CHAPTER IV

RESULTS AND DISCUSSION

Results

Effects of escalating and binge doses-METH administration on weight loss in all experimental groups

After treatment of each day, the average weight loss of animals in ED-METH group were significantly decreased on day 3-5 and day 7-14 compared with control group but there were no significant differences on day 1, 2 and day 6 ($p = 0.798$, $p = 0.652$ and $p = 0.182$, respectively). The relationship between weight loss of all experimental groups on day 1-14 was shown in Figure 16 and Table 4.

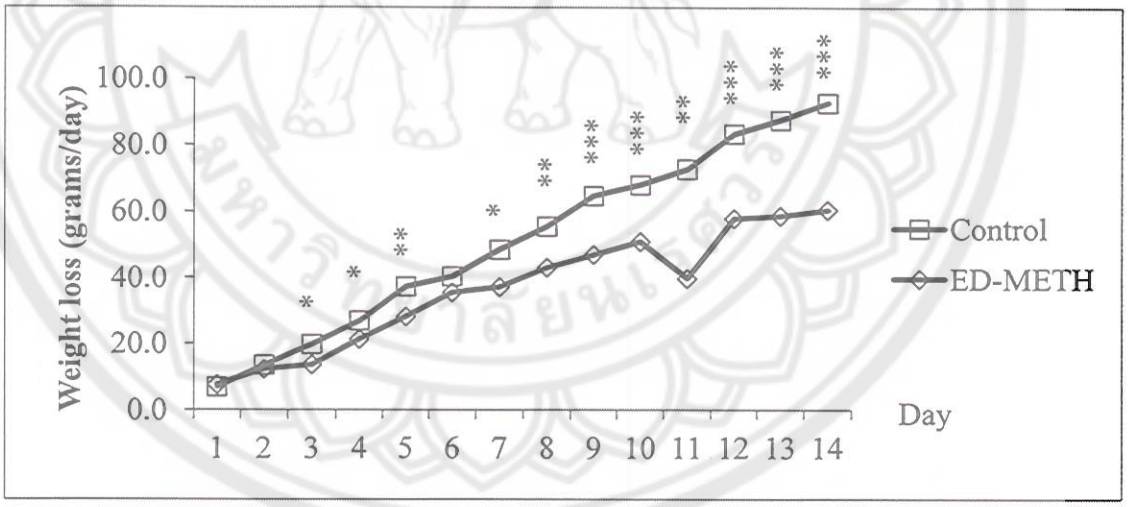

Figure 16 Weight loss of ED-METH and control groups on day 1-14

Note: Data are presented as Mean ± SEM. ED-METH group (n = 8) and control group (n = 13).

* $p < 0.05$ in comparison with control group by t-test

** $p < 0.01$ in comparison with control group by t-test

*** $p < 0.001$ in comparison with control group by t-test

The results showed that the average weight loss on day 15 was significantly decreased in both ED-METH binge and ED-METH when compared with control groups (p = 0.000). Moreover, the average weight loss was decreased in AB-METH group when compared with control group (p = 0.125). The body weight of all experimental groups on day 15 was shown in Figure 17 and Table 5.

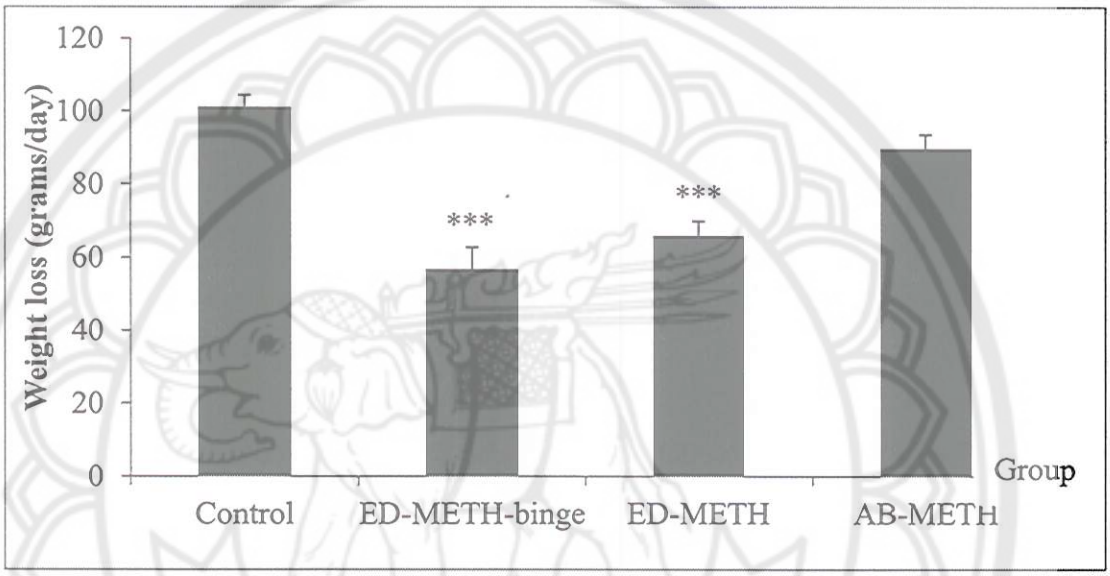

Figure 17 Weight loss of all experimental groups on day 15

Note: Data are presented as Mean ± SEM. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8). *** $p < 0.001$ in comparison with control group by ANOVA post hoc Dunnett test.

Effects of escalating and binge doses-METH administration on amount of food intake of all experimental groups

In the present study, the food remain of each group was weighted before METH administration to calculate the food that animals had taken each day (day 0-15). The results showed that amount of food intake on day 0-15 was significantly decreased in ED-METH group compared with control group. The relationship between amount of food intake of each group that received on day 0-14 and day 15 (day binge doses-METH administration) was shown in Figure 18 and Table 6.

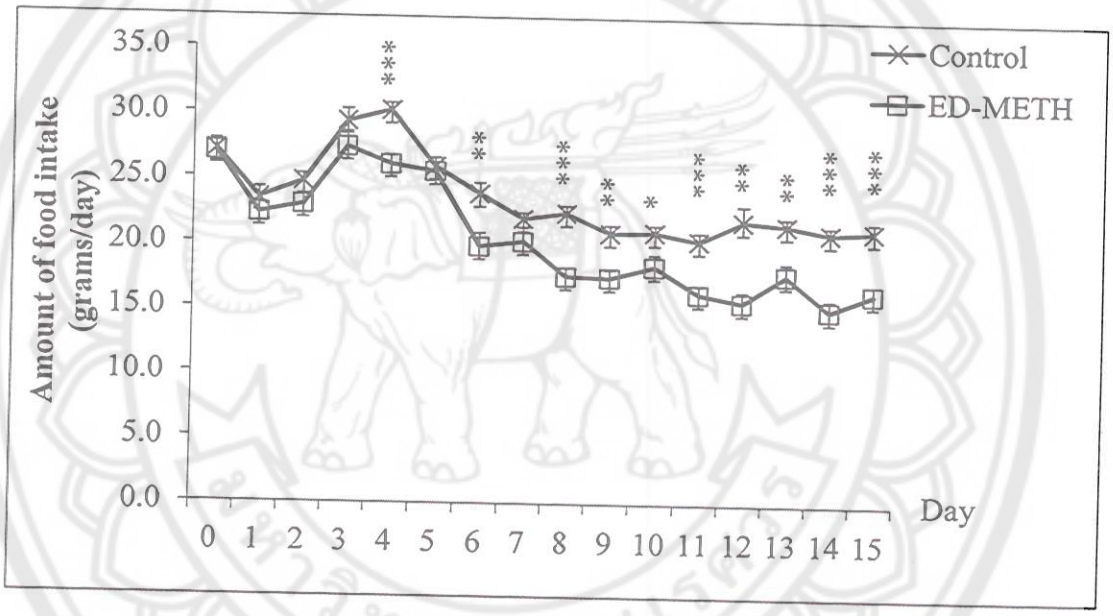

Figure 18 Amount of food intake of ED-METH and control groups on day 0-15

Note: Data are presented as Mean ± SEM. ED-METH group (n = 8) and control group (n = 13).

* $p < 0.05$ in comparison with control groups by t-test

** $p < 0.01$ in comparison with control groups by t-test

*** $p < 0.001$ in comparison with control groups by t-test

On day 15 (ED-effect), the results showed that amount of food intake was significantly decreased in escalating dose-METH group when compared with control group ($p = 0.000$ (Figure 19 and Table 7).

