Preliminary Study on Delta-9-Tetrahydrocannabinol (Δ^9-THC) and Ethanol: Hemispheric Lateralization with Behavioural Changes

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Abstract

This study is designed inspired by the fact that there is an interhemisphere asymmetry of the brain region. A lot of researches studied in demonstrating the differences between right and left hemispheres of the brain. The objective of this preliminary study is to observe scientifically the effect of delta-9-tetrahydrocannabinol (Δ^9-THC) on the hemispheric lateralization with behavioural changes. Two regions of brain are selected, prefrontal cortex and hippocampus. Behavioural tests, namely heat stress test and novel-object discrimination test (NOD), were done on day seven. The hippocampus and prefrontal cortex regions of the brain were preceded to Western Blot technique in detecting c-fos. As for behavioural tests, heat stress and NOD and *c-fos* on hippocampus did not show significant differences. Meanwhile, the prefrontal cortex shows significant difference with $p < 0.01$. With these findings, reasonable dosages of Δ^9-THC should be used to have statistically significant differences effects on behavioural tests.

1. Introduction.

Delta-9-tetrahydrocannabinol (Δ^9-THC), also known as tetrahydrocannabinol, is the main psychoactive substance which can be found in the cannabis plant. The pharmacological actions of THC are suggested involving binding to the cannabinoid receptor (CB_1) which is located in the central nervous system. In facts, Δ^9-THC has been proven to show impairment effects on variety of central effects including hypothermia, antinociception and changes of locomotor activity (Pertwee R.G. and Wickens A.P. 1997; Felder C.C., Glass M., 1998; Ameri A., 1999), immediate recall (Darley C.F. et al., 1974) , memory retrieval (Block R.I. and Ghoneim M.M., 1993), and also in working and short-term memory (Fletcher J.M. et al., 1996). For determining the behavioural changes of animal model induced by Δ^9-THC, there are many choices of tests that scientifically represent the interpretation of animal behaviour. Heat stress or hotplate test is used in order to measure acute pain induced by Δ^9-THC in term of nociception property (Anthony W. et al., 2007). To assess the ability of animal in recognizing the novel object, NOD is used (Ennaceur A. and Delacour J., 1998). This test is used widely in testing the effects of amnesic drugs observed on exploratory activity (Hammonds R. et al., 2004).

Two regions of brain focused are hippocampus and prefrontal cortex. Hippocampus is a limbic structure that plays critical roles especially in memory formation (Eichenbaum H., 2004). Meanwhile, prefrontal cortex has been acknowledged in long-term memory (LTM) of human, both for episodic and semantic memory (Cabeza R. and Nyberg L., 2000). These facts were related to the working memory task operations and cognitive control processes (Wagner A.D., 1999; Wagner A.D., 2001).

Understanding of left and right side differentiation of the brain region, or known as hemispheric lateralization, has been widely expanded in these two decades. In the 1970s, researchers have come across that hemispheric specialization of function is a gift for the human trait. It is included the language and the abilities of cognitive. After that, studies have shown that many vertebrate species have similar brain specialization (Bradshaw J.L. and Rogers L.J., 1993). Similarity of hemispheric lateralization across other vertebrate species had been published by Rogers and Andrew (2002), while information on invertebrate had been reported by Rogers and Vallortigara (2008).

Similarly, in non-human, especially in a vertebrate, there are numerous researches focused on specialization of hemispheric lateralization compared to human. The left hemisphere of the brain is reported to be related to categorization of objects, as observed on patched left eyes of the chick, and responsible in performing strategies for behaviour (Rogers L.J., 2008). A research done by Kilian (2005) has postulated that the left hemisphere of the brain is responsible for routine behaviours. In comparing to the left hemisphere, the right hemisphere of the brain has been reported to involve in novel respond and stimuli (Lippolis G. et al., 2002; Larose C. et al., 2006) and controlling the social behaviour of animals (Orsola R.S. et al., 2009). A research done by Hauser (1993) has postulated that the right hemisphere of the brain did involve in possession of intense or negative emotion of expression. The expression did relate to asymmetries of facial impression.

Molecularly, to investigate the differences between the left and right regions of brain, *c-fos* expression is used. *C-fos* in the central nervous system (CNS) is an important marker to determine the neuronal response to a painful experience (Coggeshall R.E., 2005) and is used as a neuropathic pain model. Furthermore, *c-fos* expression in spinal horn and supraspinal structures of animal models has been used to evaluate the analgesic effect following the administration of antidepressant compound.

