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Full Length Research Paper

Effects of methamphetamine on the hippocampus of rats: Behavioural and morphological approach

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This investigation examined the effects of methamphetamine (METH) on performance of rats using the Morris water maze and on the morphology of the hippocampus. Twelve adult Wistar rats were used for this study. Animals were randomly assigned into two groups (A and B) of six each. Group A rats served as control and were given normal saline. Group B animals were given single administration of METH (10 mg/kg, i.p.). Animals were subjected to behavioural studies on the water maze prior to treatment. Three days following METH administration, animals were again subjected to behavioural studies, sacrificed, brain excised and processed histologically. Results showed high level of methamphetamine-induced stereotypic movement. There was no significant difference in behavioural performance of the rats on water maze prior to treatments, but group B rats showed significantly poor performance after treatment when compared to control ($P<0.05$). Histological analysis revealed extensive neuronal destruction of the pyramidal layer of the hippocampus following methamphetamine administration. In conclusion, this study has shown the neuro-destructive effects of METH on the hippocampus and such degenerating effects could affect the learning, memory and possibly spatial navigation ability of rats during the water maze task.

Key words: Methamphetamine, hippocampus, behavioural studies, Morris water maze.

INTRODUCTION

Methamphetamine (METH) is an amphetamine (AMPH) - like compound, whose abuse is a serious problem worldwide (Tatsua et al., 2007). There is extensive evidence that METH is neurotoxic in animal models, thus the concern for the increasing use of these drugs (Escubedo et al., 2005). AMPH and METH are potent central nervous system (CNS) stimulants with additional peripheral sympathomimetic effects. They have been used clinically in the treatment of obesity, minimal brain dysfunction, narcolepsy, depression and to counter fatigue. Following the acute administration of the AMPHs, there is an increase in blood pressure, heart rate, respiration, sweating and tremor. The behavioral manifestations include species specific stereotypic behavior, anorexia, sleeplessness, hyperexcitability, and hyperactivity (Kita et al., 2003). Though it is now established that METH induces neurotoxicity in animal models, the mechanism by which it does induce neurotoxicity is still quite complex. Increasing body of evidence has shown that METH-induced neurotoxicity is dependent upon the production of reactive species, irrespective of METH mode of entry into the neuron (Davidson et al., 2001; Escubedo et al., 2005). Three basic mechanisms have been identified by which METH administration could result in production of such species; (1) Dopamine (DA) release and subsequent enzymatic oxidation; (2) DA auto-oxidation and (3) mitochondrial disruption. Aberrant release of DA can also induce oxidative stress by excitatory amino acid (EAA) excitotoxicity through increased glutamate release. (Davidson et al., 2001; Pubill et al., 2002; Kita et al., 2003; Escubedo et al., 2005; Pubill et al., 2005; Tatsua et al., 2007). Much of the study on METH-induced neurotoxicity has been focused on the DA system and the striatum, but it has been shown that METH may cause

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neurodegeneration in other parts of the brain including the parietal cortex, thalamus and hippocampus (Davidson et al., 2001; Nwoha et al., 2007).

The Morris water maze is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory, which are major functions of the hippocampus (Jarrard et al., 1984; Kesner et al., 1987). The water maze was developed by Richard Morris (Morris, 1981; Morris et al., 1982) and place navigation in the water maze is now often used as a general assay of cognitive function (Brandeis et al., 1989), for example for testing the impact of various disturbances of the nervous system (e.g. animal models of stroke (Nunn et al., 1994), aging (Gallagher and Rapp, 1997), neurodegenerative disease (Hsiao et al., 1996), or the potential impact of novel therapeutic drugs (D'Hooge and De Deyn, 2001). Most behavioral studies following administration of METH have shown hyper-locomotor and stereotypic behavior following METH administration. This study was however designed to investigate the effects of METH administration on learning and memory behavior. Though earlier studies have shown that neonatal exposure of rats to METH have deleterious effects on learning and memory when the rats become adults thus indicating negative effects of METH on the developing brain (Vorhees et al., 2000; Broening et al., 2001).

MATERIALS AND METHOD

Animals

Twelve adult male and female Wistar strain albino rats weighing between 150 and 200 g were used for this research. The rats were randomly assigned into two groups of six rats each (Groups A and B). Animals were housed in clean plastic cages under natural light and dark cycles at room temperature. Animals in all groups were fed normal laboratory chow *ad libitum* and allowed free access to water. All animals were handled in accordance with the guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publication, 1985).