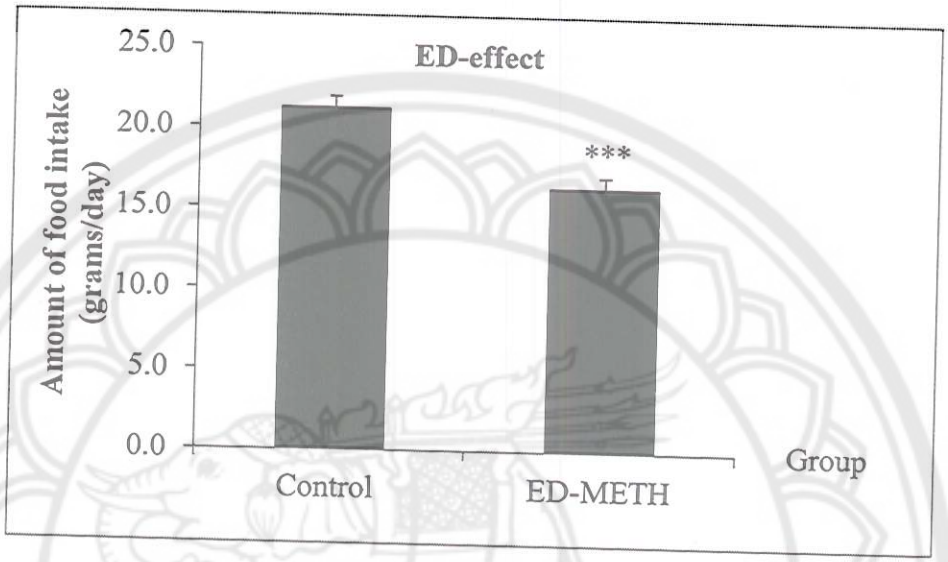

Figure 19 Effects of escalating doses-METH administration on amount of food intake

Note: Data are presented as Mean ± SEM. ED METH; escalating dose-methamphetamine group (n = 8) and control group (n = 13). *** $p < 0.001$ in comparison with control group by t-test

On day 16 (binge-effect), the results showed that amount of food intake was significantly decreased in binge-dose METH; ED-METH binge and AB-METH groups when compared with control group ($p = 0.000$) Figure 20 and Table 7.

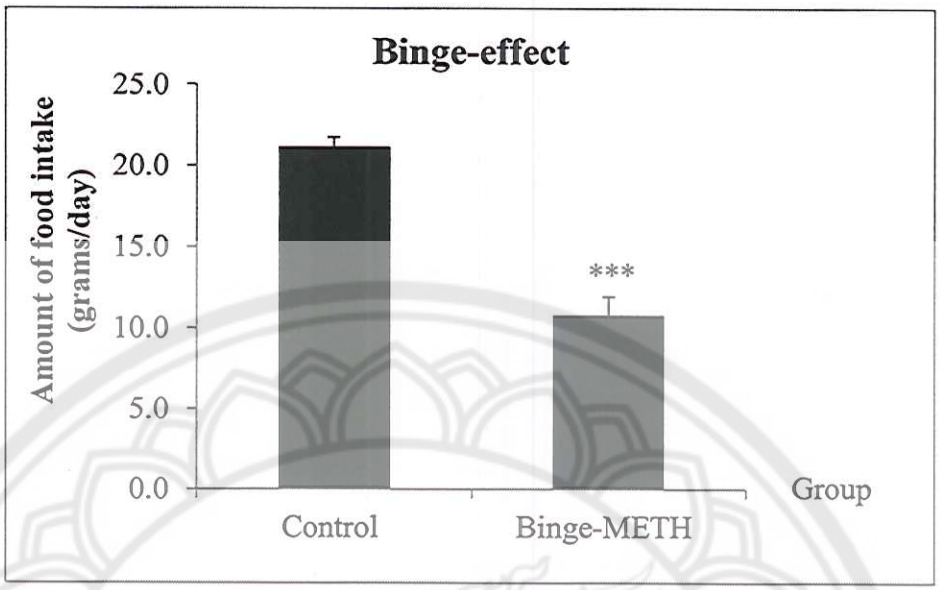

Figure 20 Effects of binge doses-METH administration on amount of food intake

Note: Data are presented as Mean ± SEM. Binge-METH; escalating dose-methamphetamine binge group and acute dose-methamphetamine binge group (n = 9) and control group (n = 12).

*** $p < 0.001$ in comparison with control group by t-test

Effects of escalating and binge doses-METH administration on core body temperature

At 1 h after the last dose injection of day 15 (day binge doses-METH administration), the results showed that the core body temperature was significantly increased in AB-METH group when compared with control group (p = 0.008). However, there were no significantly differences in ED-METH binge and ED-METH groups compared with control group (p = 0.306 and p = 0.681, respectively) (Figure 21 and Table 8).

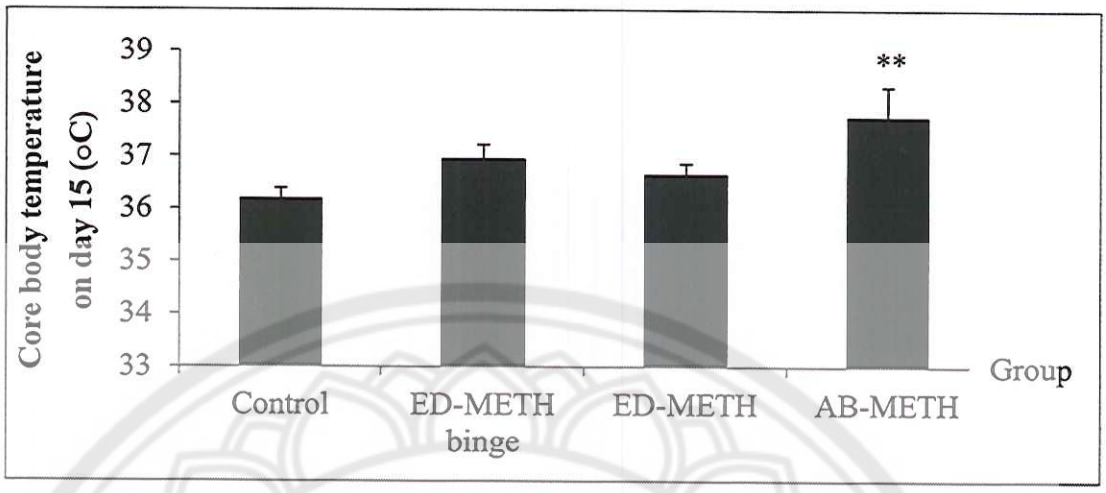

Figure 21 Effects of METH administration on core body temperature in all experimental groups on day 15

Note: Data are presented as Mean ± SEM. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8). ** $p < 0.01$ in comparison with control group by ANOVA post hoc Dunnett test

Effects of escalating and binge doses-METH administration on behavioral test

In the present study, the behaviors of animals in each group were normal active movement; sniffed or reared their home cage on day 0. On day 1, the animals in both ED-METH binge and ED-METH groups showed always sniffing of cage; rearing and walk around their home cage but in AB-METH and control groups were normal similar to day 0. On day 2-6, the animals in ED-METH binge and ED-METH groups showed hyperactivity and slow pattern of movement and always performed oral stereotype and head movement on day 7-15 and day 10-14, respectively. In contrast, animals in AB-METH group were normal on day 0-14 and performed oral stereotype and head movement on day 15, the day that animals were treated by binge doses. At the time for drug treatment of each day, rats in ED-METH binge and ED-METH groups were excited and alertness.

Effects of escalating and binge doses-METH administration on behavioral rating scale scores

On day 0, there were no significant differences in the behavioral observation of animal in all experimental groups before starting the experiment.

On day 1, the behavioral rating scale scores were significantly increased in ED-METH group compared with control group ($p < 0.001$) (Figure 22).

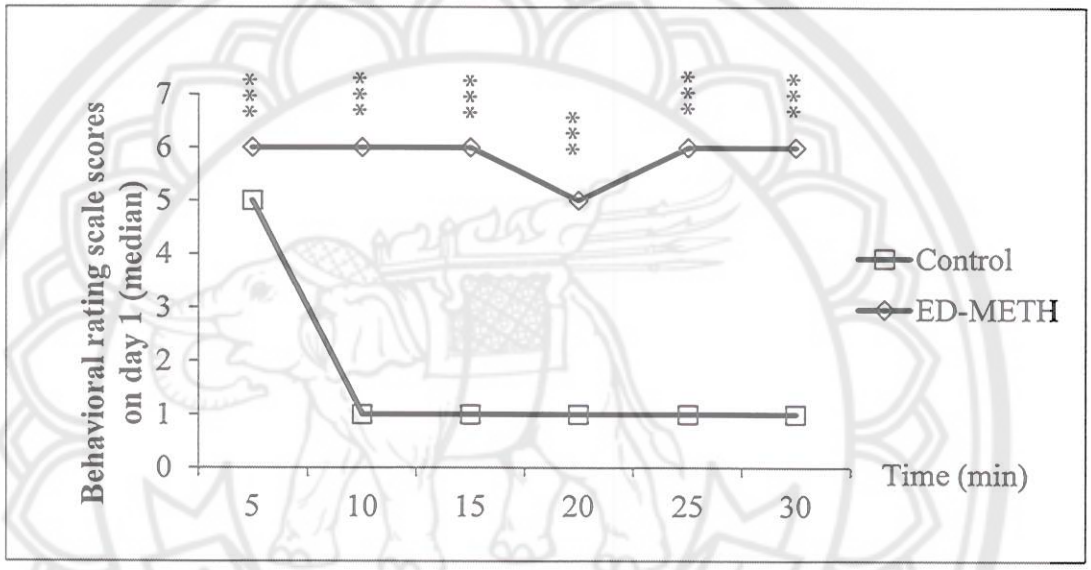

Figure 22 Behavioral rating scale scores (median) of ED-METH and control groups on day 1

Note: Data are presented as Mean ± SEM. ED-METH group (n = 8) and control group (n = 13).

