CHAPTER IV

RESULTS

In this chapter, the results were separated into 2 parts as follows:

1. The role of the 5-HT_{2A} receptor in the chronic pain model and the development of the chronic pain state
   - 1.1 The effect of CFA-induced inflammation on rats' behaviors and Fos protein expression
   - 1.2 The role of the 5-HT_{2A} receptor in the chronic pain model by using behavioral assessment and Fos protein expression
     - 1.2.1 Effect of ketanserin on rats' behavior and Fos protein expression of the control group
     - 1.2.2 Effect of ketanserin on rats' behavior and Fos protein expression in CFA-induced peripheral inflammation
2. The role of the 5-HT_{2A} receptor in the 5-HT depleted state on the changes of pain sensation
   - 2.1 The effect PCPA-induced 5-HT depletion on rats' behaviors and Fos protein expression
   - 2.2 The role of the 5-HT_{2A} receptor in the 5-HT depleted state by using behavioral assessment and Fos protein expression

1. The role of the 5-HT_{2A} receptor in the chronic pain model and the development of chronic pain state

1.1 The effect of CFA-induced inflammation on rats' behaviors and Fos protein expression

The rats expressed their nociceptive behavior immediately after CFA was injected subcutaneously in their right hind paws. They significantly expressed pain-like behavior i.e. favoring, lifting, licking and flinching their injured paws compared with the control (575.7±273.0, 0.0±0.0 seconds in a thirty-minute period respectively, $p<0.05$) and decreased their non-nociceptive behavior such as exploring, grooming, and scratching their faces compared with the control (626.3±273.1, 1307.4±359.2 seconds in a thirty-minute period respectively, $p<0.05$). In the experiments, animals also expressed other kinds of behavior such as being still but alert which was not different between control group and CFA group (325.4±134.7, 260.00±175.8 seconds in a thirty-minute period respectively, $p<0.05$). In addition, they rested and slept during this period which also is not different between both groups (188.0±278.4, 246.8±153.1 seconds in a thirty-minute period respectively, $p<0.05$). The data were summarized in table [4-1] to [4-4].

One day after the CFA injection, the time that rats expressed their nociceptive behavior was not different compared to day 0 (685.3±166.1 seconds). At Day 3 after CFA injection, nociceptive behavior was decreased significantly and was still reduced in Day 5 and Day 7 compared with Day 0 (136.3±81.6, 23.3±30.2, 18.5±37.0 seconds respectively, $p<0.05$).

Compared with the CFA group, the control-group rats did not express their nociceptive behavior during the experiment at all (Table [4-1], Figure [4-1]).

Non-nociceptive behavior in the CFA group decreased in D1 then increased in the Day 3 and the Day 5 (391.5±166.1, 1104.5±429.5, 1261.3±436.7 seconds, respectively) and decreased again in Day 7 (813.3±223.3 seconds). However, trend of the non-nociceptive behavior was increased (Figure [4-2]).

The still but alert behaviors were not found the difference among time series of each group. It was found the different between the CFA group and the control in the Day 7 groups (291.0±149.8, 71.8±82.5 seconds, respectively, $p<0.05$) Table [4-3], Figure [4-3].

The similar results were found in rest or sleep category. It was not found the difference among time series of each group. However, the differences between the CFA group and the control were found in Day 1 and Day 7 Table [4-4], Figure [4-3].

In paw withdrawal test, it was found that there was different latency in non-inflamed paw among time series (Table [4-5], [4-6], Figure [4-5], [4-6]). On the other hand, the latency of the inflamed paw in the CFA group was significantly reduced in Day 0 compared with their non-inflamed paw (4.5±0.7, 9.9±1.7 seconds, respectively, $p<0.05$).

The results showed long lasting inflammation in the follow days (Figure [4-6]). At day 1, the data showed that the paw withdrawal latency in the injured paw was slightly longer but did not significant compare with the Day 0 (6.3±1.8, seconds, $p<0.05$). In the Day 3 and the Day 5, the data showed similar result to Day 1 (5.3±1.3, 5.9±1.5 seconds, respectively, $p<0.05$). The paw

withdrawal latency was increased to a non-significant level in D7 compared with their own uninjured paw and to the control group in day 7 (8.9±1.8, 9.3±0.7 seconds, respectively, $p<0.05$).

Fos protein expression was used to determine neural activity, also in pain processing. In this research, Fos immunoreactive (Fos-IR) neurons were distributed diffusely and evenly in both hemispheres. No difference in the number of Fos-IR neurons was observed comparing medial (corresponded to hind limb area) and lateral cortical areas.

In control-group, Fos-IR neurons in both hemispheres were not different among their time series (Table [4-7], [4-8], Figure [4-7], [4-8], [4-9], [4-10]). It was found that the number of Fos-IR in CFA-induced inflammation were increased significantly at 3 days after CFA was introduced (Day 0 group) not only in contralateral but also ipsilateral sides (22±6, 24±12 positive cells per 100x100 μm^2, respectively) compared with their own control (6±2, 4±2 positive cells per 100x100 μm^2, respectively, $p<0.05$). It was also found that at Day 3 group, Fos-IR neurons were increased at the highest level in both hemispheres compared with their own time series. After that they were decreased in Day 5 and 7.

Table [4-1] The nociceptive behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group

Nociceptive Behaviors (sec)				
Group	Control	CFA-induced peripheral inflammation	Mean difference (95% CI)	p value of Control & CFA
Day 0	0.0 ±0.0	575.7 ±273.0 [a]	-575.7 (-857.4 to -294.0)	0.002
Day 1	0.0 ±0.0	685.3±138.1 [a]	-685.3 (-972.7 to -397.8)	0.003
Day 3	0.0 ±0.0	136.3 ±81.6 [b]	-136.1 (-236.1 to -36.4)	0.016
Day 5	0.0 ±0.0	23.3 ±30.2 [b]	-23.3 (-69.2 to 22.7)	0.250
Day 7	0.0 ±0.0	18.5 ±37.0 [b]	-18.5 (-63.8 to 26.8)	0.356

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

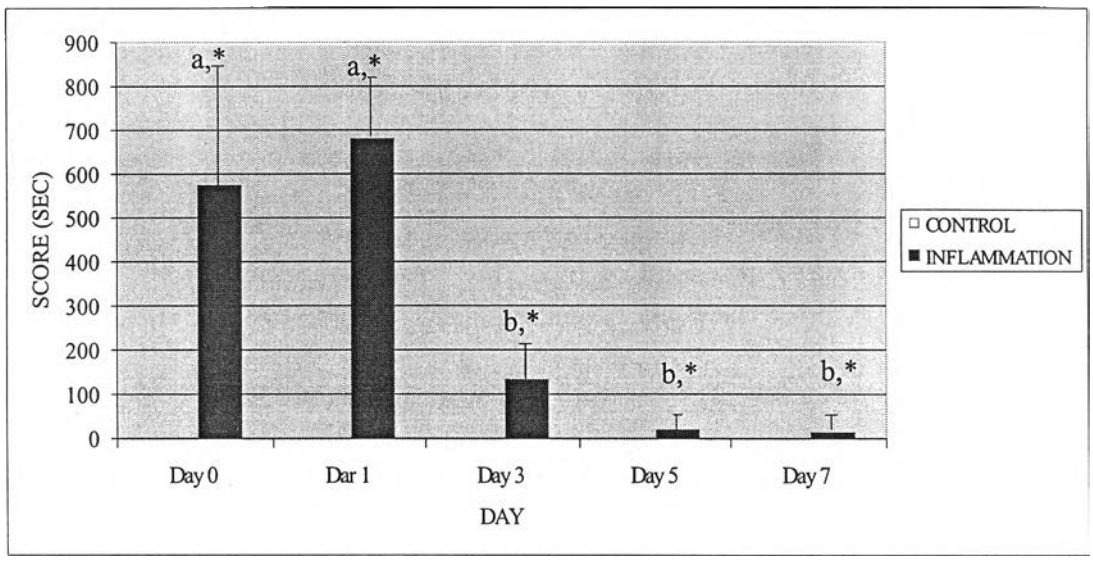

Figure [4-1] Bar graph showing the mean value ± SD of nociceptive behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-2] The non-nociceptive behaviors of the rats in the control group compare with the CFA-induced peripheral inflammation group

Non-nociceptive Behaviors (sec)				
Group	Control	CFA-induced peripheral inflammation	Mean difference (95% CI)	p value of Control & CFA
Day 0	1307.4±359.2	626.3 ±273.1 [a]	681.2 (86.0 to 1276.1)	0.031
Day 1	1522.0 ±172.5	391.5 ±166.1 [ab]	1130.5 (727.2 to 1533.8)	0.001
Day 3	1638.3 ±252.6	1104.5 ±429.6 [ac]	533.8 (-75.9 to 1143.4)	0.076
Day 5	1631.33±146.9	1261.3 ±436.9 [c]	370.1 (-318.9 to 1058.8)	0.226
Day 7	1728.3 ±82.5 [a]	813.3 ±82.5 [ac]	915.7 (623.7 to 1206.3)	0.000