Animal treatment

Animals in group A which was administered normal saline only served as control animals. Animals in group B served as experimental animals and were administered single dose of METH (neurotoxin) dissolved in normal saline, at 10 mg/kg body weight, intraperitoneally. Animals were left for three days in their cages, following administration of neurotoxin to allow for effects of the drug on the hippocampus (Nwoha et al., 2007). METH was obtained from Sigma Chemicals, USA.

Morris water maze study

The Morris water maze was used to study behavioural differences in the animals. The Morris water maze is one of the most widely used tasks in behavioral neuroscience for studying the psychological processes and neural mechanisms of spatial learning and memory. The Morris water maze has advantages over conventional mazes such as the elimination of local cues, like scent traces, and the fact that there is no fixed escape formula. Animals, usually rats or mice, are placed in a large circular pool of water and required to escape from water onto a hidden platform whose location can normally be identified only using spatial memory.

The apparatus consists of a large circular pool (tank) about 6 ft in diameter and about 3 ft deep. The inside of the tank was painted white and the outside brown. It was filled up with tap water, measuring about 25°C. A platform of 24 cm high and 10 cm in diameter was placed at one quadrant of the pool. During training, it was exposed 1 inch above the water. This teaches the rat that there is a platform, and that it is the way to get out of the water. Later, after the animal is trained and ready for testing, the escape platform was 1 inch below the water, and the water was made opaque by adding milk. Training and testing was done across a single day. Behavioural study on the water maze was carried out before treatment with METH and three days after the rats were treated with METH, with a two weeks period between the pre-treatment study and post-treatment.

Each animal underwent three consecutive trials during training. During testing each animal underwent four sessions of three consecutive trials per session. All animals completed a session before another session is carried out. The latency (time taken to reach the platform) was recorded manually with a stopwatch. If the animal failed to climb onto the platform within 60 s, the trial was stopped and the animal was removed from the water and placed on the platform. The inter-trial interval was 10 s, the animal remaining on the platform during this period before beginning the new trial. At the end of the three trials, the animal was removed and placed under a lamp for warmth. The inter-session interval was 1 h. One probe trial was performed after testing on the same day, in which the platform is removed from the pool. The probe trial was performed to verify that the animal understands the location of the platform and the strategy that the animal follows when it discovers the platform is not there (Pierre and Debouzie, 2000). The number of times the animal crossed the position of the removed platform was counted as probe trial value.

Histological analysis

After undergoing the water maze testing on the third day following neurotoxin administration, animals were weighed. The animals were then sacrificed by cervical dislocation. The skulls were opened so that the whole brain can be excised. They were fixed in 10% formal calcium (pH 7.1), for twenty-four hours for histological procedures. Brains were routinely processed for paraffin wax embedding. Then, 6 µm thick paraffin cross sections containing hippocampal tissue were mounted on slides and stained using routine haematoxylin and eosin (Drury and Wallington, 1980) method. Microscopy was conducted on an Olympus microscope (Tokyo, Japan) and images were captured and processed by an attached eyepiece camera.

Statistical analysis

One-way ANOVA was used to analyze data obtained from behavioural studies. Primer for windows (McGraw-Hill, version 4.0.0.0) was the statistical package used to analyse data. Results are expressed as mean ± standard error of mean. $P<0.05$ was taken as accepted level of significant difference.