*** $p < 0.001$ in comparison with saline group by Kruskal–Wallis test

On day 14, the behavioral rating scale scores were significantly increased in ED-METH group when compared with control group ($p < 0.001$) (Figure 23)

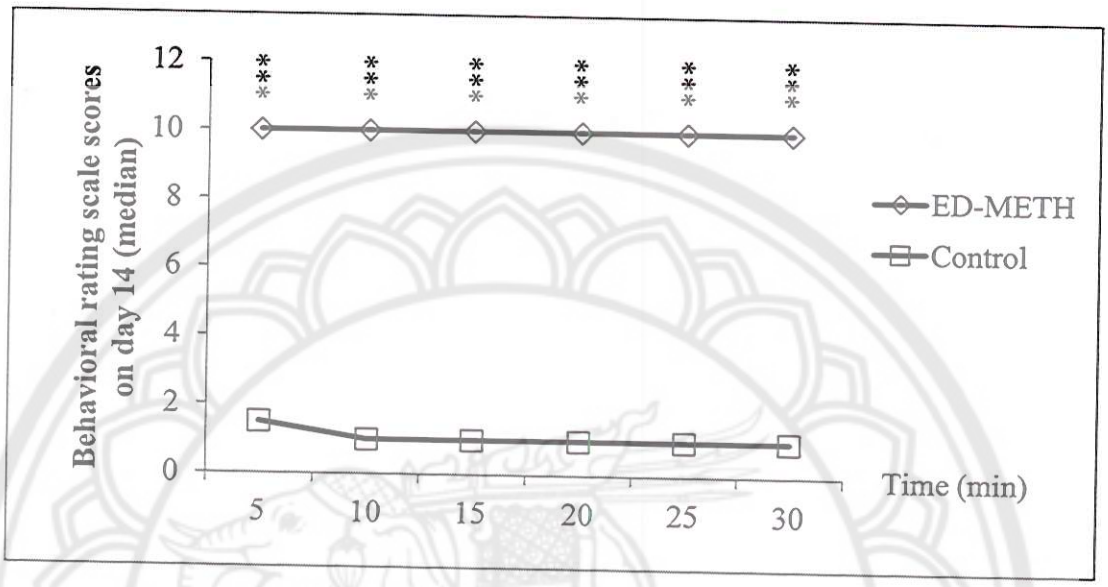

Figure 23 Behavioral rating scale scores (median) in ED-METH and control groups on day 14

Note: Data are presented as Mean ± SEM. ED-METH group (n = 8) and control group (n = 13).

*** $p < 0.001$ in comparison with saline group by Kruskal–Wallis test

The results showed that the scores of behavioral rating scale on day 15 was significantly increased in both ED-METH binge and AB-METH groups compared with control group ($p < 0.001$) (Figure 24). Moreover, the behavioral rating scale scores were significantly increased in AB-METH groups when compared with day 14 (before binge doses treated) ($p < 0.001$) (Figure 25A) but there were no significant differences in ED-METH binge group when compared at day 14 (Figure 25B).

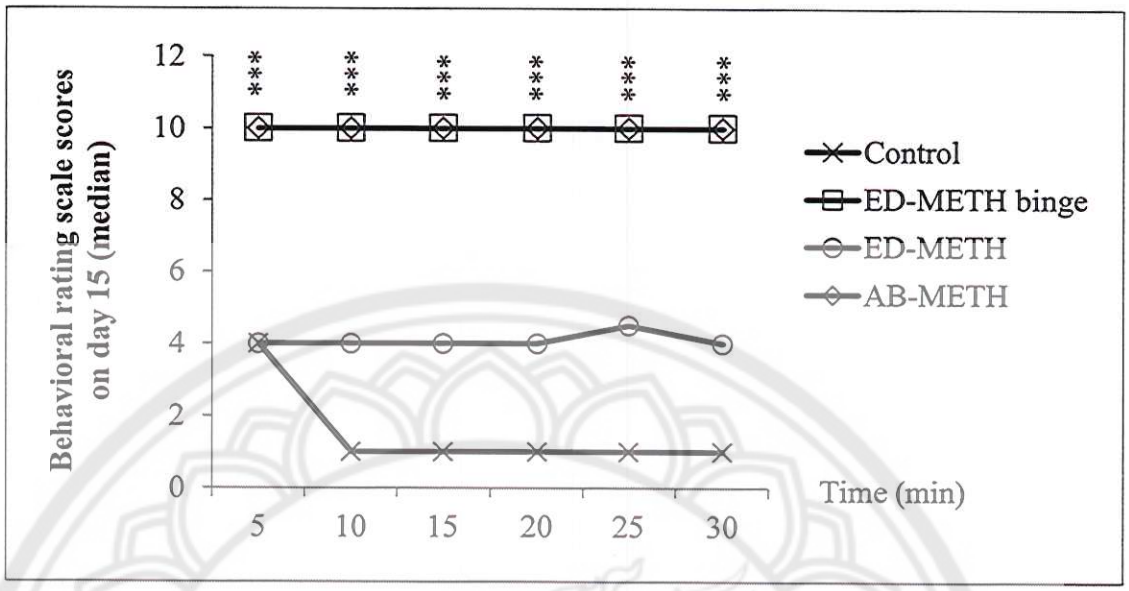

Figure 24 Behavioral rating scale scores (median) in all experimental groups on day 15

Note: Data are presented as Mean ± SEM. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8). *** $p < 0.001$ in comparison with control group by Kruskal–Wallis one-way ANOVA on ranks with post hoc Dunn's test

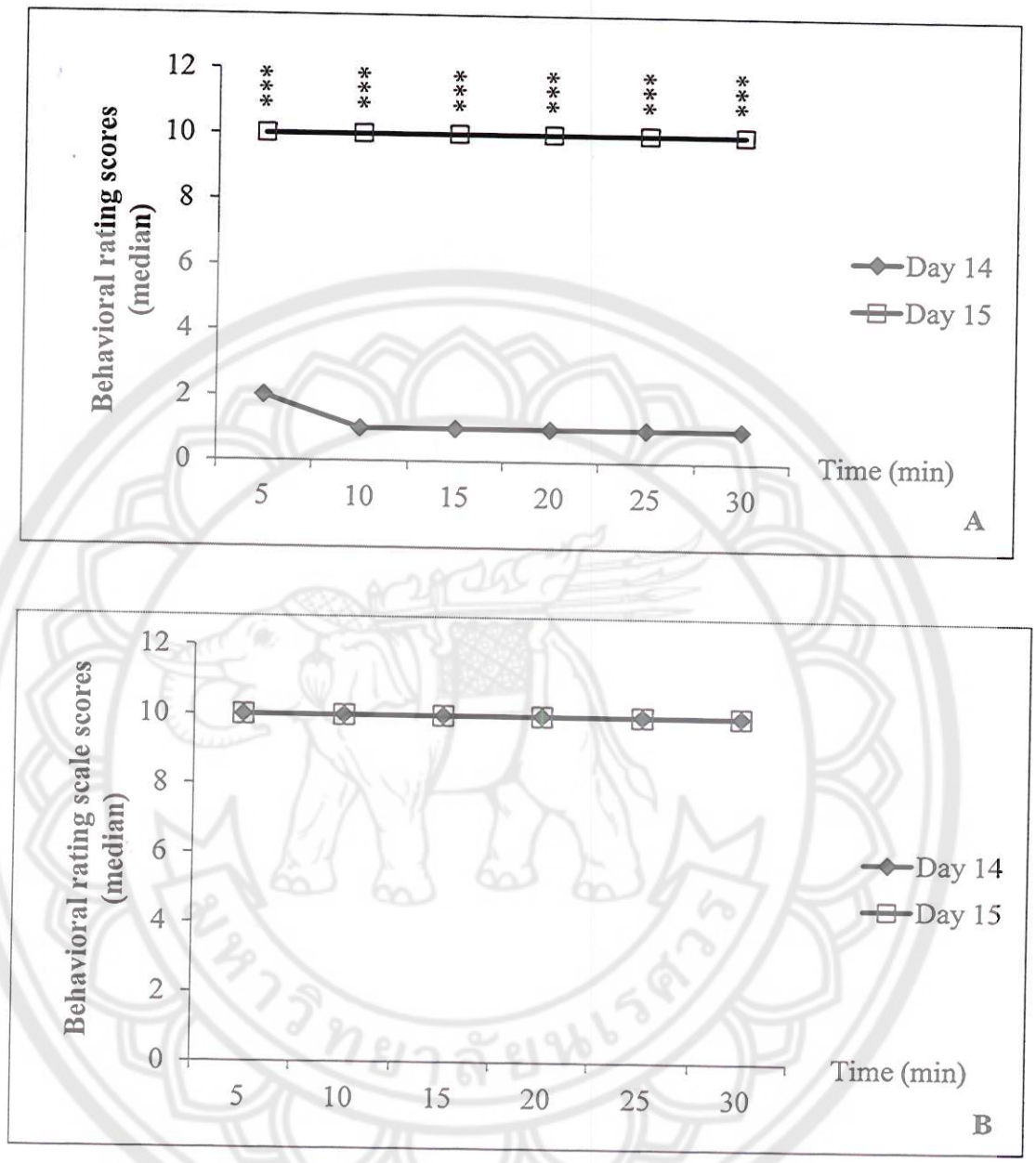

Figure 25 Behavioral rating scale scores (median) in AB-METH group (A) and ED-METH binge (after binge dose treated) (B) on day 14 and day 15

Note: Data are presented as Mean ± SEM. ED-METH binge = escalating dose-methamphetamine binge group (n = 4), AB-METH = Acute dose-methamphetamine binge group (n = 5).

