

Inhibitory effects of caffeine on hippocampal neurogenesis and function

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Received 15 February 2007

Available online 26 March 2007

Abstract

Caffeine is one of the most extensively consumed psychostimulants in the world. However, compared to short-term effects of caffeine, the long-term effects of caffeine consumption on learning and memory are poorly characterized. The present study found that long-term consumption of low dose caffeine (0.3 g/L) slowed hippocampus-dependent learning and impaired long-term memory. Caffeine consumption for 4 weeks also significantly reduced hippocampal neurogenesis compared to controls. From these results, we concluded that long-term consumption of caffeine could inhibit hippocampus-dependent learning and memory partially through inhibition of hippocampal neurogenesis.

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Keywords: Caffeine; Neurogenesis; Learning; Memory

Caffeine has been shown to ameliorate amnesia in humans, particularly in cases of age-related cognitive decline [1] and scopolamine-induced amnesia [2]. Interpretations of these results may be difficult due to interference by previous caffeine consumption habits and the heterogeneity of samples. The beneficial effect of caffeine on learning and memory in animal models has been reported since the 1960s but the results of these animal studies are also contradictory [2–5]. Most of these studies have examined the acute effects of caffeine. The effects of long-term caffeine consumption on learning and memory has been poorly characterized.

This study focused on the long-term effects of low dose caffeine consumption because it is more representative of general human consumption. We found that long-term consumption of low dose caffeine reduced neurogenesis in

the hippocampus and modulated hippocampus-dependent learning and memory.

Materials and methods

Animals. Male Sprague–Dawley rats (body weight 280–320 g) were caged in an air-conditioned room that was maintained at $22 \pm 2^\circ\text{C}$, with a relative humidity of $50 \pm 10\%$, and a 12/12 h light/dark cycle. Procedures related to animal care were in accord with the guidelines in ‘Guide for the Care and Use of Laboratory Animals’ [6]. Caffeine (0.3 g/L) was included in drinking water for the indicated time.

Immunohistochemistry. Immunohistochemistry was performed as previously described [7]. The following primary antibodies were used; anti-neuron specific nuclear protein (NeuN) (1:500, Chemicon) and anti-bromodeoxyuridine (BrdU) (1:500, Megabase).

BrdU immunohistochemistry. Rats were injected with BrdU (50 mg/kg) in two experimental paradigms, the first was designed to determine the influence of caffeine on the rate of cell proliferation in the hippocampus and the second as a cell marker to follow cell survival. To follow cell proliferation, BrdU was injected once every 2 h over a 10-h period to label proliferating cells and the animals were sacrificed for immunohistochemical processing. To determine cell survival rats were injected with BrdU at the beginning of the experiment to follow the survival of the labeled cells

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after long-term caffeine exposure. Three or four rats were used per group. The remainder of the procedure was performed as previously described [7].

Morphological analysis. A modified unbiased stereological protocol was used to quantitate BrdU labeling in the hippocampus. BrdU-labeled cells were counted in every 6th section at 200 $\times$. The results were presented as the average number of cells in each section, taking the average of between three and four rats per treatment. In all cases, labeled cells were counted by an investigator blinded to the coded slides. In those experiments focusing on the SGZ of the dentate gyrus, a cell was counted if it touched or was within two cell diameters of the SGZ. To confirm the double labeling, a confocal microscope (Leica TCS SP2) was used.

Morris water maze (MWM) test. Morris water maze test (MWM) to check hippocampal-dependent spatial learning ability was performed as previously described with minor modifications [7]. For a non-spatial, but cued, version of the water maze test, a visible platform was used. During the cue training, the visible platform was randomly moved to one of four quadrants on each trial. Latency to reach the platform was timed, and the swim path was videotaped. Rats were trained for 4 days with four trials per day. The inter-trial interval was 5 min.

Data analysis. Data are presented as means $\pm$ SD. Differences between mean values were evaluated using the Student's *t*-test (unpaired comparison). *P* values of <0.05 were considered statistically significant.