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

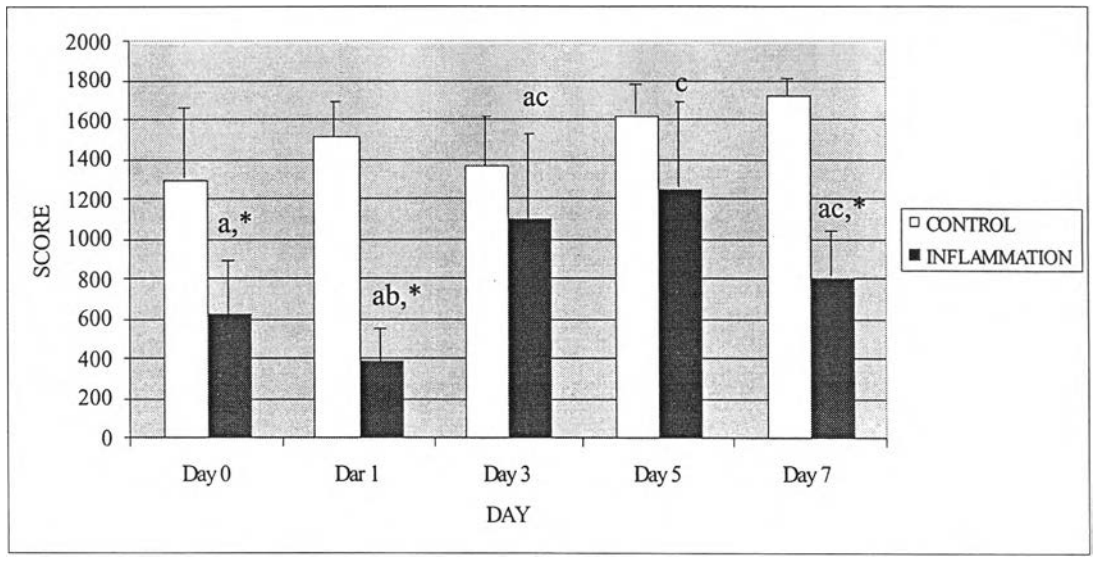

Figure [4-2] Bar graph showing the mean value ± SD of non-nociceptive behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-3] The still but alert behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group

Still but Alert (sec)				
Group	Control	CFA-induced peripheral inflammation	Mean difference (95% CI)	p value of Control & CFA
Day 0	325.4 ±134.7	269.0±273.0	56.4 (-210.9 to 323.8)	0.624
Day 1	217.5 ±86.9	157.5 ±46.6	51.39 (-82.7 to 202.7)	0.308
Day 3	161.8 ±252.6	286.3 ±118.2	139.41 (-465.6 to 216.6)	0.406
Day 5	168.8 ±146.9	222.0 ±121.5	101.01 (-312.9 to 206.3)	0.620
Day 7	71.8 ±82.4	291.0 ±149.8	-219.25 (-428.5 to -10.0)	0.043

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

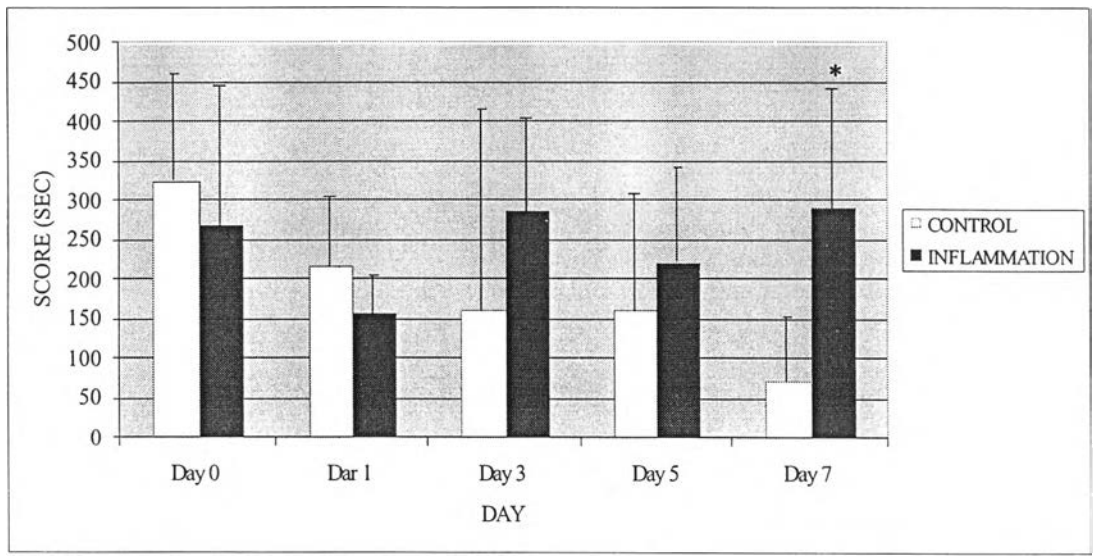

Figure [4-3] Bar graph showing the mean value ± SD of still but alert behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-4] The rest or sleep behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group

Rest or Sleep (sec)				
Group	Control	CFA-induced peripheral inflammation	Mean difference (95% CI)	p value of Control & CFA
Day 0	188.2 ±278.4	246.8 ±153.1	-141.0 (-576.9 to 294.9)	0.459
Day 1	47.0 ±66.5	565.8 ±163.2	-518.75 (-867.8 to -169.7)	0.015
Day 3	0.0 ±0.0	273.0 ±370.5	-273.00 (-726.3 to -180.3)	0.191
Day 5	0.0 ±0.0	222.0 ±352.5	-293.5 (-829.5 to 242.5)	0.218
Day 7	0.0 ±0.0	291.0 ±252.7	126.35 (-986.4 to -386.1)	0.002

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

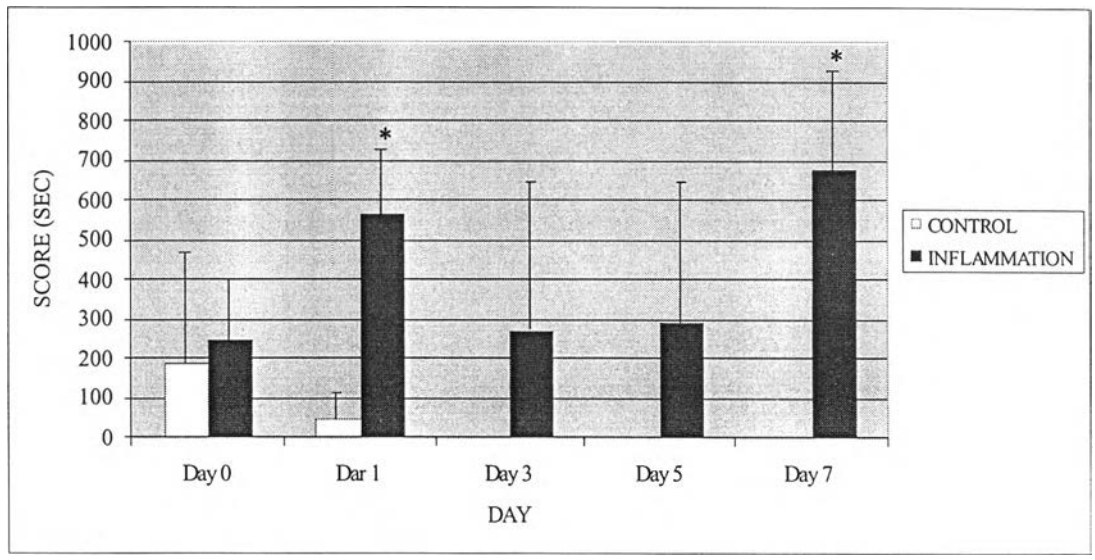

Figure [4-4] Bar graph showing the mean value ± SD of rest or sleep behaviors of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-5] The paw withdrawal latency in contralateral side of the CFA-induced peripheral inflammation compare with the control group

Group	Latency (Sec)		Mean difference (95% CI)	p value of Control & CFA
	Control	CFA-induced peripheral inflammation		
Day 0	9.9 ±2.9	10.9 ±4.0 [ab]	-1.02 (-6.06 to 4.02)	0.653
Day 1	10.0 ±1.9	12.7 ±2.8 [ab]	-2.70 (-6.22 to 0.82)	0.271
Day 3	9.2 ±1.4	9.4 ±0.9 [a]	-0.20 (-1.90 to 1.50)	0.805
Day 5	9.3 ±0.6	9.3 ±1.4 [a]	0.04 (-1.68 to 1.76)	0.961
Day 7	10.9 ±0.8	13.2 ±2.8 [b]	-2.41 (-5.51 to 0.81)	0.086