*** $p < 0.001$ in comparison with day 14 by Kruskal–Wallis test

Effects of escalating and binge doses-METH administration on locomotor activity

In the present study, there were no significant differences of locomotor activity in ED-METH and control groups on day 0-1. However, the results showed that the locomotor activity was significantly increased in ED-METH group when compared with control group on day 2-14 (Figure 26 and Table 9).

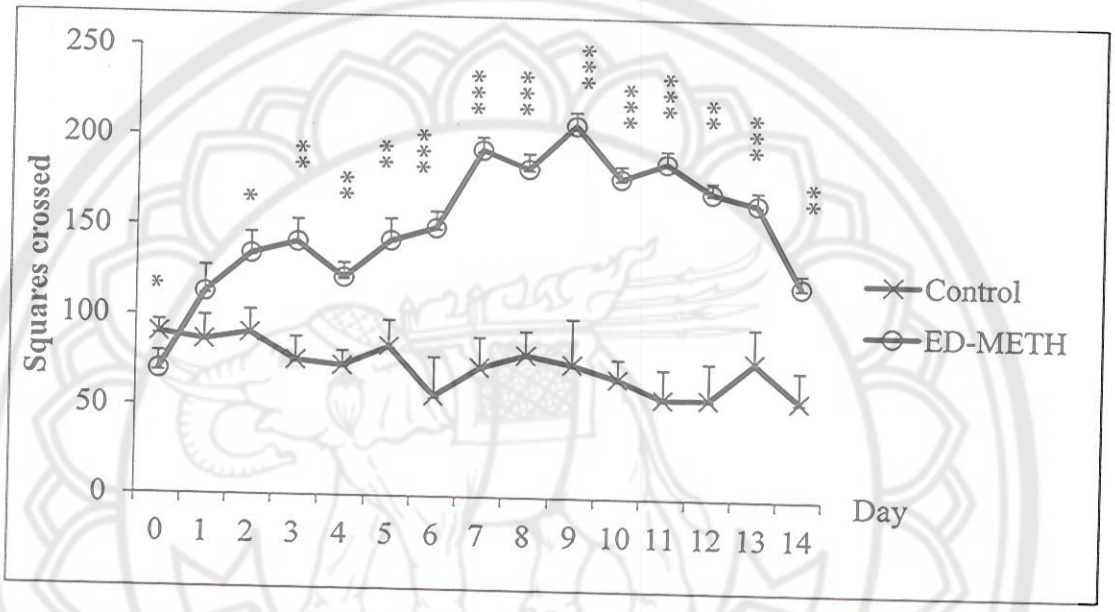

Figure 26 Effects of methamphetamine on locomotor activity on day 0-14

Note: Data are presented as Mean ± SEM. ED-METH group (n = 8) and control group (n = 13).

* $p < 0.05$ in comparison with control group by t-test

** $p < 0.01$ in comparison with control group by by t-test

*** $p < 0.001$ in comparison with control group by by t-test

Moreover, the locomotor activity was significantly decreased in AB-METH group when compared with control group (p = 0.005) but no significant differences were found in both ED-METH binge and ED-METH groups (p = 0.275 and p = 0.913, respectively) (Figure 27 and Table 10).

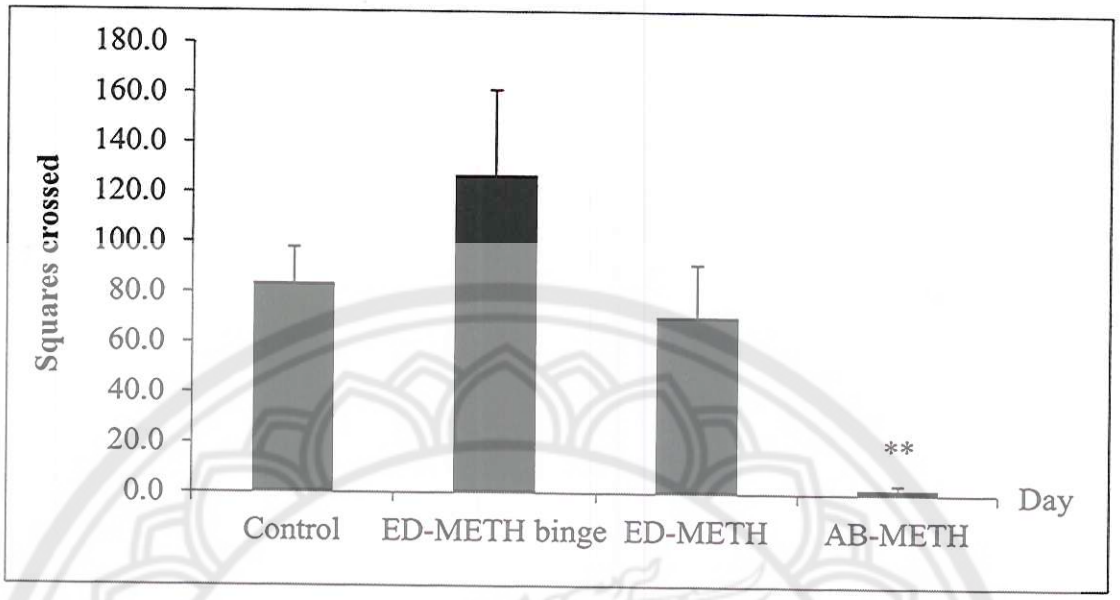

Figure 27 Effects of METH administration on locomotor activity on day 15

Note: Data are presented as Mean ± SEM. ED-METH binge; escalating dose-methamphetamine binge group (n = 3), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8). ** $p < 0.01$ in comparison with saline-treated group by ANOVA post hoc Dunnett test

Effects of escalating and binge doses-METH administration on novel object recognition test (NOR)

The novel object recognition test was demonstrated the memory of animals in all experimental groups after escalating and binge doses-METH administration. In this study, the novel object recognition test is included the short-term (1.30 h after training session), and long-term (24 h after training session) memory recognition tests.

In this study, the novelty index in training session was no significant difference in all experimental groups when compared with control group; ED-METH group (p = 0.658), ED-METH binge group (p = 0.736) and AB-METH group (p = 0.912).

In short-term test, the novelty index was significantly decreased in all experimental groups when compared with control group (ED-METH group; $p = 0.006$, ED-METH binge group; $p = 0.030$ and AB-METH group; $p = 0.020$).

The novelty index of long-term memory was significantly decreased in ED-METH binge group when compared with control group ($p = 0.010$) but there were no significant differences in ED-METH and AB-METH groups when compared with control group ($p = 0.861$ and $p = 0.110$, respectively). The novelty index of novel object recognition test of each group was shown in Table 3.

Table 3 The novelty index of novel object recognition test after escalating and binge doses-METH administration

Group	Training Mean±SEM	Short-term test Mean±SEM	Long-term test Mean±SEM
Control	0.51 ± 0.03	0.56 ± 0.02	0.45 ± 0.04
ED-METH	0.49 ± 0.04	0.40 ± 0.07	0.44 ± 0.04
p	0.658	0.021*	0.861
Control	0.62 ± 0.03	0.60 ± 0.03	0.64 ± 0.05
ED- METH binge	0.64 ± 0.04	0.49 ± 0.04	0.40 ± 0.04
p	0.736	0.030*	0.010*
Control	0.62 ± 0.03	0.60 ± 0.03	0.64 ± 0.05
AB-METH	0.62 ± 0.06	0.45 ± 0.06	0.47 ± 0.10
p	0.912	0.020*	0.110

Note: Animals were treated with saline (control) or saline on day 1-14 and 4x6.0 mg/kg METH at 2 h intervals on day 15 (AB-METH) or gradually increasing three doses of METH (0.1-3.9 mg/kg/day) on day 1-13 and 3x4.0 mg/kg METH on day 14 and saline on day 15 (ED-METH) or gradually increasing three doses of METH (0.1-3.9 mg/kg/day) on day 1-13 and 3x4.0 mg/kg METH on day 14 and four consecutive injections of 6.0 mg/kg METH at 2 h intervals on day 15 (ED-METH binge). Data were presented as Mean ± SEM. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating

dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 4) and control group (n = 8).

* $p < 0.05$ in comparison with control group by t- test.

Expression of proliferating cell nuclear antigen (PCNA)

The immunohistochemistry demonstrated PCNA immunoreactive (PCNA-IR) cells in all neuronal cells of subgranular zone of dentate gyrus and subventricular zone. PCNA-IR were strongly limited to proliferative cells but there were less in mature neurons, astrocyte and oligodendrocyte.

Expression of PCNA-IR cells in subgranular zone after escalating and binge doses-METH administration

In this study, the results showed that the PCNA-IR cells were significantly decreased in both ED-METH binge and ED-METH groups when compared with control group (p = 0.011 and p = 0.021, respectively) but there was no significant difference in AB-METH group when compared with control group (p = 0.145) (Figure 28 and Table 11).

