav Immun 89: 133–144.

Kliper E, Bashat DB, Bornstein NM, Shenhar-Tsarfaty S, Hallevi H, Auriel E, et al. (2013) Cognitive decline after stroke: relation to inflammatory biomarkers and hippocampal volume. Stroke 44: 1433–1435.

Kolahdouzan M, Hamadeh MJ (2017) The neuroprotective effects of caffeine in neurodegenerative diseases. CNS Neurosci Ther 23: 272–290.

Kucheryavykh LY, Kucheryavykh YV, Washington AV, Inyushin MY (2018) Amyloid beta peptide is released during thrombosis in the skin. Int J Mol Sci 19: 1705.

Kulesh A, Drobakha V, Kuklina E, Nekrasova I, Shestakov V (2018) Cytokine response, tract-specific fractional anisotropy, and brain morphometry in post-stroke cognitive impairment. J Stroke Cerebrovasc Dis 27: 1752–1759.

Lee J, Lee JE, Kim Y (2017) Relationship between coffee consumption and stroke risk in Korean population: the Health Examinees (HEXA) Study. Nutr J 16: 7.

Leite MR, Wilhelm EA, Jesse CR, Brandão R, Nogueira CW (2011) Protective effect of caffeine and a selective A2A receptor antagonist on impairment of memory and oxidative stress of aged rats. Exp Gerontol 46: 309–315.

Li W, Liu J, Chen JR, Zhu YM, Gao X, Ni Y, et al. (2018) Neuroprotective effects of DTIO, a novel analog of Nec-1, in acute and chronic stages after ischemic stroke. Neuroscience 390: 12–29.

Liu J, Li C, Peng H, Yu K, Tao J, Lin R, et al. (2017) Electroacupuncture attenuates learning and memory impairment via activation of α7nAChR-mediated anti-inflammatory activity in focal cerebral ischemia/reperfusion injured rats. Exp Ther Med 14: 939–946.

Liu N, Chen R, Du H, Wang J, Zhang Y, Wen J (2009) Expression of IL-10 and TNF-alpha in rats with cerebral infarction after transplantation with mesenchymal stem cells. Cell Mol Immunol 6: 207–213.

Lonze BE, Ginty DD (2002) Function and regulation of CREB family transcription factors in the nervous system. Neuron 35: 605–623.

Ma CY, Yin L (2016) Neuroprotective effect of angiotensin II type 2 receptor during cerebral ischemia/reperfusion. Neural Regen Res 11: 1102–1107.

Madeira MH, Boia R, Ambrósio AF, Santiago AR (2017) Having a coffee break: the impact of caffeine consumption on microglia-mediated in-

flammation in neurodegenerative diseases. Mediators Inflamm 2017: 4761081.

Mahdi S, Almosawi S, Baksh H, Qareeballa A, Alsaleh B, Falamarzi F, et al. (2019) Effect of chronic administration and withdrawal of caffeine on motor function, cognitive functions, anxiety, and the social behavior of BLC57 mice. Int J Health Sci 13: 10–16.

Martins A, Schimidt HL, Garcia A, Colletta Altermann CD, Santos FW, Carpes FP, et al. (2017) Supplementation with different teas from Camellia sinensis prevents memory deficits and hippocampus oxidative stress in ischemia-reperfusion. Neurochem Int 108: 287–295.

McLellan TM, Caldwell JA, Lieberman HR (2016) A review of caffeine's effects on cognitive, physical and occupational performance. Neurosci Biobehav Rev 71: 294–312.

Moy S, Nadler J, Perez A, Barbaro R, Johns J, Magnuson T, et al. (2004) Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes Brain Behav 3: 287–302.

Muhammad M, El-Ta'aluEl-Ta'alu AB, Mohammed MA, Yarube IU, Nuhu JM, Yusuf I, et al. (2018) Effect of caffeine on serum tumour necrosis factor alpha and lactate dehydrogenase in wistar rats exposed to cerebral ischaemia-reperfusion injury. Niger J Physiol Sci 33: 1–8.