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

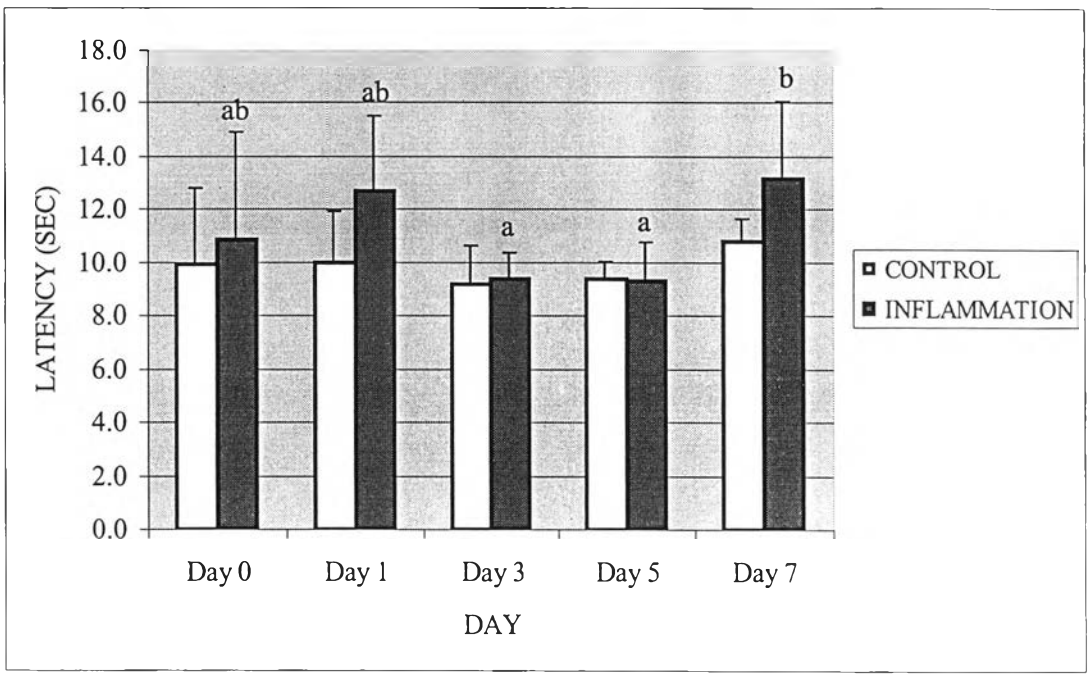

Figure [4-5] Bar graph showing the mean value ± SD of paw withdrawal latency of the rats in the control group, contralateral side, compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-6] The paw withdrawal latency in ipsilateral side of the CFA-induced peripheral inflammation compared with the control group

Group	Latency (Sec)		Mean difference (95% CI)	p value of Control & CFA
	Control	CFA-induced peripheral inflammation		
Day 0	9.9 ±1.7	4.5 ±0.7 [a]	5.32 (3.42 to 7.23)	0.000
Day 1	9.5 ±2.4	6.3 ±1.8 [a]	3.21 (0.35 to 6.05)	0.030
Day 3	8.3 ±0.6	5.3 ±1.3 [a]	2.96 (1.34 to 4.58)	0.001
Day 5	9.3 ±0.8	5.9 ±1.5 [a]	3.46 (1.70 to 5.22)	0.002
Day 7	9.3 ±0.7	8.9 ±1.8 [b]	0.58 (-1.41 to 2.62)	0.518

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

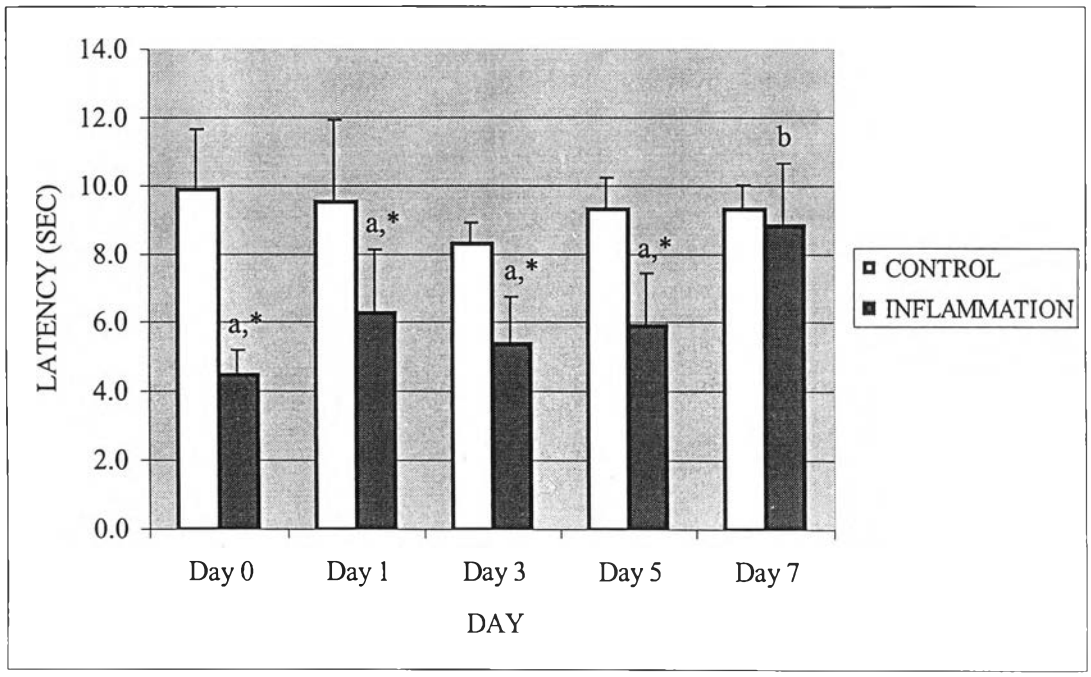

Figure [4-6] Bar graph showing the mean value ± SD of paw withdrawal latency in ipsilateral side of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-7] The number of Fos-IR neurons in contralateral side of the CFA-induced peripheral inflammation in somato sensory cortex compared with the control group

Group	Fos positive cells		Mean difference (95% CI)	p value of Control & CFA
	Control	CFA-induced peripheral inflammation		
Day 0	7 ±3	8 ± 2 [a]	-1.50 (-6.39 to 3.39)	0.466
Day 1	5 ±1	11 ± 5 [ab]	-6.17 (-18.40 to 6.06)	0.207
Day 3	6 ±2	22 ± 6 [b]	-16.00 (-25.57 to -6.42)	0.010
Day 5	7 ±3	16 ±7 [ab]	-9.33 (-21.68 to 30.1)	0104
Day 7	7 ±3	16 ±7 [ab]	-9.33 (-21.68 to 30.1)	0.104

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

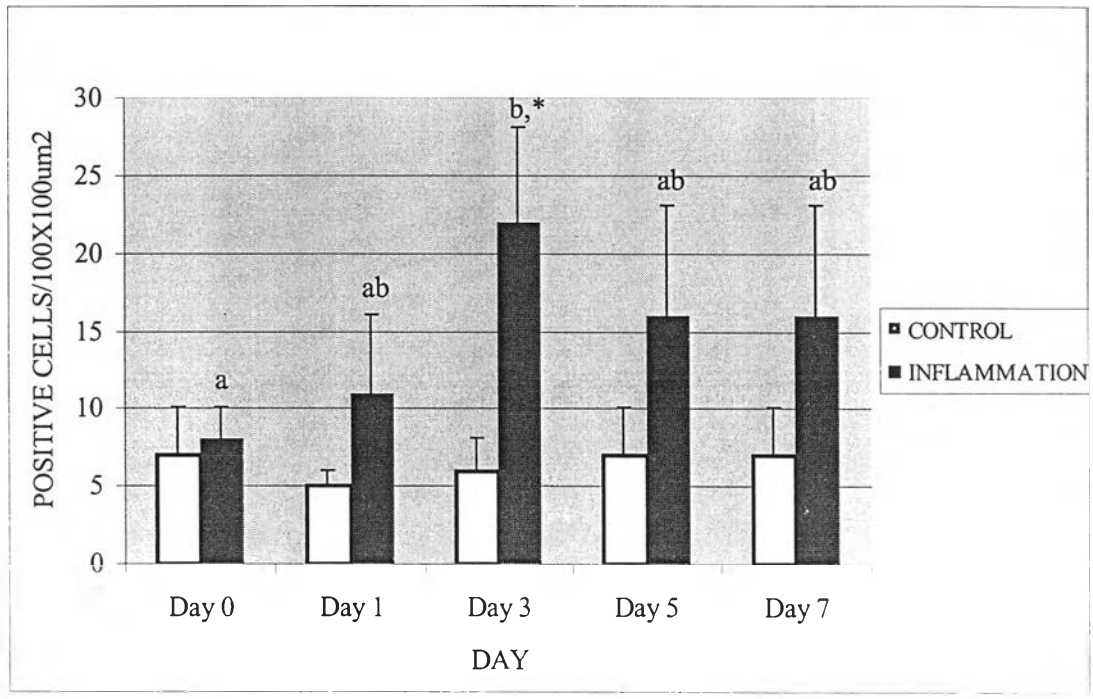

Figure [4-7] Bar graph showing the mean value ± SD of number of Fos-IR neurons in contralateral side of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-8] The number of Fos-IR neurons in ipsilateral side of the CFA-induced peripheral inflammation in somato sensory cortex compared with the control group