This study is conducted in order to observe the influence of Δ^9-THC on the interhemispheric asymmetry of prefrontal cortex and hippocampus observed by changes in c-fos expression together with the alteration of behavioural performances observed through heat stress test and NOD tests.

2. Materials and methods.

2.1. Animals.

Briefly, 7 Sprague Dawley rats aged between 8 to 9 weeks with average weight 250 – 300 g was purchased from the animal house of Faculty of Medicine and Health Sciences, University Putra Malaysia. The rats were given 2 mg/kg of Δ^9-THC for seven days continuously, intraperitoneally. For negative control, 0.9% normal saline with 2% ethanol was given intraperitoneally.

2.2. Behavioural tests.

At day seven, all the rats underwent two behavioural tests, heat stress and NOD tests. After treatment, rats were acclimatized in the behavioural room for one hour prior to testing. The paws of rats were given heat at 42 ^{0}C ± 0.1 for 20 minutes. Then, the rats are placed in the Perspex box for 30 minutes where locomotor activities are counted.

For NOD test, the rats are exposed to two familiar objects at the first three minutes, E1 and the time spent on each object, A1 and A2, are counted. The rats are given second three minutes exposure, E2, with one familiar object and one novel object. The time spent on each object, A3 and B1 respectively, are counted. Based on the record of time, D1, and D2, are calculated;

Exposure 1, E1 = A1 + A2
Exposure 2, E2 = A3 + B1
Discrimination ratio, D1 = B1 – A3
Discrimination index, D2 = D1 / (B1 + A3)

2.3. Western Blot.

Hippocampus and prefrontal cortex of rats were collected at day eight by decapitation. Both parts of the brain undergo homogenization with sucrose lysis, protease inhibitor and phosphatase inhibitor, before it is centrifuged at 13 000 rpm for 10 minutes at 4 ^{0}C. Cytosolic fraction obtained, undergo protein determination using Bradford reagent and been aliquot at 1 mg/ml using sucrose lysis.

10% of resolving gel with 4% of stacking gel is prepared. Sample added with Laemmli buffer is loaded in the well and the electrophoresis is run for one and a half hour before proceeding to transfer process. The membranes used undergo blocking stage using skim milk for two hours. Next the membrane is incubated with *c-fos* (1:500, Abcam) for overnight before incubating with horse-radish peroxidase antibody as secondary antibody (1:5000, Abcam) for two hours. The membrane is then viewed using chemiluminesence under gel documentation. The image obtained is measured using Image J before proceeded to the analysis process.

2.4. Analysis.

Readings of behavioural tests are analyzed using SPSS 16.0 and Tukey comparison test. The measure obtained from Image J was corrected by measurement of β-actin of each sample. The corrected measure was then analyzed using SPSS 16.0 and Tukey comparison test.

3. Result.

3.1. Behavioural tests

In the heat stress test, the nociception stimulus is measured through the number of crossings in locomotor activity observed for 20 minutes. The means of treatments (2.0 mg/kg Δ^9-THC and negative control) are stated in **Table 1**. The table showed no significant difference between Δ^9-THC and control with $p > 0.05$.

Table 1. Mean of crossing with standard error of mean (S.E.M) in locomotor activity after giving heat stress.

Treatment	Mean of crossing ± S.E.M
2.0 mg/kg Δ^9-THC	140.333 ± 52.000
Control	100.000 ± 31.000

[1]

In NOD, E1 (A1 + A2) and E2 (A3 + B1) are calculated (**Table 2**) to notify the total time spent on both exposures. The table showed no significant difference between the two exposures of both control and Δ^9-THC with $p > 0.05$. This finding did emphasizes the explore behaviours of the rats were same on both familiar and novel objects.

Table 2. A means total time spent, second, on both novel-object in E1 and E2 with standard error of mean (S.E.M).

[1] Both 2.0 mg/kg Δ^9-THC and control did not showed significant different with $p > 0.05$.

Treatment	Mean of E1 ± S.E.M	Mean of E2 ± S.E.M
2.0 mg/kg Δ^9-THC	12.300 ± 0.229	9.703 ± 3.284
Control	10.515 ± 3.505	9.345 ± 4.135

[2]

In interpreting NOD, both discrimination ratios, D1 (**Figure 1**), and discrimination index, D2 (**Figure 2**), were calculated before proceeding with the analysis. Both D1 and D2 showed no significant different between the Δ^9-THC and control at $p > 0.05$.