Results

Effects of long-term caffeine consumption on learning and memory

To investigate the effects of long-term caffeine consumption on learning and memory, we performed a spatial version of the MWM test with rats 4 weeks after they began to drink caffeinated water (0.3 g/L). Repeated measurements revealed that caffeine-fed rats were slower to find the hidden platform than control rats during the initial testing period, although the groups were similar at the later period (Fig. 1A). To test the effect of caffeine on long-term memory we gave rats a probe trial at 1, 2, and 3 weeks after training. There was no performance difference between the caffeine-fed and the control groups on the week 1 probe trial. However, caffeine-fed rats performed significantly worse than control rats on the week 2 and week 3 probe trials and spent only a little more time than that expected by chance in the target quadrant (Fig. 1B).

Next, we performed a cue version of the MWM test 4 weeks after drinking caffeinated water. The spatial version of the MWM test is dependent on the hippocampus; however, the cue version of the MWM test is dependent on the striatum, not the hippocampus. As in the spatial task, both groups learned the task gradually. Repeated measurements showed no significant difference between the two groups (Fig. 1C).

Effects of long-term caffeine consumption on hippocampal neurogenesis

Several reports have suggested that hippocampal neurogenesis is necessary to support memory and learning [8,9]. To examine the effects of long-term caffeine use on the proliferation of neural stem cells at the subgranular zone (SGZ) of the hippocampus, nuclear uptake of BrdU was determined by immunohistochemistry in tissue sections as