Group	Fos positive cells		Mean difference (95% CI)	p value of Control & CFA
	Control	CFA-induced peripheral inflammation		
Day 0	7 ±7	7 ± 2 [a]	-0.08 (-10.74 to 10.57)	0.985
Day 1	4 ±1	8 ± 3 [a]	-4.50 (-10.89 to 1.89)	0.111
Day 3	4 ±2	24 ± 12 [b]	-20.67 (-40.15 to -1.19)	0.042
Day 5	9 ±5	12 ±6 [a]	-3.00 (-14.63 to 8.63)	0.514
Day 7	9 ±5	12 ±6 [a]	-3.00 (-14.63 to 8.63)	0.514

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

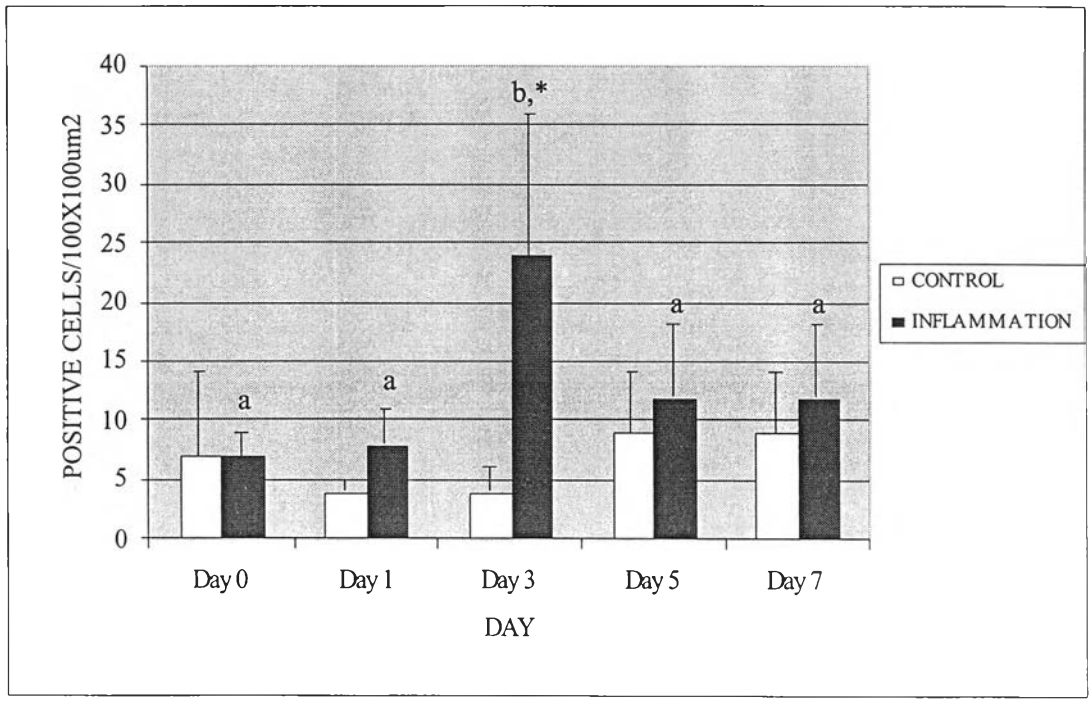

Figure [4-8] Bar graph showing the mean value ± SD of number of Fos-IR neurons in ipsilateral side of the rats in the control group compared with the CFA-induced peripheral inflammation group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

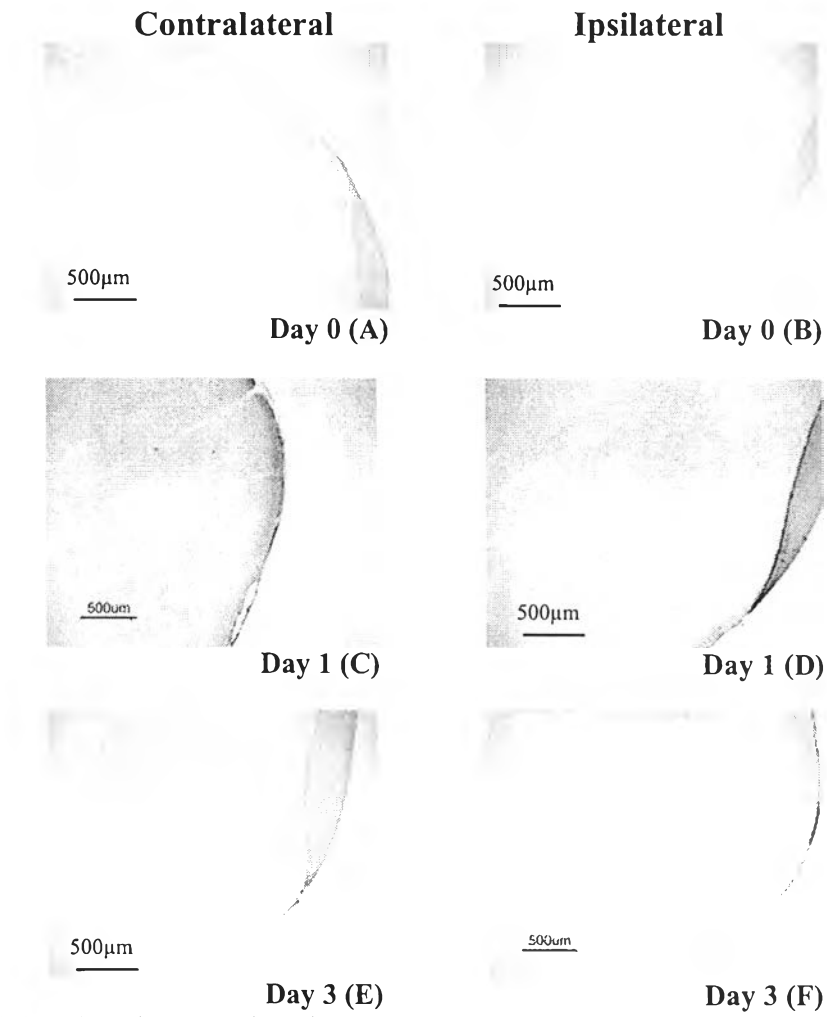

Figure [4-9] The pictures showing

(A)the expression of Fos-IR neurons in contralateral side of the control group, Day 0

(B)the expression of Fos-IR neurons in ipsilateral side of the control group, Day 0

(C)the expression of Fos-IR neurons in contralateral side of the control group, Day 1

(D)the expression of Fos-IR neurons in ipsilateral side of the control group, Day 1

(E) the expression of Fos-IR neurons in contralateral side of the control group, Day 3

(F) the expression of Fos-IR neurons in ipsilateral side of the control group, Day 3

Bar = 500 μm

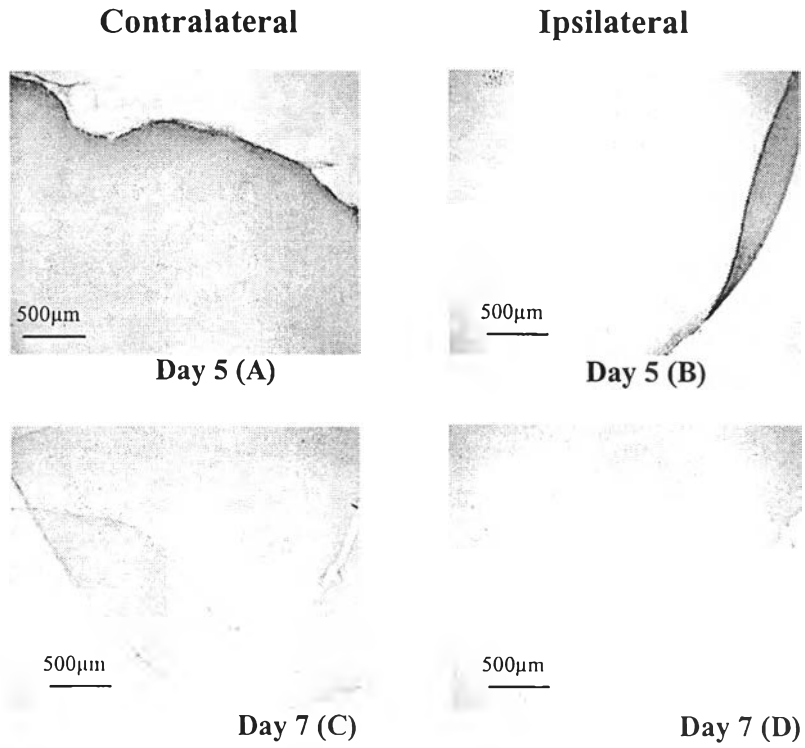

Figure [4-10] The pictures showing

(A)the expression of Fos-IR neurons in contralateral side of the control group, Day 5

(B)the expression of Fos-IR neurons in ipsilateral side of the control group, Day 5

(C)the expression of Fos-IR neurons in contralateral side of the control group, Day 7

(D)the expression of Fos-IR neurons in ipsilateral side of the control group, Day 7