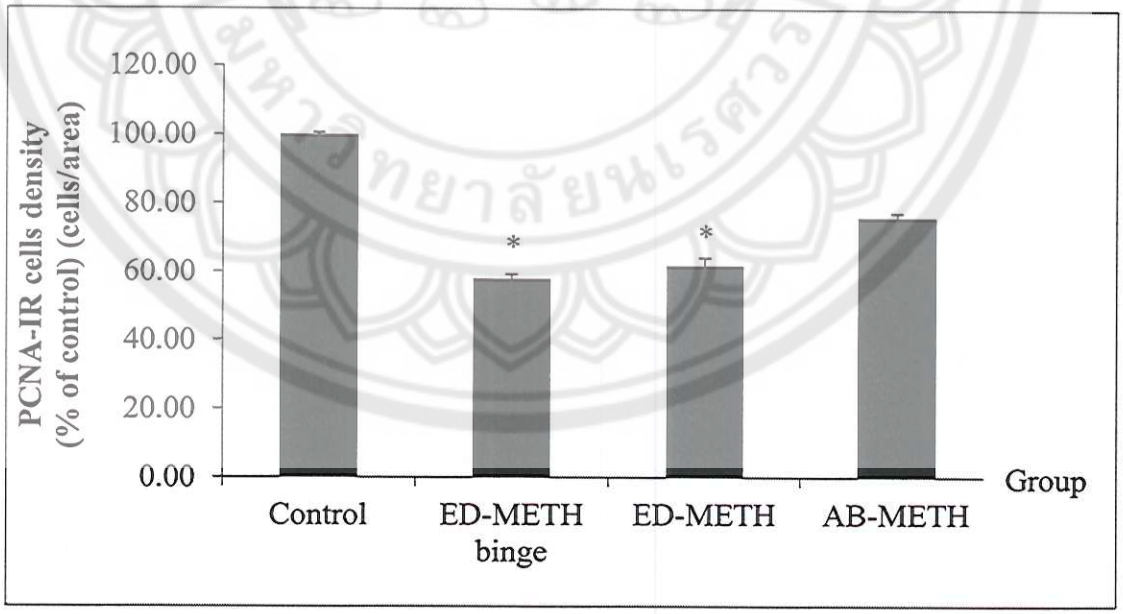

Figure 28 Expression of PCNA-IR cells in subgranular zone after escalating and binge doses-METH administration

Note: The PCNA-IR cells were observed in subgranular zone under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

* $p < 0.05$ in comparison with control group by ANOVA post hoc Dunnett test.

Figure 29 Expression of PCNA-IR cells in subgranular zone after escalating and binge doses-METH administration in coronal section stained with monoclonal antibody against proliferating cell nuclear antigen (1:100) at 40x magnification. Black arrows indicate PCNA-IR cells. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute dose-methamphetamine binge group (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 10 μm

Expression of PCNA-IR cells in subventricular zone after escalating and binge doses-METH administration

In this study, the results showed that the PCNA-IR cells were significantly decreased in all experimental groups when compared with control group (ED-METH binge; p = 0.000, ED-METH; p = 0.000 and AB-METH; p = 0.011 (Figure 30 and Table 12).

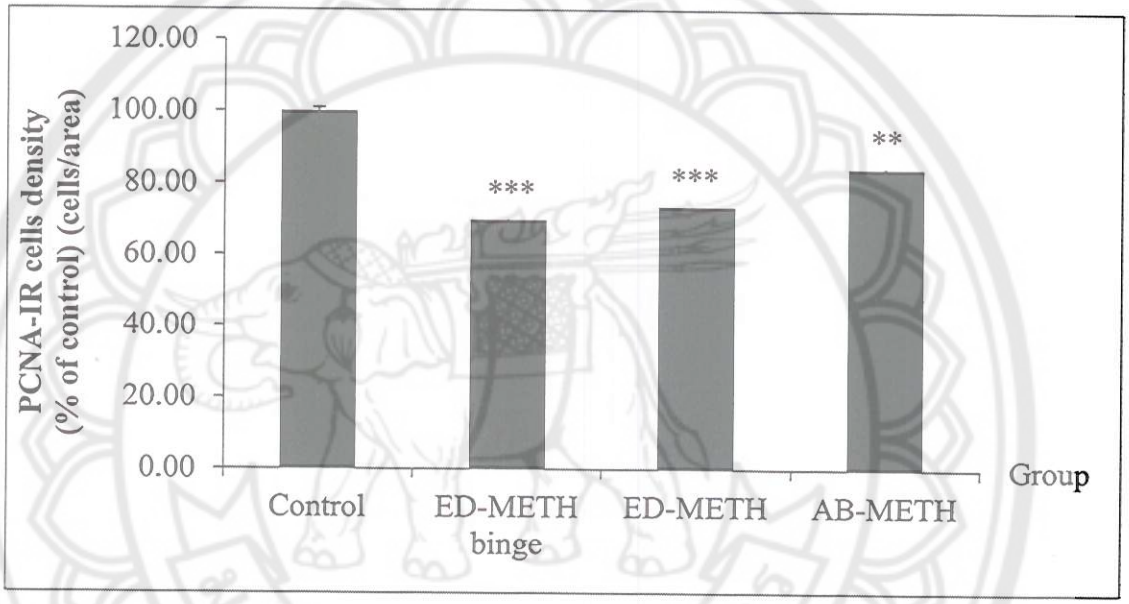

Figure 30 Expression of PCNA-IR cells in subventricular zone after escalating and binge doses-METH administration

Note: The PCNA-IR cells were observed in subventricular zone under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

** $p < 0.01$ in comparison with control group by ANOVA post hoc Dunnett test.

*** $p < 0.001$ in comparison with control group by ANOVA post hoc Dunnett test.

Figure 31 **Expression of PCNA-IR cells in subventricular zone after escalating and binge doses-METH administration in coronal section stained with monoclonal antibody against proliferating cell nuclear antigen (1:100) at 40x magnification. Black arrows indicate PCNA-IR cells. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 10 μm**

Expression of microtubule-associated protein 2 (MAP2)

The immunohistochemistry demonstrated MAP2 immunoreactive (MAP2-IR) cells in all neuronal cells. MAP2-IR were strongly limited to mature neuronal cells but there were less in astrocyte and oligodendrocyte.

Expression of MAP2-IR cells in subgranular zone after escalating and binge doses-METH administration

In the present study, MAP2-IR cells were significantly decreased in both ED-METH binge and ED-METH groups when compared with control group ($p = 0.000$ and $p = 0.000$, respectively). However, no significant differences of MAP2-IR cells were observed in AB-METH group when compared with control group ($p = 0.180$) (Figure 32 and Table 13).

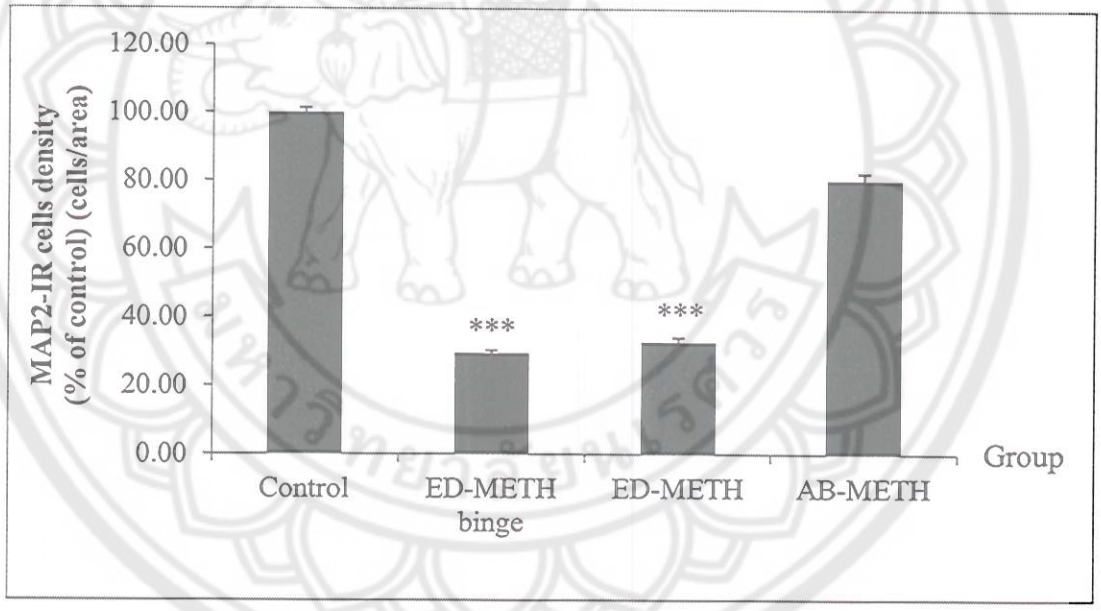

Figure 32 Expression of MAP2-IR cells in subgranular zone after escalating and binge doses-METH administration

Note: The MAP2-IR cells were observed in subgranular zone under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4),

AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

*** $p < 0.001$ in comparison with control group by ANOVA post hoc Dunnett test.

Figure 33 Expression of MAP2-IR cells in subgranular zone after escalating and binge doses-METH administration in coronal section stained with polyclonal antibody against microtubule-associated protein 2 (1:750) at 40x magnification. Yellow arrows indicate MAP2-IR cells. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 10 μm

Expression of MAP2-IR cells in subventricular zone after escalating and binge doses-METH administration

In the present study, MAP2-IR cells were significantly decreased in all groups when compared with control group (ED-METH binge; p = 0.002, ED-METH; p = 0.005 and AB-METH; p = 0.030) (Figure 34 and Table 14).