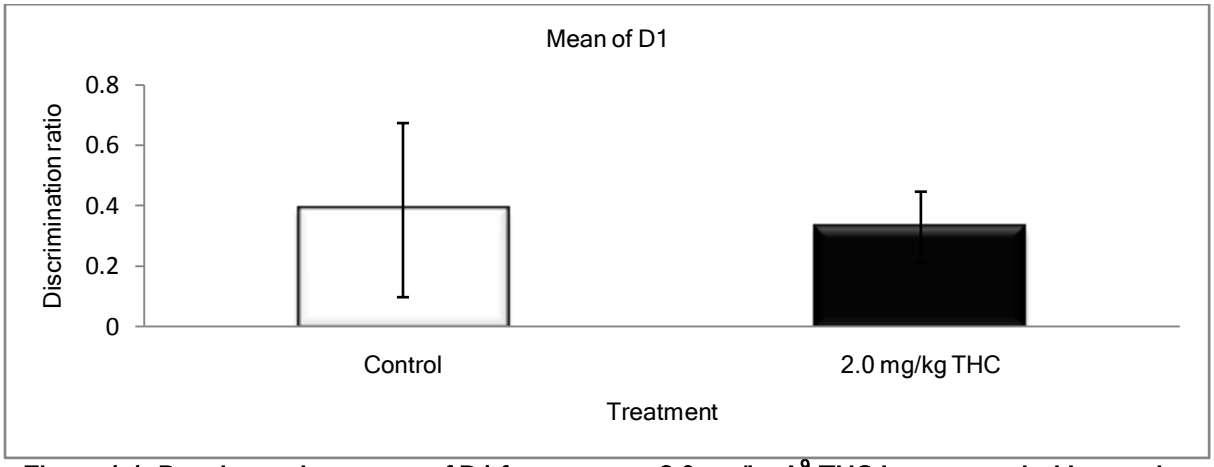

Figure 1.1: Bar charts show mean of D1 for treatment 2.0 mg/kg Δ^9-THC in compared with negative control. Figure shows no significant difference at $p > 0.05$.

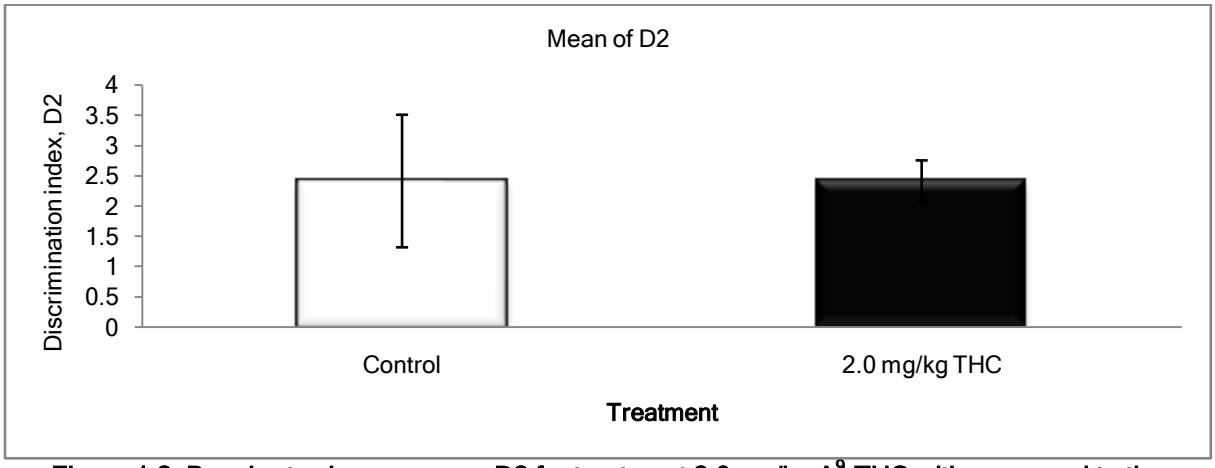

Figure 1.2: Bar charts show mean on D2 for treatment 2.0 mg/kg Δ^9-THC with compared to the negative control. There is no significant difference between those two treatments, with $p > 0.05$.

3.2. C-fos.

In analyzing *c-fos* through Western Blot technique, the values of protein are corrected with values of β-actin antibody. To fulfill the objective of this study, left and right hemispheres of prefrontal cortex and hippocampus will be evaluated. **Table 3** below shows the means of left and right hemispheres of prefrontal cortex and hippocampus compared with the control and 2.0 mg/kg Δ^9-THC.

[2] Both 2.0 mg/kg Δ^9-THC and control did not showed significant different with $p > 0.05$.

Figure 3 did show the comparison between control and 2.0 mg/kg Δ^9-THC for prefrontal cortex, while **Figure 4** was simplified the hippocampus.

Table 3. Table showed mean density of *c-fos*.