Bar = 500 μm

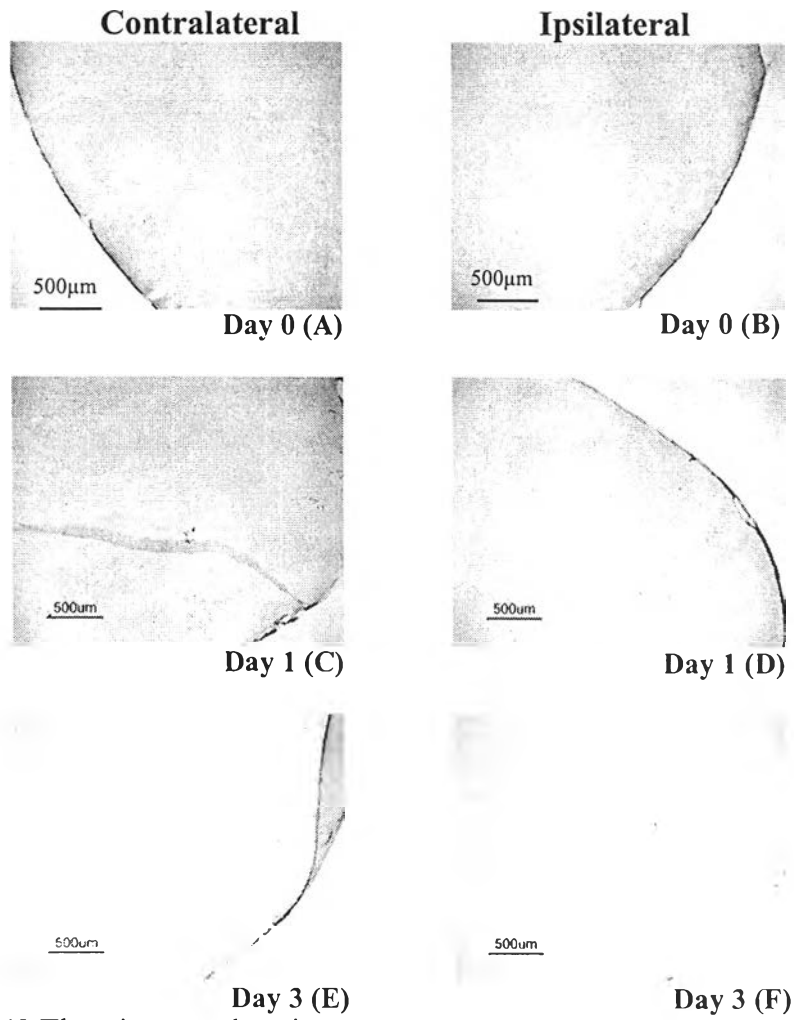

Figure [4-11] The pictures showing

(A)the expression of Fos-IR neurons in contralateral side of the CFA group, Day 0

(B)the expression of Fos-IR neurons in ipsilateral side of the CFA group, Day 0

(C)the expression of Fos-IR neurons in contralateral side of the CFA group, Day 1

(D)the expression of Fos-IR neurons in ipsilateral side of the CFA group, Day 1

(E) the expression of Fos-IR neurons in contralateral side of the CFA group, Day 3

(F) the expression of Fos-IR neurons in ipsilateral side of the CFA group, Day 3

Bar = 500 μm

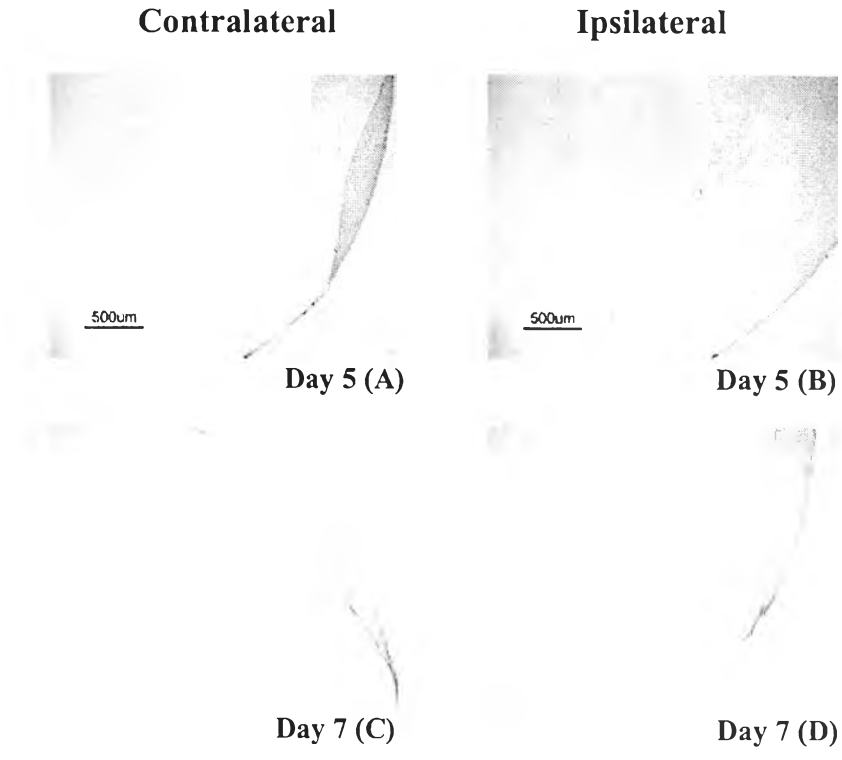

Figure [4-12] The pictures showing

(A)the expression of Fos-IR neurons in contralateral side of the CFA group, Day 5

(B)the expression of Fos-IR neurons in ipsilateral side of the CFA group, Day 5

(C)the expression of Fos-IR neurons in contralateral side of the CFA group, Day 7

(D)the expression of Fos-IR neurons in ipsilateral side of the CFA group, Day 7

Bar = 500 μm

1.2 The role of 5-HT_{2A} receptor in chronic pain model by using behavioral assessment and Fos protein expression

1.2.1 Effect of ketanserin on rats' behavior and Fos protein expression of the control group

The Day 3 groups were selected to study the effect of ketanserin, 5-HT_{2A} antagonist. It was found that ketanserin reduced non-nociceptive behavior compared with the control group. (1108.0±383.3, 1638.3±252.6 seconds in a thirty-minute period, respectively, $p<0.05$). However, the effect on the still but alert and the rest and sleep category between both groups was not different Table [4-9], Figure [4-13].

For the paw withdrawal test, no latency-different was found among the groups or both sides of paws neither Table [4-10], Figure [4-14].

It was also found that ketanserin did not affect the number of Fos protein expressions in both hemispheres Table [4-11], Figure [4-15], [4-16].

Table [4-9] The effect of Ketanserin on observed behaviors of the rats'

Behaviors (sec)	Group		Mean difference (95% CI)	p-value of control & control+ ketanserin
	Control from Day 3	Control with ketanserin		
Nociceptive	0.0±0.0	0.0 ± 0.0	0.0 (0.0 to 0.0)	1.000
Non-nociceptive	1638.3±252.3	1108.0±383.3	501.3 (97.1 to905.5)	0.022
Still but Alert	161.8±252.6	692.0 ±383.3	-281.00 (-593.5 to 30.8)	0.071
Rest or Sleep	153.8±151.1	375.2 ±308.4	-221.45 (-623.2 to 150.3)	0.234

- In the same row, * indicated the significantly different between groups.

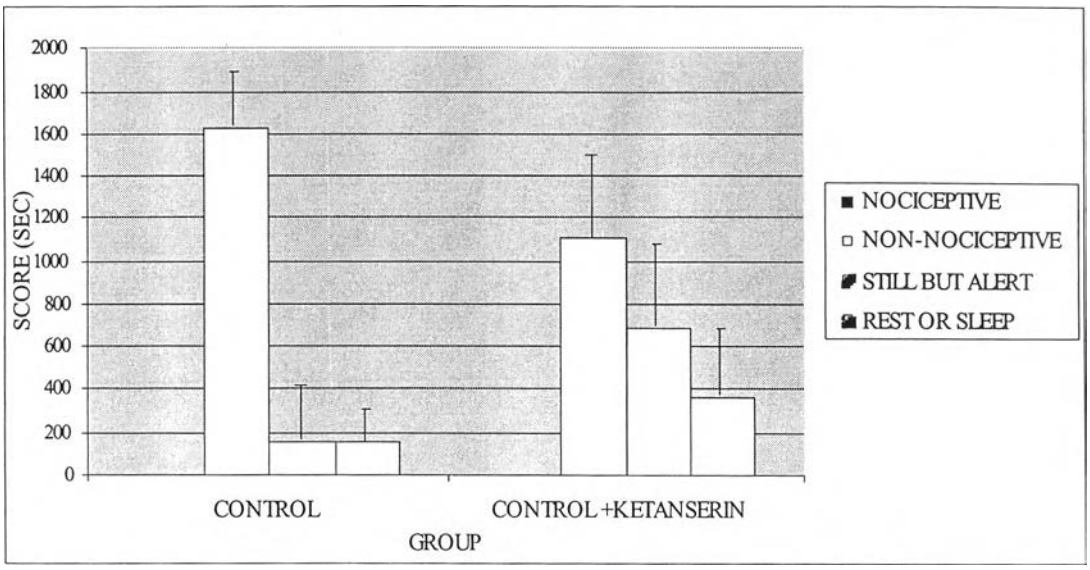

Figure [4-13] Bar graph showing the mean value ± SD of observed behaviors of the rats in the control group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. * indicated the significantly difference between groups.