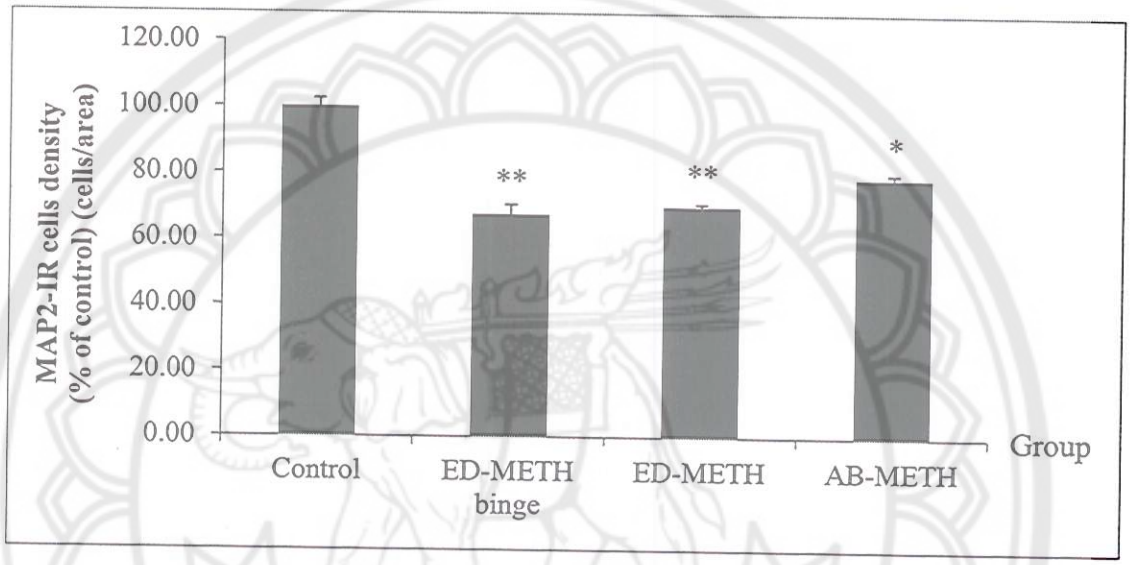

Figure 34 Expression of MAP2-IR cells in subventricular zone after escalating and binge doses-METH administration

Note: The MAP2-IR cells were observed in subventricular zone under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

* $p < 0.05$ in comparison with control group by ANOVA post hoc Dunnett test.

** $p < 0.01$ in comparison with control group by ANOVA post hoc Dunnett test.

Figure 35 Expression of MAP2-IR cells in subventricular zone after escalating and binge doses-METH administration in coronal section stained with polyclonal antibody against microtubule-associated protein 2 (1:750) at 40x magnification. Yellow arrows indicate MAP2-IR cells. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 10 µm

Expression of glial fibrillary acidic protein (GFAP)

The immunohistochemistry demonstrated GFAP immunoreactive (GFAP-IR) cells in an intermediate filament protein class III, a main component in astrocytes. GFAP-IR were strongly limited to astrocytes but there were less in neuronal cells and white matter.

Expression of GFAP-IR cells in subgranular zone after escalating and binge doses-METH administration

In the present study, the results showed that the GFAP-IR cells were significantly increased in ED-METH binge, ED-METH and AB-METH groups when compared with control group ($p = 0.006$, 0.027 and $p = 0.005$, respectively) (Figure 36 and Table 15).

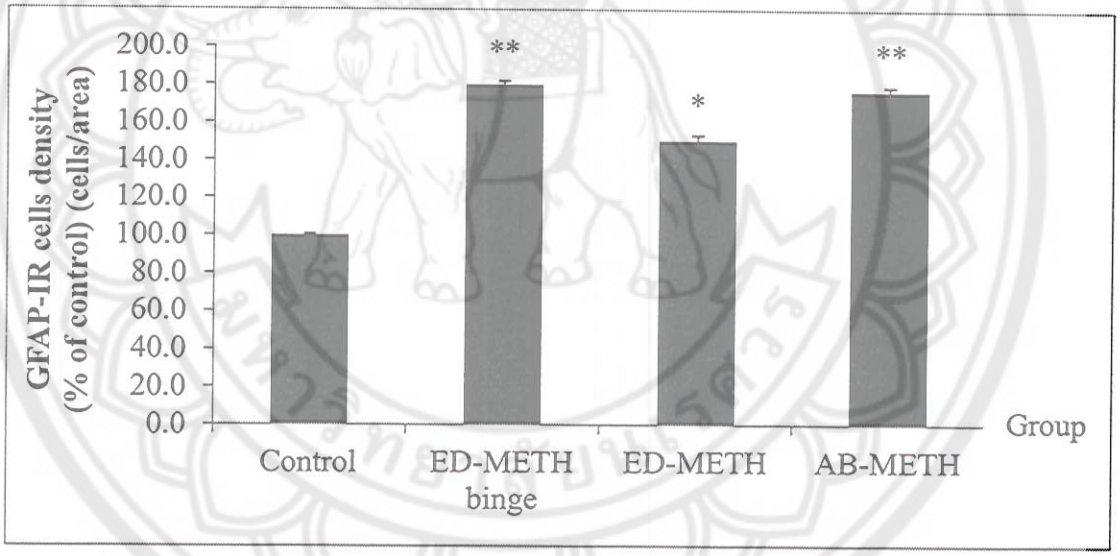

Figure 35 Expression of GFAP-IR cells in subgranular zone after escalating and binge doses-METH administration

Note: The GFAP-IR cells were observed in dentate gyrus under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

* $p < 0.05$ in comparison with control group by t-test.

** $p < 0.01$ in comparison with control group by ANOVA post hoc Dunnett test.

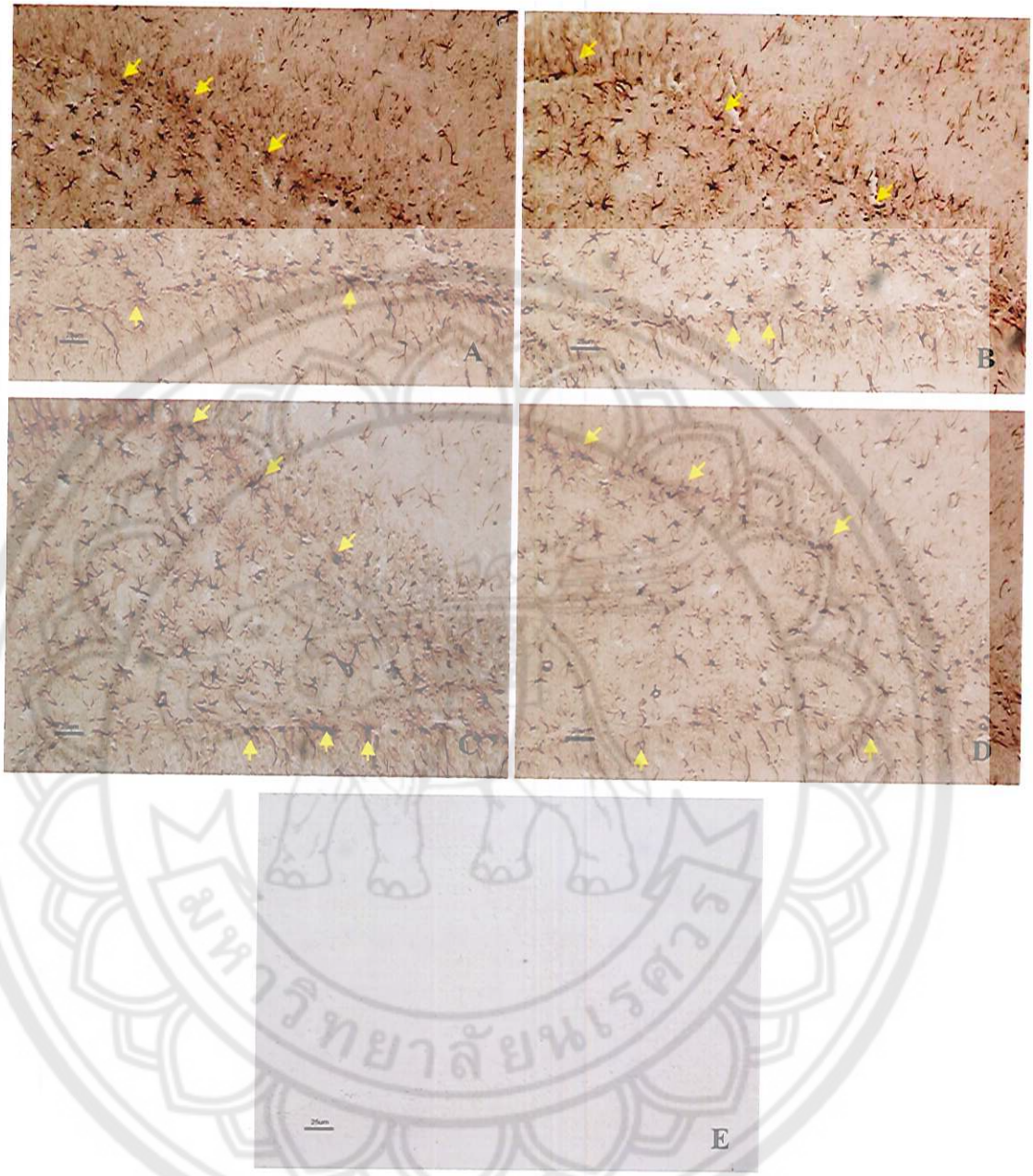

Figure 37 Expression of GFAP-IR cells in subgranular zone after escalating and binge doses-METH administration in coronal section stained with monoclonal antibody against glial fibrillary acidic protein (1:1000) at 20x magnification. Yellow arrows indicate GFAP-IR cells. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n = 8); (E) Negative control. Scale bar = 25 µm

Expression of GFAP-IR cells in subventricular zone after escalating and binge doses-METH administration

In the present study, the results showed that the GFAP-IR cells were significantly increased in both ED-METH binge and AB-METH groups when compared with control group ($p = 0.044$ and $p = 0.000$, respectively) but there was no significant difference in ED-METH group when compared with control group ($p = 0.444$) (Figure 38 and Table 16).