RESULTS

Physical observations

METH-induced stereotypic movements were observed in

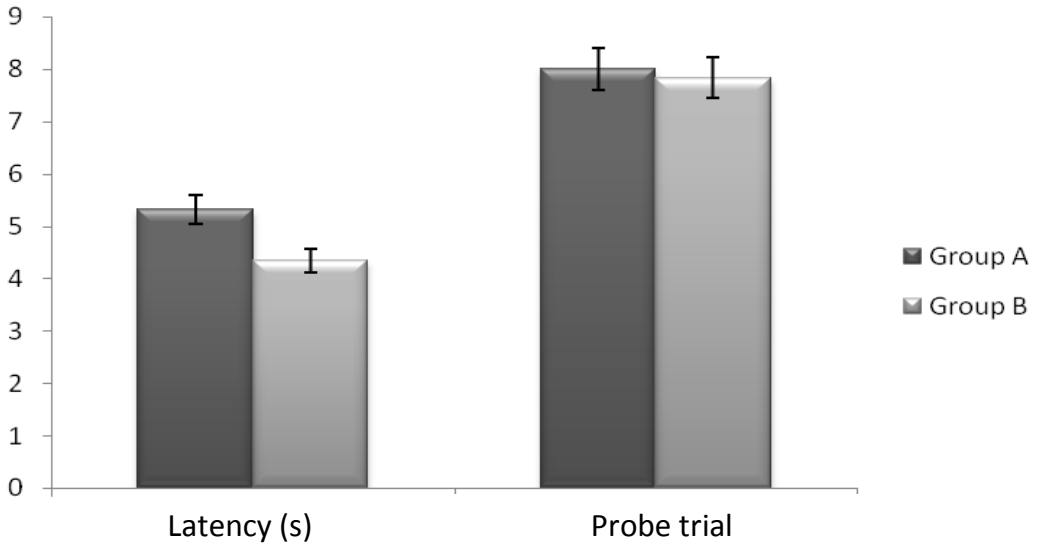

Figure 1. Pre-treatment behaviour of rats on the water maze. Each bar represents Mean ±SEM.
* P<0.05 compared to control. n = 6.

all six animals treated with METH in Group B. The stereotypic movement observed included circling, nail and/ or wood-chip biting, sniffing, repetitive rearing, vigorous and compulsive grooming. The onset of this symptom was observed about 20 min following injection with METH in Group B rats. The symptom lasted for just 3 to 4 h in the rats except for two rats in Group B, where even on the third day following injection, circling and continuous sniffing were still observed for a few minutes. 24 h after METH administration, the rats in Group B showed signs of sluggishness and excessive sleeping. There was a reduction in these symptoms 48 h after METH administration. None of these symptoms were observed in Group A rats, as they received no METH.

Pre-treatment behavioural studies

As shown in Figure 1, there was no significant difference between control and treated groups, in the duration it took animals to locate the platform (latency) and the probe trial.

Post-treatment behavioural studies

Following treatment with METH (Figure 2), animals in Group B had significantly longer duration to locate the platform (latency) when compared to control group. Group B animals also had a significantly lower probe trial values when compared to control group, as the control animals crossed the position of the platform significantly more times than Group B animals in the probe trial.

H&E staining

The normal histology of the hippocampus for the control group showed three distinct layers as shown in Figure 3. They are stratum oriens, stratum pyramidale and stratum radiatum. In the control group (Figure 3a1 to a4), the cells were intact with well stained nuclei. The regions of the principal cell layer of the hippocampus proper (pyramidal layer) were also intact through CA1 to CA4 and well demonstrated in this group. The dentate gyrus (DG) was intact and well stained. Its principal cell layer, the granular layer was well demonstrated. As shown in Figure 3b1 to b4, the layers of the hippocampus were shown to have histological alteration in Group B (METH treated rats). The pyramidal layer of the hippocampus was observed with extensive vacuolations of the cells as compared to the control group, thus indicating neuronal cell death. Figures 3b1 to b4 shows that neuronal cell death was more in the CA1 and CA2 region than CA3. The cells of the CA4 region, the hilus of DG and granular cell layer of DG, were least affected. The stratum radiatum of treated group also shows extensive degeneration of neuronal fibres in the CA1 and CA2 regions (Figure 3b1 to b2) when compared with the control (Figure 3a1).

DISCUSSION

METH administration at low to high doses has been known to induce stereotypic movements in laboratory rats and mice (Kita et al., 1998; Canales and Graybiel, 2000; Escubedo et al., 2005; Tatsuta et al., 2007; Kristen et al., 2010). In the present study, METH-induced stereotypy was