Table [4-10] The effect of Ketanserin on paw withdrawal latency

Latency (Sec)	Group		Mean difference (95% CI)	p-value of control & control+ ketanserin
	Control from Day 3	Control with ketanserin		
Contralateral side	9.2 ±1.4	11.2 ±2.6	-2.00 (-5.01 to 1.01)	0.164
Ipsilateral side	8.3 ±0.6	9.3 ±1.0	-0.96 (-2.20 to 0.28)	0.112

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

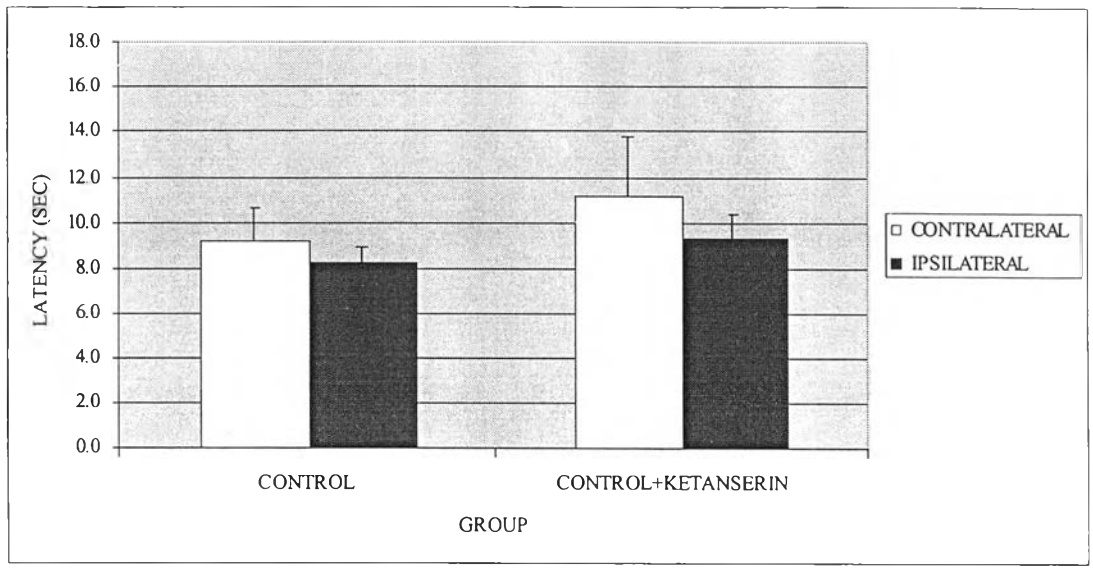

Figure [4-14] Bar graph showing the mean value ± SD of paw withdrawal latency of the rats in the control group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. . [a] indicates the significantly difference between the difference between the limbs. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-11] The effect of Ketanserin on Fos positive cells

Fos positive cells	Group		Mean difference (95% CI)	p-value of control & control+ ketanserin
	Control from Day 3	Control with ketanserin		
Contralateral side	6 ±2	5 ±1	-0.67 (-2.26 to 3.59)	0.561
Ipsilateral side	4 ±2	5 ±1	-1.00 (-4.07 to 2.07)	0.417

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

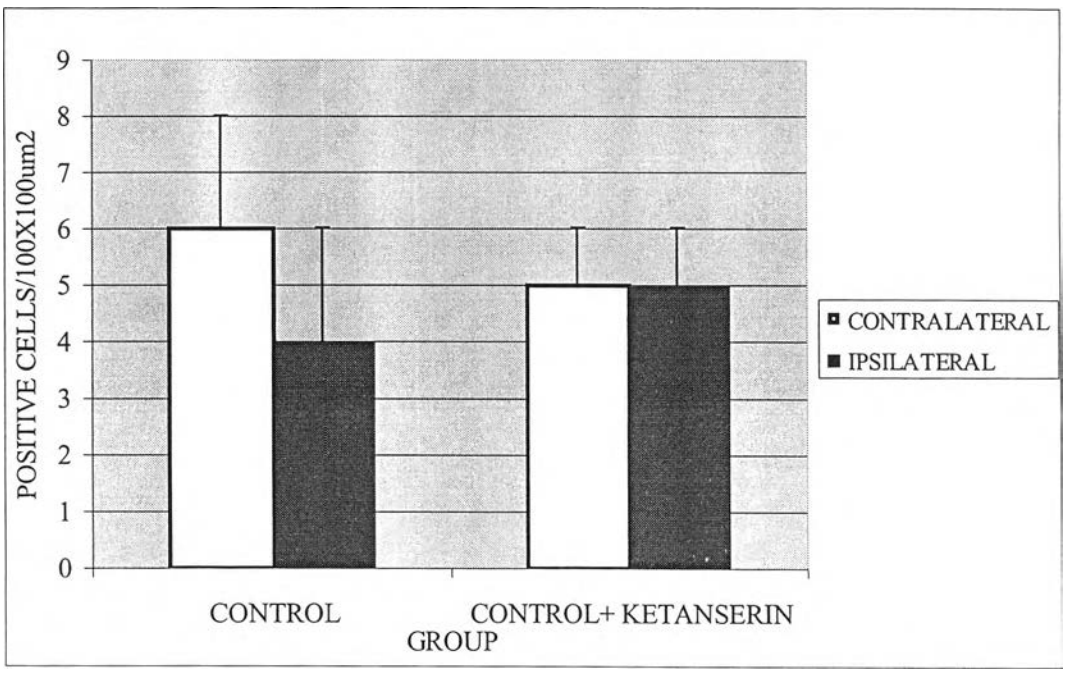

Figure [4-15] Bar graph showing the mean value ± SD of the number of Fos-IR neurons of the rats in the control group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. Significant difference of different treatment was assessed with independent sample t-test. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

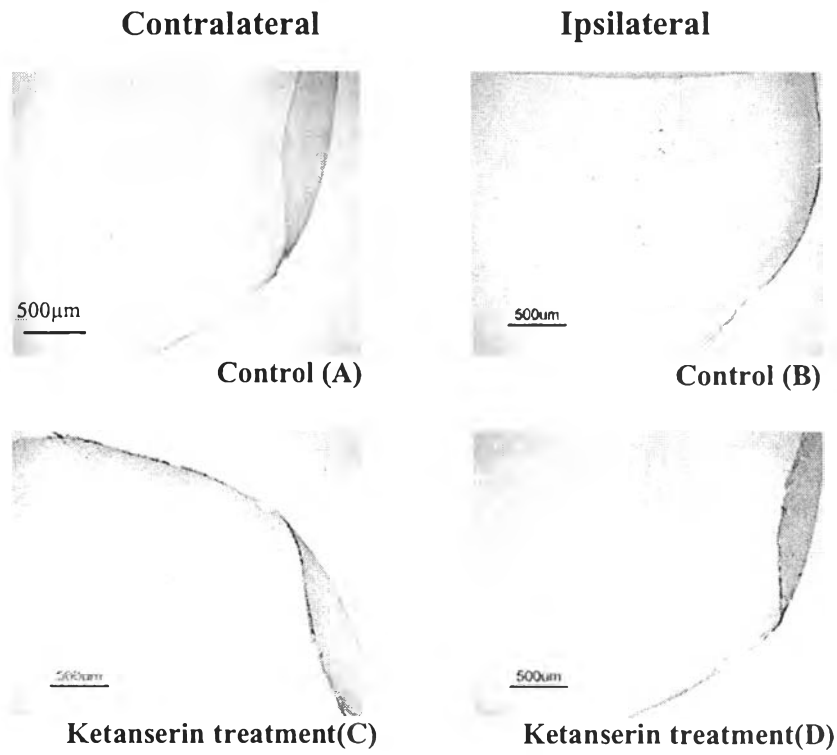

Figure [4-16] The pictures showing

(A)the expression of Fos-IR neurons in contralateral side of the control group

(B)the expression of Fos-IR neurons in ipsilateral side of the control group

(C)the expression of Fos-IR neurons in contralateral side of the control group with ketanserin treated

(D)the expression of Fos-IR neurons in ipsilateral side of the control group with ketanserin treated

Bar = 500 μm

1.2.2 Effect of ketanserin on rats' behavior and Fos protein expression in CFA-induced peripheral inflammation

According to the effect of CFA-induced peripheral inflammation, it was found that ketanserin could reduce nociceptive behaviors. But, it was not altering other behaviors Table [4-12], Figure [4-17].

The paw withdrawal latency of CFA with ketanserin-treatment was not different, compared with the CFA alone group. Though, it was found that ketanserin could lengthen the paw withdrawal latency in the ipsilateral side, compared with its control (14.3±2.0, 5.3±1.3 seconds, respectively) Table [4-13], Figure [4-18].

From the results of the two experiments above, it was not surprising that number of Fos-IR neurons were reduced by ketanserin in contralateral hemisphere (7±2 positive cells per 100x100 μm^2) of the somatosensory cortex, compared with the CFA alone group (22±6 positive cells per 100x100 μm^2). And it was a trend to reduced the immunoreactive neurons in the ipsilateral hemisphere (7±3 positive cells per 100x100 μm^2) compared with CFA alone group (24±12 positive cells per 100x100 μm^2) Table [4-14], Figure [4-19], [4-20].