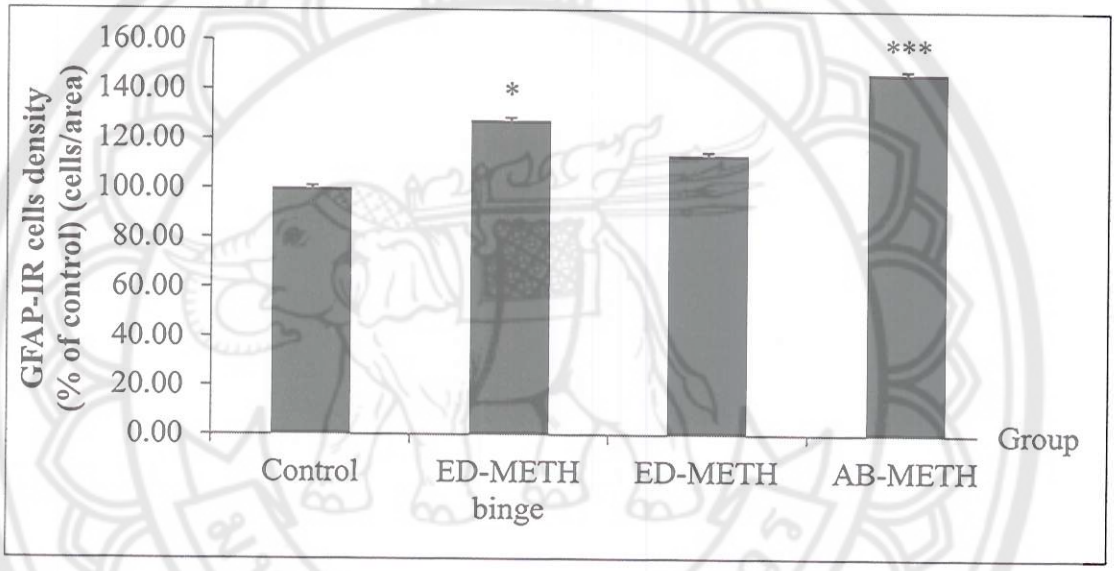

Figure 38 Expression of GFAP-IR cells in subventricular zone after escalating and binge doses-METH administration

Note: The GFAP-IR cells were observed in dentate gyrus under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

* $p < 0.05$ in comparison with control group by ANOVA post hoc Dunnett test.

*** $p < 0.01$ in comparison with control group by ANOVA post hoc Dunnett test.

Figure 39 Expression of GFAP-IR cells in subventricular zone after escalating and binge doses-METH administration in coronal section stained with monoclonal antibody against glial fibrillary acidic protein (1:1000) at 20x magnification. Black arrows indicate GFAP-IR cells. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 25 µm

Expression of myelin basic protein (MBP)

The optical density demonstrated MBP immunoreactivity (MBP-IR) in myelin sheaths that envelop around the axon of the neurons. MBP-IR was strongly limited in cingulate cortex and white matter which are enriching of the axon. MBP-IR was less in neuronal cells.

Expression of MBP-IR in cingulate cortex after escalating and binge doses-METH administration

In this study, MBP-IR was significantly decreased in cingulate cortex in ED-METH binge and AB-METH when compared with control group ($p = 0.039$ and $p = 0.032$, respectively) but there was no significant difference in ED-METH group when compared with control group ($p = 0.140$) (Figure 40 and Table 17).

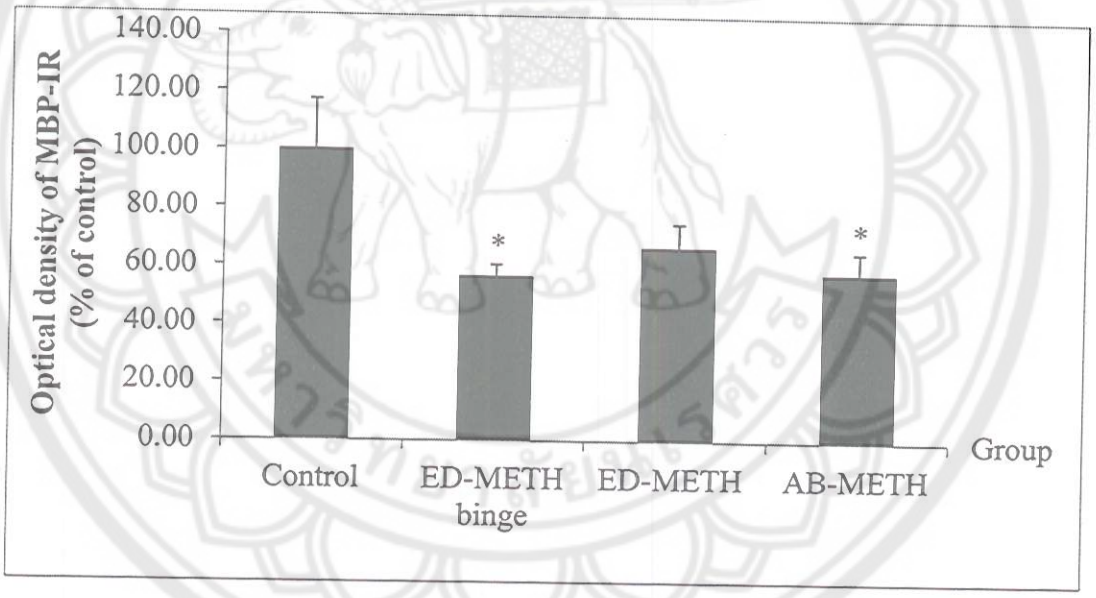

Figure 40 Density of MBP-IR in cingulate cortex after escalating and binge doses-METH administration

Note: The optical density of myelin sheath in cingulate cortex were investigated under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating

dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8). * $p < 0.05$ in comparison with control group by ANOVA post hoc Dunnett test.

Figure 41 Expression of MBP-IR in cingulate cortex after escalating and binge doses-METH administration in coronal section stained with monoclonal antibody against myelin basic protein (1:250) at 20x magnification. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 10 μm

Expression of MBP-IR in white matter after escalating and binge doses-METH administration

The results showed that there were no significant differences of MBP-IR were observed in white matter in all experimental groups (ED-METH binge group; p = 0.763, ED-METH group; p = 0.989 and AB-METH group; p = 0.407 (Figure 42 and Table 18).

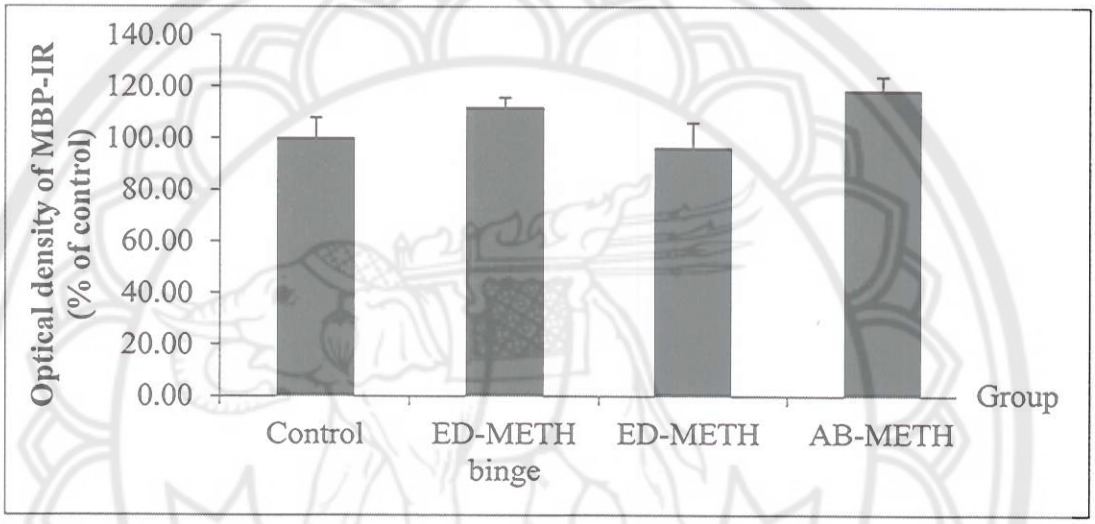

Figure 42 Density of MBP-IR in white matter after escalating and binge doses-METH administration

Note: The optical density of myelin sheath in white matter was investigated under a light microscope at 20x magnification. Data are presented as Mean ± S.E.M. ED-METH binge; escalating dose-methamphetamine binge group (n = 4), ED-METH; escalating dose-methamphetamine group (n = 4), AB-METH; acute dose-methamphetamine binge group (n = 5) and control group (n = 8).