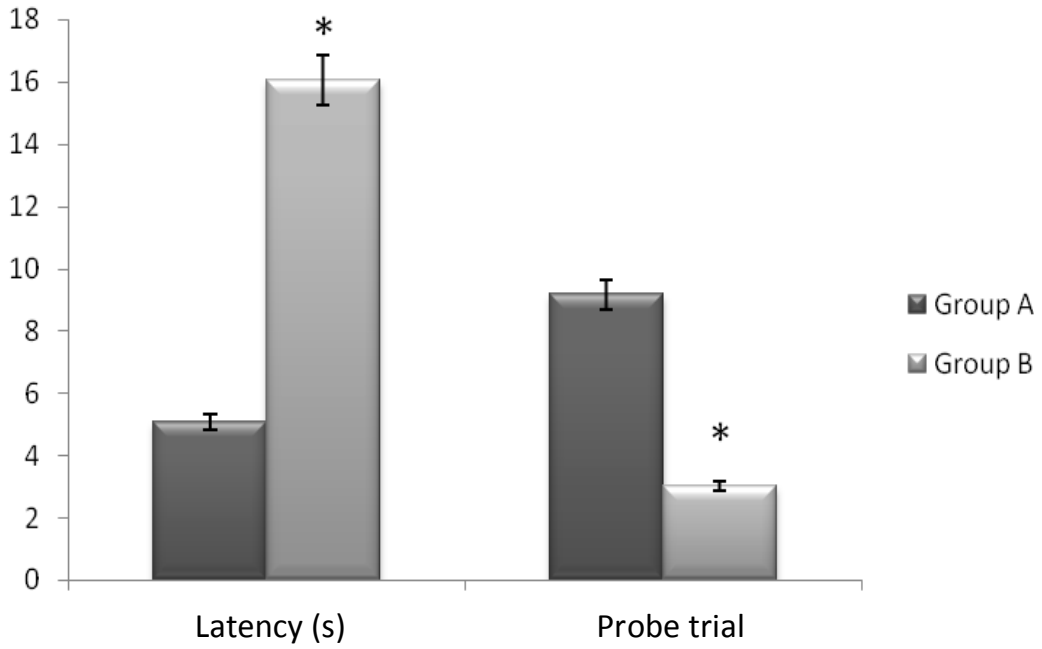

Figure 2. Post-treatment behaviour of rats on the water maze. Each bar represents Mean ±SEM. * P<0.05 compared to control. n = 6.

was observed in all animals following administration.

Stereotypy is defined as the development of abnormally repetitive motor actions that coincides with an inability to initiate normal adaptive responses (Canales and Graybiel, 2000; Kristen et al., 2010). Stereotypy is a cardinal feature of a broad range of neurologic and neuropsychiatric disorders and is a major component of the behavioral syndrome induced by psychomotor stimulant drugs (Canales and Graybiel, 2000) of which METH is one of such drugs. Motor stereotypies can be induced by dopaminergic stimulation of the striatum and can be abolished by intrastriatal blockade of dopaminergic transmission. Clinical evidence also implicates basal ganglia dysfunction in complex behavioral and cognitive stereotypies (Canales and Graybiel, 2000). Excessive sleeping is known to characterize METH withdrawal (McGregor et al., 2005) and this could possibly be the reason for this observed symptom a day following the single dose administration of METH.

Behavioural studies using the water maze showed that METH significantly affected the performance of Wistar rats on the maze. Animals administered with METH alone showed significantly longer latency and reduced number of times they located the platform during the probe trial after administration. This indicates poorer learning and memory capacity. This supports earlier evidence that showed METH negatively affects learning and memory as seen after neonatal exposure of rats to METH (Vorhees et al., 2000; Broening et al., 2001). The water maze is used to study the psychological processes and neural mechanisms of spatial learning and memory. The present behavioural studies have shown that METH negatively affects learning and memory and this indicates an injury to the hippocampus which is involved in spatial/relational memory (Jarrard et al., 1984; Kesner et al., 1987). The hippocampus of rats is now known to have some pyramidal neurons that help the animals navigate better through their environment as the firing of these cells indicates a specific location in the environment of the rats. These cells are called “place cells” and they probably help in the place navigation ability of rats which can now be studied in the water maze (Brandeis et al., 1989; Nakazawa et al., 2004). Extensive destruction of hippocampal pyramidal cells by METH would have affected the ability of exposed rats to correctly navigate in the maze and this could also have contributed to their poor performance on the maze.

Histological results of this study clearly support earlier reports that METH extensively destroys hippocampal neurons (Nwoha et al., 2007) and that METH induces neurotoxicity in the brain. High doses of METH disrupt blood brain barrier in the limbic regions producing extensive neuronal degeneration in the hippocampus, leading to learning and memory loss (Nwoha et al., 2007). Also METH, has been reported to induce neurotoxicity by generating reactive oxygen species (Kita et al., 2003; Escubedo et al.,2005) and these free radicals cause oxidative stress that result in brain neuronal damage (Anderson, 2004). Histological findings in this study showed higher destruction of cells in the CA1 and CA2 regions than the CA3 region of the pyramidal cell layer. The cells in the CA4 region, the hilus of the DG and