Table [4-12] The effect of ketanserin on behaviors of the rats in the CFA-induced peripheral inflammation group compared with the CFA alone group.

Behaviors (sec)	Group		Mean difference (95% CI)	p-value of CFA & CFA+ ketanserin
	CFA from Day 3	CFA with ketanserin		
Nociceptive	136.3 ±81.6	5.6 ±12.5	130.7 (44.6 to 216.7)	0.009
Non-nociceptive	1104.5±429.6	920.6±373.9	183.9 (-448.5 to 816.3)	0.514
Still but Alert	286.3± 118.2	433.6±436.1	-147.4 (-371.2 to 76.5)	0.163
Rest or Sleep	273.0± 370.5	440.2±436.1	-167.2 (-816.4 to 482.0)	0.562

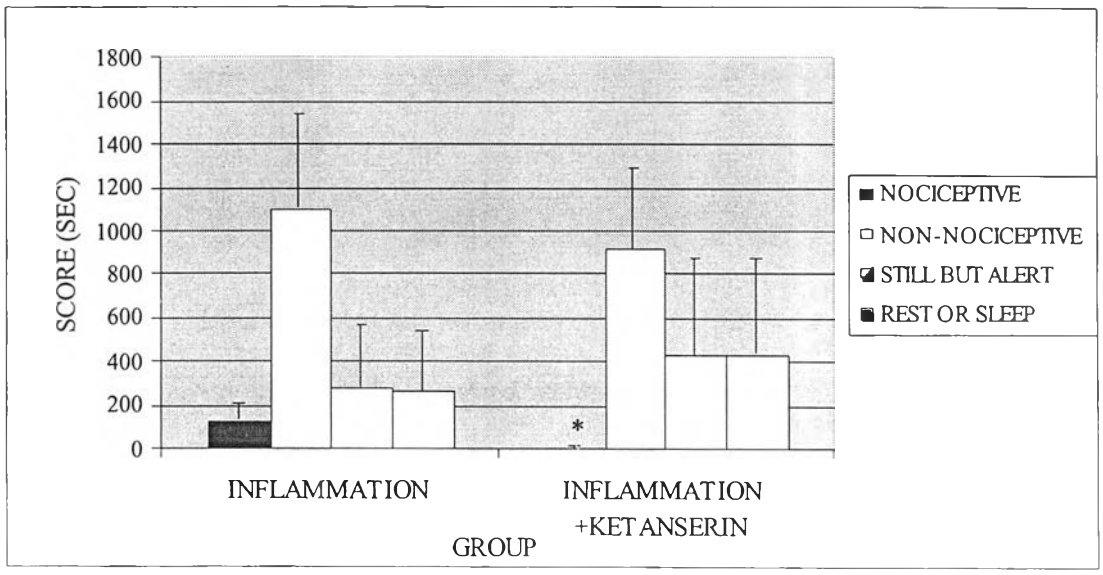

Figure [4-17] Bar graph showing the mean value ± SD of observed behaviors of the rats in the CFA-induced inflammation group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. * indicated the significantly difference between groups.

Table [4-13] The effect of ketanserin on paw withdrawal latency of the CFA-induced peripheral inflammation group compared with the CFA alone group.

Latency (Sec)	Group		Mean difference (95% CI)	p-value of CFA & CFA+ ketanserin
	CFA from Day 3	CFA with ketanserin		
Contralateral side	9.4±0.9 [a]	9.2±1.1 [a]	0.18 (-1.29 to 1.65)	0.785
Ipsilateral side	5.3±1.3 [b]	14.3±2.0 [b]	-8.96 (-11.48 to -6.51)	<0.001

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

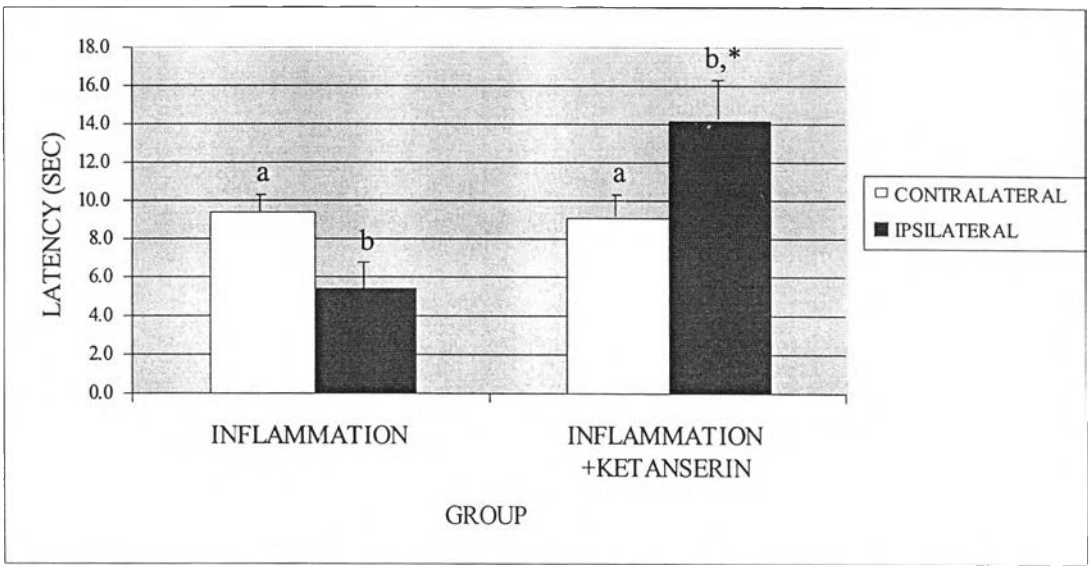

Figure [4-18] Bar graph showing the mean value ± SD of paw withdrawal latency of the rats in the CFA-induced inflammation group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. [a] indicates the significantly difference between the difference between the limbs. * indicated the significantly difference between groups.

Table [4-14] The effect of ketanserin on the number of Fos positive cells of the CFA-induced peripheral inflammation group compared with the CFA alone group.

Fos positive cells	Group		Mean difference (95% CI)	p-value of CFA & CFA+ ketanserin
	CFA-induced peripheral inflammation	CFA with ketanserin		
Contralateral side	22 ±6	7 ±2	14.33 (-4.76 to 23.91)	0.014
Ipsilateral side	24 ±12	7 ±3	17.67 (-2.08 to 37.41)	0.068

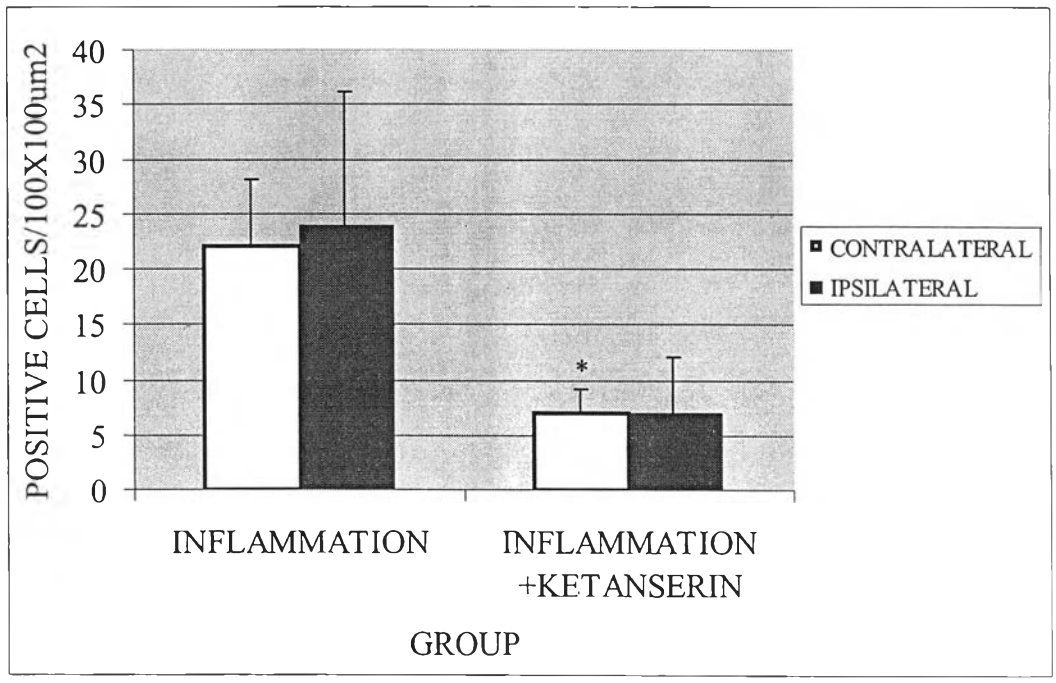

Figure [4-19] Bar graph showing the mean value ± SD of number of the number of Fos-IR positive cells of the rats in CFA-induced inflammation group compare, with ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. * indicated the significantly difference between groups.