Figure 43 Expression of MBP-IR in white matter after escalating and binge doses-METH administration in coronal section stained with monoclonal antibody against myelin basic protein (1:250) at 20x magnification. (A) ED-METH binge; escalating dose-methamphetamine binge group (n = 4); (B) ED-METH; escalating dose-methamphetamine group (n = 4); (C) AB-METH; acute binge METH (n = 5); (D) Control (n =8); (E) Negative control. Scale bar = 10 μm

Discussion

The present study demonstrated the alterations of neuronal stem cells, oligodendrocytes, astrocytes and mature neurons in the hippocampal neurogenesis and gliogenesis after escalating and binge doses methamphetamine administration in rats. The subgranular zone of hippocampus and subventricular zone were chosen for a number of reasons. Firstly, previous studies suggested that the subgranular zone of hippocampus and subventricular zone are areas to generate new neuronal stem cells (Mandyam, et al., 2007; Mandyam, et al., 2008; Lai, et al., 2010). Secondly, many studies have provided evidence that methamphetamine has neurotoxic effects (Riddle, et al., 2005) on neurodegeneration in hippocampus (Guilarte, et al., 2003) and induce neuronal cells death leading to diminish process of neurogenesis (Mandyam, et al., 2008; Schaefers, et al., 2009). In addition, there has been reported that the methamphetamine has effects on cognitive function impairments especially learning and memory (Costa, et al., 2008; Bortolato, et al., 2010). Therefore, this study was to investigate the effects of escalating and binge doses-METH administration on the alterations of neurogenesis and gliogenesis in subgranular and subventricular zones. Moreover, behavioral profiles and cognitive performance have also been observed.

Effects of METH on amount of food intake and weight loss

After treatment in each day, the average weight loss in ED-METH group were significantly decreased on day 3-5 and day 7-14 when compared with control group but no significant differences were found on day 1, 2 and day 6. The results showed that amount of food intake on day 0-15 were significantly decreased in ED-METH group when compared with control group. Moreover, on day 16 (after binge doses-METH administration), the results showed that amount of food intake were significantly decreased in both ED-METH binge and AB-METH binge groups when compared with control group but no significant differences was found in ED-METH group.

The present results showed effects of METH on amount of food intake and weight loss after escalating and binge doses-METH administration. The results of this study were in agreement with a previous report that showed a decrease of body weight after taking METH in anorexia (Ginawi, et al., 2005). Moreover, it has been reported

that METH has effects on hypothalamus especially arcuate hypothalamic nucleus which play an important role in controlling of appetite (Ginawi, et al., 2005). A previous study has reported that METH can change neuronal and hormonal signals in hypothalamus such as a reduction of leptin, an anorexigenic peptide synthesized in adipocyte cells, a decrease of insulin, and an increase of ghrelin (Crowley, et al., 2005). Moreover, the reduction of leptin causes a decrease of signals in anorectic neurons leading to a decrease in appetite (Goltz et al., 2010). In addition, lesions of lateral hypothalamic nucleus can cause a loss of appetite which leads to a decrease of body weight (Crowley, et al., 2005; Wynne and Bloom, 2006; Suzuki, et al., 2012). Therefore, the results of this study confirm an effect of METH on weight loss.

Effect of escalating and binge doses-METH on core body temperature

At 1 h after the last dose injection of day 15, the results showed that the core body temperature was significantly increased in AB-METH group when compared with control group. However, there were no significantly differences in ED-METH binge and ED-METH groups when compared with control group.

The present study showed effects of METH on core body temperature following escalating and binge dose-METH administration. The results of this study were in agreement with a previous report that an increased core body temperature was found after METH administration (Segal, et al., 2003). Several studies were found an increase of neurochemical dopamine which leads to hyperthermia following acute high dose METH administration (Segal, et al., 2003; Devidson, et al., 2005). An increase of core body temperature in AB-METH group may a result from a decrease of dopamine, dopamine transporter, and vesicular monoamine transporter 2 (VMAT2) following an acute high dose administration. An increase of core body temperature was also found in ED-METH binge and ED-METH groups. However, the core body temperature of ED-METH binge and ED-METH groups were not shown a high temperature as seen in AB-METH group. This is because the doses in ED-METH binge and ED-METH groups were increased gradually from low dose to high dose reflecting core body temperature. In addition, METH has been reported to induce neurotoxic effects on anterior hypothalamic nucleus which plays an important role in thermoregulation of the body. In normal stage, neurons in anterior hypothalamus send discharge impulses to respiratory and cardiovascular centers of

brainstem and spinal cord via descending hypothalamic efferent and induce vasodilation leading to rapid heat dissipation. The lesion of anterior hypothalamic nucleus causes hyperthermia as a result of damaged nerve signals that control dissipation of heat. Therefore, the results of this study indicate that an increase of core body temperature after acute high dose METH administration may affect from METH-induced hyperthermia.

Effects of escalating and binge dose-METH administration on behavioral profiles

Locomotor test was performed to investigate the effects of escalating and binge doses-METH to confirm the behavior of drug dependence. The result of this study suggests that drug dose 0.1-0.3 mg/kg METH can cause behavioral changes. The result on day 2-14 also suggests that METH induce hyperlocomotion and increase behavioral responses. This study was in agreement with the several reports that found METH-induced hyperactivity (Devidson, et al., 2005; Fujii, et al., 2007). A previous study has reported an elevation of extracellular dopamine concentrations in the caudate nucleus and nucleus accumbens leading to hyperlocomotion after METH administration (Fujii, et al., 2007). Moreover, previous studies have reported that an increase of behavioral responses was found after escalating and binge-dose METH administrations in rats (Segal, et al., 2003; Davidson, et al., 2005). However on day 15, AB-METH group was significantly decreased in locomotor activity while animals had shown oral stereotype and head movement. It may be because of an acute high dose of METH which induce stereotype rather than hyperlocomotion. Therefore, an increase of behavioral responses and hyperlocomotion of this study suggest neurotoxic effects of METH.

Effect of escalating and binge doses-METH on novel object recognition

Novel object recognition (NOR) test is most common used to demonstrate the neurobiological mechanism of learning and memory especially recognition memory. NOR test is a non-spatial memory which is related with hippocampus and prefrontal cortex. Both areas play an important role in learning and memory. In this study, NOR test was performed to investigate the effects of escalating and binge doses-METH administration on an impairment of cognitive performance of animal in both short-term and long-term memories after METH dependence.

In this study, we found a significant decrease of novelty index in all treatment groups in short-term test. In long-term test, novelty index was significantly decreased in ED-METH binge group. The results suggest that escalating and binge doses-METH induce learning and memory impairments.

The results of this study was in agreement with several studies reported METH can cause cognitive functions impairment especially learning and memory (Simões, et al., 2007; Lee, et al., 2011). The glutamatergic system plays a major role in cognitive functions especially learning and memory (Riedel, et al., 2003). Previous study reported METH can increase extracellular glutamate concentrations that activate glutamate receptor and cause overactivation and excitotoxicity leading to neuronal apoptosis and neuronal cell death (Cadet, et al., 2003). Therefore, the impairment of cognitive performance of this study may be a result of METH-induced excitotoxicity leading to a reduction of glutamate NMDA receptor and glutamatergic dysfunction.

Effect of escalating and binge doses-METH on hippocampal neurogenesis and gliogenesis

In this study, the proliferative nuclear antigen (PCNA) was studied as a marker of proliferative cell and microtubule associated protein 2 (MAP2) was also observed as a marker of mature neuron. The results of this study demonstrated a decrease of neuronal proliferative cell in subgranular and subventricular zones, and a decrease of mature neuron in both subgranular and subventricular zones after escalating and binge doses-METH administration. Several studies have reported a diminish process of generating new neuronal cells following METH administration (Mandyam, et al., 2008; Schaefers, et al., 2009). PCNA is a protein which, plays a role in a processivity factor for DNA polymerase δ in eukaryotic cells. From the result, it can be interpreted that escalating and binge doses-METH administration induce a disruption of cell cycle processespecially DNA replication. This is, because PCNA is normally expressed in nuclei during DNA synthesis. Moreover, a decrease of mature neuron may be a result of METH-induced a disminish of neuronal stem cell. The results were consistent with a previous report which found a loss of mature neurons following METH administration (Kuczenski, et al., 2007; Mandyam, et al., 2009).

In this study, we found a significant increase of GFAP immunoreactive (GFAP-IR) cells following METH administration especially acute high dose of

METH. A previous study has suggested METH-induced neurotoxicity that can cause an increase of astrocytes for maintaining brain homeostasis (Gold, et al., 2009). In AB-METH group, a number of GFAP-IR cell was found to be increased in a higher number than other experimental groups which it may be a result of a high dose of METH administration and elevation of astrocytes may be compensatory responses to injury (Gold et al., 2009). The results of this study were in agreement with the previous reports that an increase the expression of GFAP-IR cells were found after high dose of METH administration in rats (Guilarte, et al., 2003; Kuczenski, et al., 2007).

Our finding also demonstrated that, MBP immunoreactivity (MBP-IR) was significantly decreased in cingulate cortex in ED-METH binge and AB-METH groups but there was no significant difference in ED-METH group. A decrease of MBP expression provides evidence to support that METH-induced neurotoxicity which leads to myelinated fibers damage (Sharma, et al., 2009). Moreover, there has been reported that METH can induce myelination changes and degeneration of myelinated axons which cause a decrease of MBP and mean diameter of myelin sheath (Melo, et al., 2006; Sharma, et al., 2009). These results suggest that, a decrease of MBP-IR in cingulate cortex was shown effects of METH on diminish of myelin sheaths which cause a disruption of nerve signals to hippocampus, leading to an impairment of learning and memory.