Park EH, O'Reilly KC, Taborga D, Nicholas K, Ahmed AS, Ruiz N, et al. (2019) Is the rat prefrontal cortex crucial for cognitive control during spatial cognition? BioRxiv doi.org/10.1101/2019.12.20.884262.

Pawluk H, Woźniak A, Grześk G, Kołodziejska R, Kozakiewicz M, Kopkowska E, et al. (2020) The role of selected pro-inflammatory cytokines in pathogenesis of ischemic stroke. Clin Interv Aging 15: 469–484.

Piriyawat P, Labiche LA, Burgin WS, Aronowski JA, Grotta JC (2003) Pilot dose-escalation study of caffeine plus ethanol (caffeinol) in acute ischemic stroke. Stroke 34: 1242–1245.

Pleli T, Mondorf A, Ferreiros N, Thomas D, Dvorak K, Biondi RM, et al. (2018) Activation of adenylyl cyclase causes stimulation of adenosine receptors. Cell Physiol Biochem 45: 2516–2528.

Prediger RD, Batista LC, Takahashi RN (2005) Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats. Involvement of adenosine A1 and A2A receptors. Neurobiol Aging 26: 957–964.

Ran H, Yuan J, Huang J, Wang J, Chen K, Zhou Z (2020) Adenosine A(2A) Receptors in bone marrow-derived cells attenuate cognitive impairment in mice after chronic hypoperfusion white matter injury. Transl Stroke Res 11: 1028–1040.

Reyhani-Rad S, Mahmoudi J (2016) Effect of adenosine A2A receptor antagonists on motor disorders induced by 6-hydroxydopamine in rat1. Acta Cir Bras 31: 133–137.

Rothenburg LS, Herrmann N, Swardfager W, Black SE, Tennen G, Kiss A, et al. (2010) The relationship between inflammatory markers and post stroke cognitive impairment. J Geriatr Psychiatry Neurol 23: 199–205.

Sadigh-Eteghad S, Geranmayeh MH, Majdi A, Salehpour F, Mahmoudi J, Farhoudi M (2018) Intranasal cerebrolysin improves cognitive function and structural synaptic plasticity in photothrombotic mouse model of medial prefrontal cortex ischemia. Neuropeptides 71: 61–69.

Strong R, Grotta J, Aronowski J (2000) Combination of low dose ethanol and caffeine protects brain from damage produced by focal ischemia in rats. Neuropharmacology 39: 515–522.

Sun L, Tian X, Gou L, Ling X, Wang L, Feng Y, et al. (2013) Beneficial synergistic effects of concurrent treatment with theanine and caffeine against cerebral ischemia-reperfusion injury in rats. Can J Physiol Pharmacol 91: 562–569.

Wadhwa M, Chauhan G, Roy K, Sahu S, Deep S, Jain V, et al. (2018) Caffeine and modafinil ameliorate the neuroinflammation and anxious behavior in rats during sleep deprivation by inhibiting the microglia activation. Front Cell Neurosci 12: 49.

Waer FB, Laatar R, Jouira G, Srihi S, Rebai H, Sahli S (2021) Functional and cognitive responses to caffeine intake in middle-aged women are dose depending. Behav Brain Res 397: 112956.

Waje-Andreassen U, Kråkenes J, Ulvestad E, Thomassen L, Myhr KM, Aarseth J, et al. (2005) IL-6: an early marker for outcome in acute ischemic stroke. Acta Neurol Scand 111: 360–365.

Xie G, Huang X, Li H, Wang P, Huang P (2021) Caffeine-related effects on cognitive performance: Roles of apoptosis in rat hippocampus following sleep deprivation. Biochem Biophys Res Commun 534: 632–638.

Xu P, Chen A, Li Y, Xing X, Lu H (2019) Medial prefrontal cortex in neurological diseases. Physiol Genomics 51: 432–442.

Zhou LYY, Wright TE, Clarkson AN (2016) Prefrontal cortex stroke induces delayed impairment in spatial memory. Behav Brain Res 296: 373–378.