Brain of region	Treatment	Hemisphere of brain	
		Left	Right
Prefrontal cortex	Control	100.00 ± 0.000	100.00 ± 0.000
	2.0 mg/kg Δ^9-THC	95.992 ± 2.868	133.018 ± 4.906 ***
Hippocampus	Control	100.00 ± 0.000	100.00 ± 0.000
	2.0 mg/kg Δ^9-THC	88.158 ± 0.824 *	45.000 ± 2.589 ***

[3]

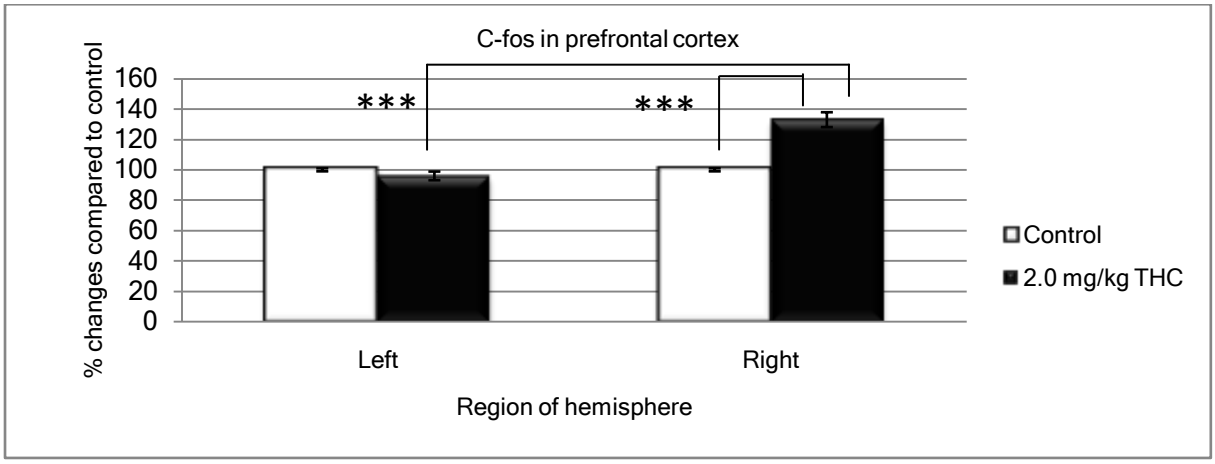

Figure 3. Bar charts above show percentage changes of *c-fos* in prefrontal cortex compared to control. Left region of the prefrontal cortex for both control and 2.0 mg/kg Δ^9-THC does not show significant difference where $p > 0.05$. Compared to right region of the prefrontal cortex, there is a significant difference between control and 2.0 mg/kg Δ^9-THC with $p < 0.001$ (***). Prefrontal of Δ^9-THC treated showed significant observable different as comparing the left and right hemispheres at p < 0.001 (***).

[3] Both right and left hemispheres of prefrontal cortex and hippocampus for treatment of 2.0 mg/kg Δ^9-THC showed significant differences with $p < 0.001$ compared to when comparing with negative control.