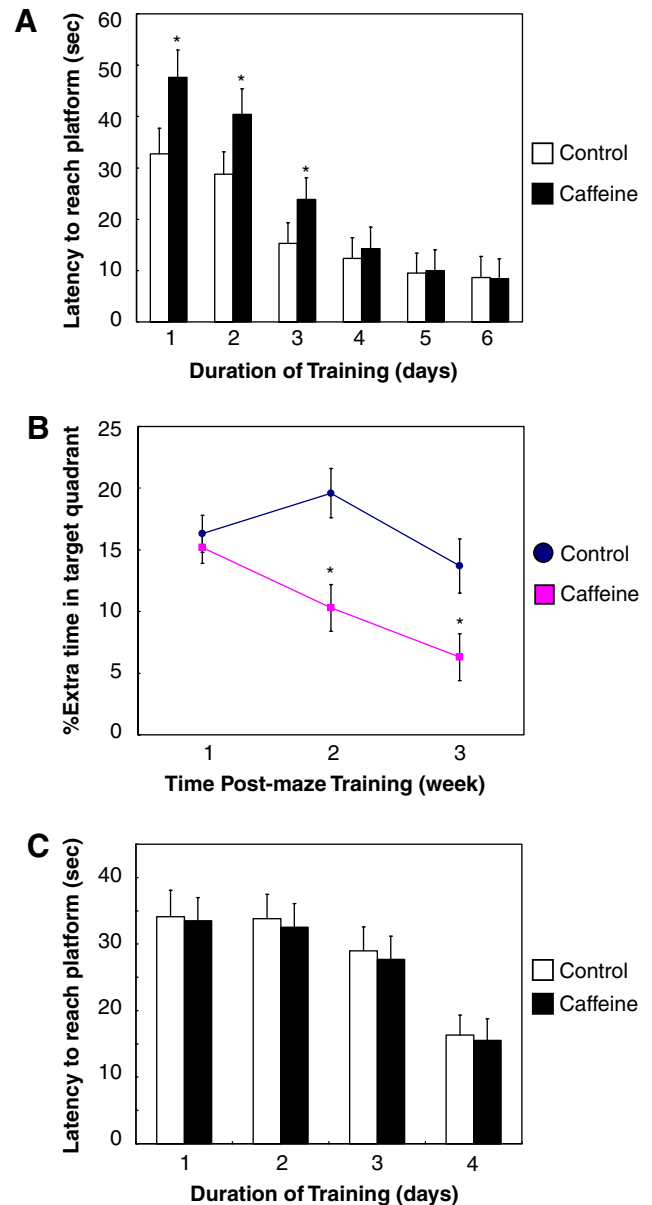


Fig. 1. Impaired hippocampal learning and memory in caffeine-fed rats in the MWM test. (A) The acquisition of spatial information at the initial period of the water maze was delayed in caffeine-fed rats ($n=10$) compared to control rats ($n=10$) in a spatial version of MWM test. (B) There was no performance difference between caffeine-fed and control rats in the 1 week probe trial. The Y axis shows the difference between the % time spent in the quadrant formerly containing the hidden platform (target) and the mean % time spent in the other three quadrants. At the 1 week probe trial, both groups spent a significant time in the target quadrant, indicating an intact reference memory. However, caffeine-fed rats performed significantly worse than control rats on 2 and 3 week probe trials. Data are means $\pm$ SD. * $P < 0.05$. (C) There was no significant difference between caffeine-fed and control rats in a cue training test.

described in Materials and methods. After 1 week of caffeine exposure (0.3 g/L), no significant change in proliferation in the SGZ was found. However, when caffeine exposure was extended to 2 weeks, a significant decrease in proliferation was observed. By week 4, proliferation had declined by 34.4% (Figs. 2 and 3).

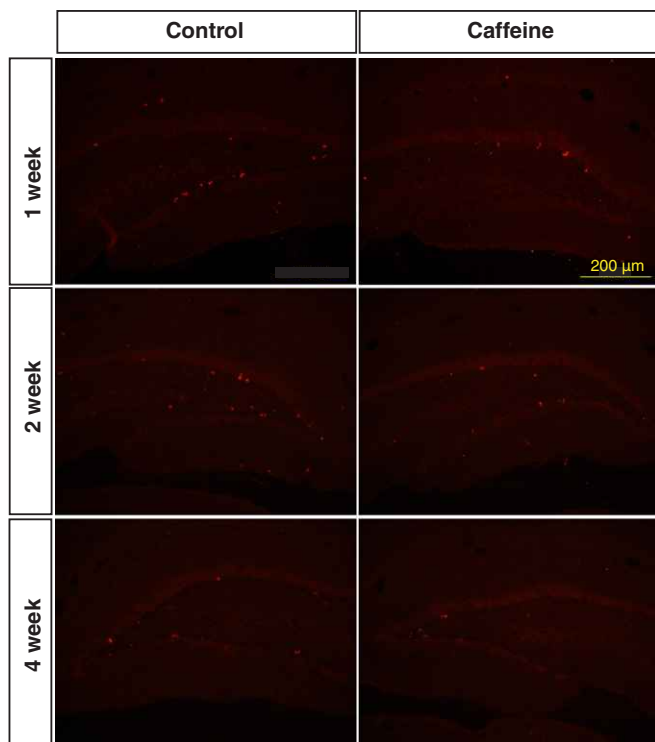


Fig. 2. Decline in the proliferation of neural stem cells in the SGZ. The injection schedule of BrdU was described in Materials and methods. Brain sections were stained with anti-BrdU antibody. One week of exposure to caffeine (0.3 g/L) resulted in no significant difference in BrdU-positive cells between caffeine-fed and control rats in the SGZ. However by 2 weeks of treatment, a significant decline was evident. By 4 weeks of treatment, reduction of proliferation at the SGZ was more pronounced.

Next, we examined whether long-term caffeine exposure can affect the survival of the newly divided cell. Rats were pulsed with BrdU and then treated with caffeine for 4 weeks, as described in Materials and methods. Cell survival was checked by counting the number of BrdU-positive cells in the SGZ. Long-term caffeine exposure slightly decreased the number of BrdU-positive cells compared to controls by 23.8% in the SGZ (Fig. 4). For further confirmation, the number of newly generated neurons was counted using confocal microscopy. To identify newly generated neurons, the sections were doubly stained with anti-BrdU and anti-NeuN antibodies. The number of new neurons was significantly decreased in caffeine-fed rats compared to control rats (Fig. 4), suggesting that long-term caffeine exposure can modulate the survival of newly divided cells.

Discussion

In this study, we chose a low dose of caffeine (0.3 g/L) for two main reasons: First, it leads to a plasma level of caffeine representative of regular daily human consumption; and second, in a model of neonatal ischemia, this low dose of 0.3 g/L was more protective than a higher dose, 0.8 g/L [10]. However, it would be of interest to extend the present study to other doses of caffeine.