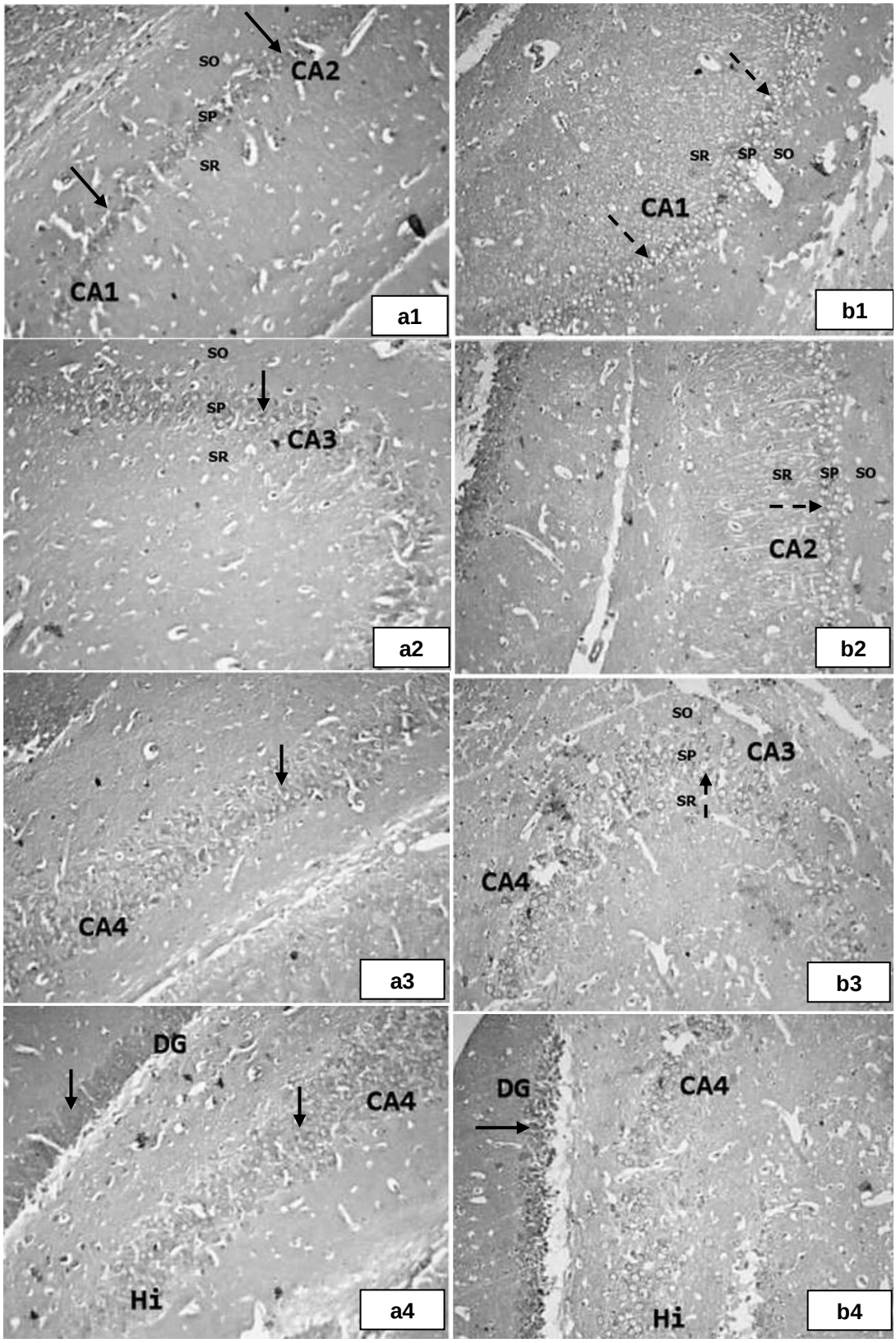

Figure 3. Light micrographs of different layers and parts hippocampus of control (a) and treated (b) rats. H&E ×100. CA, Cornu ammonis; DG, dentate gyrus; Hi, hilus of DG; SO, stratum oriens; SP, stratum pyramidale (Pyramidal layer); SR, stratum radiatum (Straight arrows- intact neurons; dashed arrows-vacuolations).

the DG, were least affected by the effect of METH.

In conclusion, this study has shown the neuro-destructive effects of METH on the hippocampus and that such degenerating effects could affect some behaviour such as learning, memory and possibly the spatial navigation ability of rats during the water maze task.

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