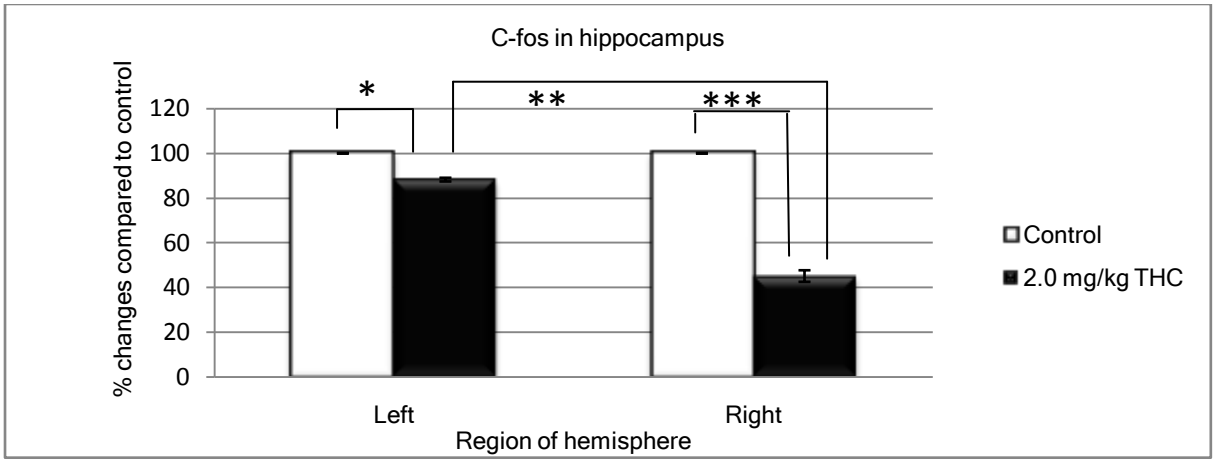

Figure 4. Bar chart above shows the percentage of change in *c-fos* through Western Blot for hippocampus region. Left hemisphere of control and 2.0 mg/kg Δ^9-THC does have significant difference with $p < 0.05$, while in right hemisphere of 2.0 mg/kg Δ^9-THC, there is significant difference, where $p < 0.001$. Hippocampus of Δ^9-THC treated showed an observable effect between the left and right hemispheres that significant at $p < 0.01$ (**).

4. Discussion.

Through behavioral test, heat stress, there is no significant difference between treatments given, normal saline with 2 % ethanol as negative control and 2.0 mg/kg Δ^9-THC. There is increasing number of crossing observed in locomotor activity in heat stress test indicated the nociception effects of the Δ^9-THC although statistically not proven. The large scale of S.E.M. was suggested due to the huge range of body weight of animals used. An increasing number of locomotor activity of Δ^9-THC is incomparable with nociceptin and comparable with morphine (Sebastien F. et al., 1996). In the early 40s, Δ^9THC has been reported to have analgesic and nociception effects observed in human (Walton R.P., 1938). Numerous researches have been done in proving the effects of tetrahydrocannabinol in animals which lead to varying postulation depending on the species of animal model used (Buxbaum D.M., 1972).

The dosage used, 2.0 mg/kg of Δ^9-THC did not seem to be recommended to give significant effects on nociception properties. Further investigation should be planned for reasonable dosages in order to have valuable effects on animals and humans. In NOD, time spent on novel and familiar objects in E1 and E2, show no significant differences. It proves that animal model used have similar reactivity toward both objects upon the time allotted. D1 was used to measure the time spent on the novel to familiar objects. The result shows no significant differences on D1. D2 was used in order to measure the difference between novel and familiar objects over total time exposure of both objects. There is no significant difference in E2 and D1, and also in D2. On the bar chart of D2, there is slightly increased in time spent to differentiate novel and familiar objects. It leads to postulation that treatment of 2.0 mg/kg Δ^9-THC did increase the memory property since less time is spent on re-exposed object compared to the new object. Data analyzed for D1 and D2 proved that treatments of 2.0 mg/kg Δ^9-THC does not cause memory impairment nor improvement. The physiological evidence of working memory in animals has typically come from studies in which animals are given a brief cue to hold in memory during a delay period of a few seconds and then required to make some choice (Meltzer H.Y., 1991).

Again, the dosage used in this study, 2.0 mg/kg Δ^9-THC does not seem to be high enough to produce significant differences in memory and cognitive performances. For further exploration, a higher dosage of Δ^9-THC is recommended in order to have clear cut of the dosage and effects.

For this study, *c-fos* had been used in order to determine the hemispheric lateralization of prefrontal cortex and hippocampus. *C-fos* is widely used as a marker of neuronal activation (daCosta A. et al., 1997). Through Western Blot in measuring the amount of *c-fos* in prefrontal cortex, there are significant differences between the control and 2.0 mg/kg Δ^9-THC. Even more, both right and left regions showed significant differences regardless of treatments. In hippocampus, there are significant differences between the control and 2.0 mg/kg Δ^9-THC, with no significant differences between right and left hemispheres of the region. Activation on the right hemisphere of the brain has a dominant role in acute and chronic stress, and also modulated in the processing of pain (Carrasquillo Y. and Gereau R.W., 2008). This can be seen in bar chart presenting the differences between left and right hemispheres of prefrontal cortex where right region shows higher measurement of protein compared to left. The left hemisphere of brains seems to concentrate on controlling the positive cognitive bias (Rogers L.J., 2010). Higher measurement of *c-fos* in left hemisphere observed in the hippocampus which is significantly different in comparison with the right side. Thus, it can be postulated to the slight increase in D2 measured in NOD.

5. Conclusion.

In conclusion, from this study, the dosage of 2.0 mg/kg of Δ^9-THC is able to give clear cut on measuring the difference between the right and left hemispheres of prefrontal cortex and hippocampus. Both hemispheres give different values in protein of interest which leads to postulation that different hemisphere of the brain is responsible for different tasks and controlling different systems in animal and human body. In further, both hemispheres of the brain should be recommended in order to give an overall measurement of proteins of interest.

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