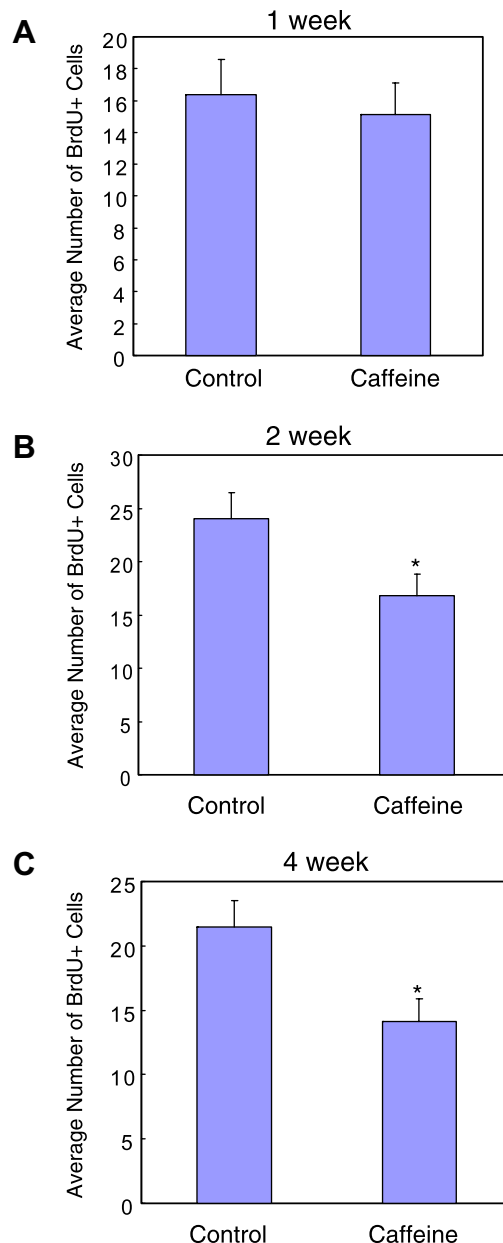


Fig. 3. Decline in the proliferation of neural stem cells at the SGZ. BrdU-positive cells in Fig. 2 were counted in a stereological method. One week of exposure to caffeine (0.3 g/L) resulted in no significant difference in the average number of BrdU-positive cells in the SGZ between caffeine-fed and control rats (A). However, by 2 weeks of treatment a significant decline was evident (B), reaching a reduction of 34.4% by 4 weeks (C). Data are means $\pm$ SD per section from three independent experiments. * $P < 0.05$.

Effects of long-term caffeine consumption on learning and memory

Extensive studies concerning the effects of caffeine on memory have been conducted. Most of these studies have suggested that caffeine improves memory [1,2,11,12]. However, the present study showed that long-term consumption of caffeine could impair memory (Fig. 1). One of the differences between the present study and previous studies is that

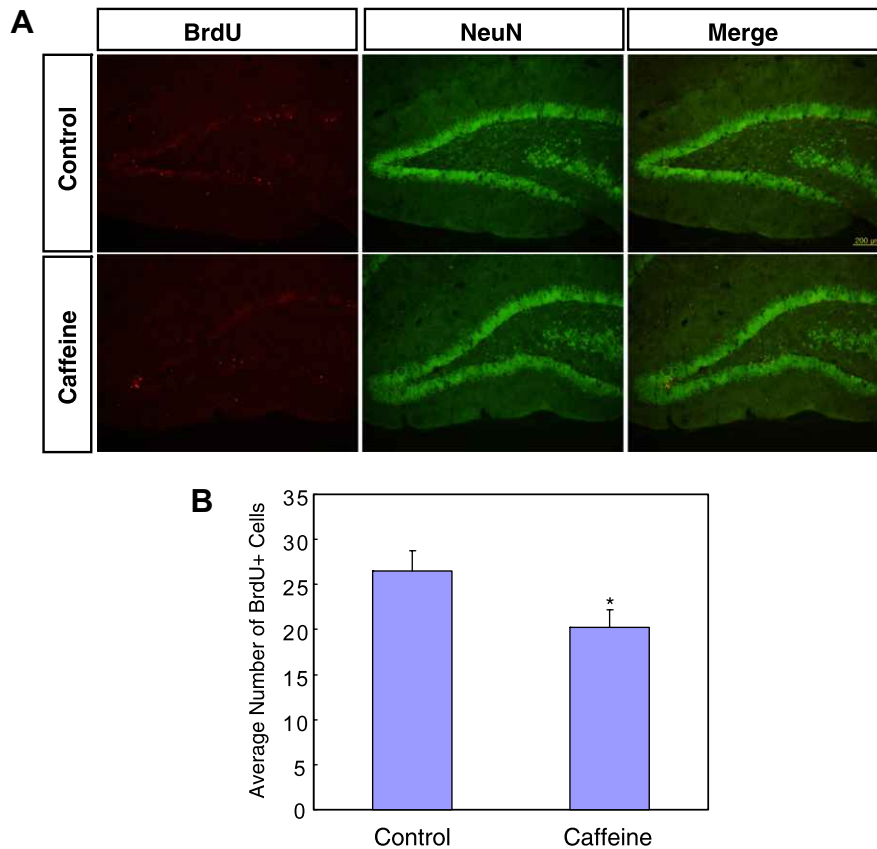


Fig. 4. Survival rate of newly divided cells was significantly reduced in caffeine-fed rats. The injection schedule of BrdU was described in Materials and methods. (A) The brain sections were stained with anti-BrdU (red) and anti-NeuN (green) antibodies to identify newly generated neurons. (B) BrdU-positive cells in (A) were counted in a stereological method. Data are means $\pm$ SD per section from three independent experiments. * $P < 0.05$.

most of the previous studies were focused on the acute effects of caffeine. Acute administration of caffeine decreased the threshold for convulsants [13,14]. In contrast, chronic administration of caffeine increased it [15,16]. Moreover, acute administration of caffeine worsened ischemia-induced damage [17], however, chronic administration of caffeine reduced it [18,19].

Effects of long-term caffeine consumption on neurogenesis at hippocampus

Many studies have shown that the neurogenesis at hippocampus can be regulated physiologically and pharmacologically [20]. However, the effects of long-term caffeine consumption on hippocampal neurogenesis have never been previously investigated. In the present study, we showed that long-term caffeine consumption could inhibit hippocampal neurogenesis for the first time.

Previous reports have supported that hippocampal neurogenesis is associated with memory [8,9]. So, reduction of hippocampal neurogenesis by caffeine in the present study (Figs. 2–4) can be one of the reasons why memory was impaired in the caffeine-fed rats. However, learning ability was also impaired in the caffeine-fed rats in this study

(Fig. 1). The effect of caffeine on learning cannot be explained by reduction of hippocampal neurogenesis because inhibition of neurogenesis in the hippocampus by irradiation only impaired memory retention and learning was spared [7]. So, other unknown mechanisms may be involved in the caffeine-induced learning impairment.

Modulation of cell proliferation by caffeine has been described in various cells. In particular, oral administration of caffeine has long been known to reduce cancer growth [21]. Several p53-dependent or -independent mechanisms underlying the modulation of cell proliferation by caffeine have been suggested [22,23]. In addition to the intracellular mechanisms of caffeine that affect cell proliferation, the adenosine receptor, which has also been shown to be regulated by caffeine [24], may also be involved. The activation of the adenosine A2a receptor is antagonistic to the dopamine D2 receptor, which has been shown to enhance neurogenesis [25].

In conclusion, the present study showed that long-term consumption of low dose caffeine, representative of normal human intake, can inhibit hippocampal neurogenesis and hippocampus-dependent learning and memory. In future studies, the underlying mechanism that explains the inhibition of neurogenesis by caffeine needs to be fully elucidated.

Acknowledgments

This study was supported by Medical Research Institute Grant (2003-31), Pusan National University and MRC program of MOST/KOSEF (R13-2005-009).

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