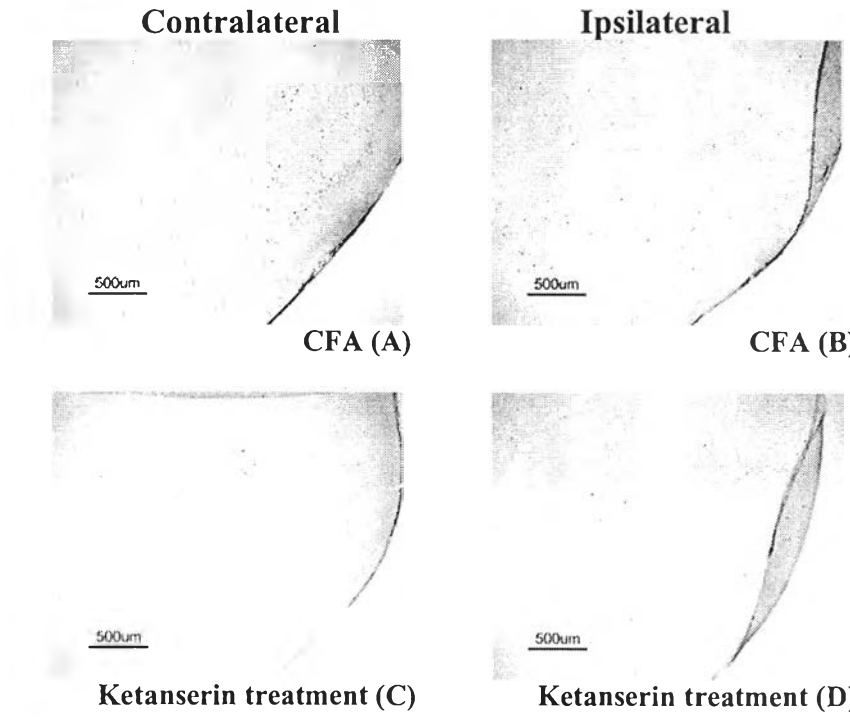

Figure [4-20] The pictures showing

(A)the expression of Fos-IR neurons in contralateral side of the CFA group

(B)the expression of Fos-IR neurons in ipsilateral side of the CFA group

(C)the expression of Fos-IR neurons in contralateral side of the CFA group with ketanserin treated

(D)the expression of Fos-IR neurons in ipsilateral side of the CFA group with ketanserin treated

Bar = 500 μm

2. The role of the 5-HT_{2A} receptor in 5-HT depleted state on the changes of pain sensation

2.1 The effect of PCPA-induced 5-HT depletion on rats' behaviors and Fos protein expression

In the control group, non-nociceptive behavior slowly increased by the time. The statistics detected that Day 0 rats exhibited non-nociceptive behavior less than Day 7 (1307.4±359.2, 1728.3±82.5 seconds in a thirty-minute period, respectively, $p<0.05$). However, during the days in between, non-nociceptive behavior did not different from both Day 0 and Day 7 (1522.0±172.5, 1638.3±252.6, 1631.3±146.9 seconds, $p<0.05$) Table [4-15], Figure [4-21]. On the other hand, it was found that still but alert time in D7 was lower than D0 (71.8±82.5, 325.4±134.7 seconds, respectively, $p<0.05$) Table [4-16], Figure [4-22]. In addition, the time that the rats rested and slept in each day did not difference Table [4-17], Figure [4-23].

In the PCPA-induced 5-HT depletion group, the data showed that in Day 1, the rats expressed their non-nociceptive behavior less than during the other days. (Day 0; 1686.4±72.9, Day 1; 1158.0±368.0, Day 3; 1478.5±248.5, Day 5; 1702.3±96.7, Day 7; 1565.5±92.4 seconds in a thirty-minute period, respectively, $p<0.05$) Table [4-15].

The comparison between both groups found that non-nociceptive behavior within the Day 7 group, rats in the control group expressed this behavior greater than the PCPA group (1728.3±82.3, 1565.5±92.4 seconds in the thirty-minute period respectively, $p<0.05$) Table [4-15], figure [4-21].

For the still but alert category, there was no difference found between both groups Table [4-16], Figure [4-22]. In these experimental groups, rats did not express nociceptive behavior at all Table [4-15].

In the rest or sleep category, it was found that rats in the PCPA group expressed these behaviors significantly greater than in the control group (147.8±110.4, 0±0 seconds in the thirty-minute period respectively, $p<0.05$).

As described above, rats in the control group did not withdraw their paws in different latency among time series Table [4-6], [4-7], Figure [4-5], [4-6].

In the PCPA-induced 5-HT depletion group, it was found that D3 group has the highest latency in left side of the hind paw (10.9±1.7 seconds, $p<0.05$). And it was detected in a significant level compared with Day 0 and Day 7 (7.9±0.7, 7.4±1.3 seconds, respectively, $p<0.05$). The other days' latencies were not different among groups. The ipsilateral side of the hind paw-latency in Day 1 and Day 3 was significantly higher than in Day 7 (8.9±1.7, 8.7±1.4, 6.8±1.8 seconds, respectively, $p<0.05$). The other days' latencies were not different among groups.

For the comparison of the control groups with the PCPA groups, the data showed that in Day 0 paw withdrawal latency of the right side of the hind paw of the control group was higher than that in the PCPA group (9.9±1.7, 7.0±1.2 seconds, respectively, $p<0.05$). In addition, the left hind paw in Day 7, the control group-latency was greater than that of the PCPA group (10.9±0.8, 7.4±1.3 seconds, respectively, $p<0.05$) Table [4-18], [4-19], Figure [4-24], [4-25].

The paw withdrawal latencies in Day 1 and Day 5 were not different among groups and time series.

It was found that PCPA did not alter the expression of Fos protein in the left hemisphere cortex neither compared with its control nor time series Table [4-20], Figure [4-27]. However, the statistics detected the difference among time series in the right hemisphere. It was found that 3 days after PCPA-administration (Day 0) group had the greatest number of positive neurons (9±4 positive cells per area). Thus, no difference was found between the PCPA group and their own control groups Table [4-21], Figure [4-27], [4-28], [4-29].

Table [4-15] The non-nociceptive behaviors of the rats in the control group compared with the PCPA-induced 5-HT depleted group

Non-nociceptive Behaviors (sec)				
Group	Control	PCPA-induced 5-HT depletion	Mean difference (95% CI)	p-value of control & PCPA
Day 0	1307.4±359.2	1686.4 ±72.9 [a]	-379.0 (-757.0 to -0.9)	0.050
Day 1	1522.0±172.5	1158.0 ±368.0 [b]	364.00 (-429.9 to 1157.9)	0.272
Day 3	1638.3 ±252.6	1478.5 ±248.5 [a]	159.75 (-273.8 to 593.2)	0.402
Day 5	1631.3 ±146.9	1702.3 ±96.7 [a]	-70.9 (-305.3 to 163.5)	0.472
Day 7	1728.3 ±82.5	1565.5 ±92.4 [a]	162.8 (11.3 to 314.3)	0.039

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

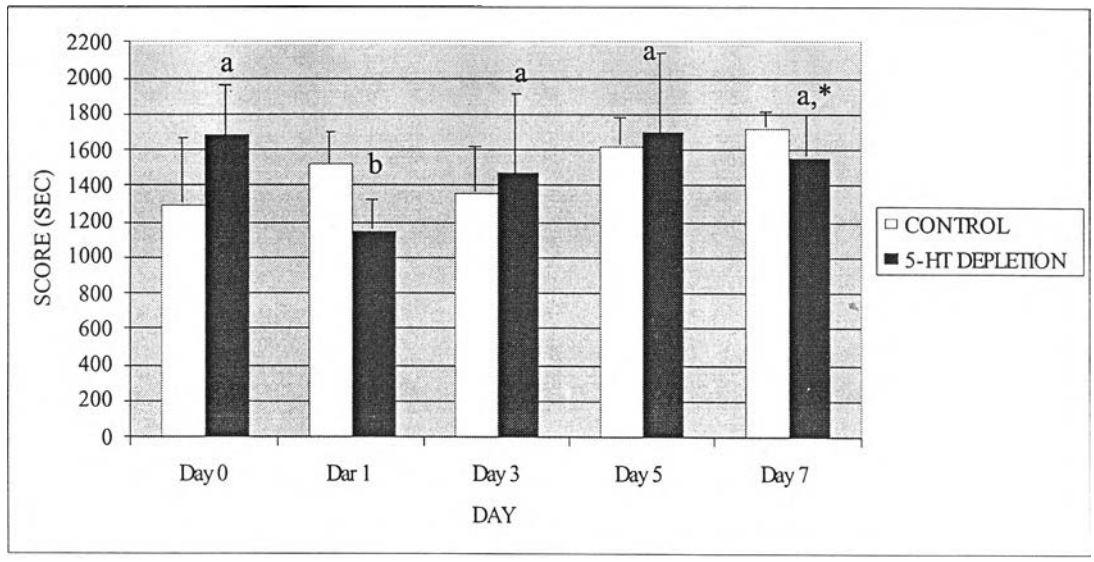

Figure [4-21] Bar graph showing the mean value ± SD of non-nociceptive behaviors of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-16] The still but alert behaviors of the rats in the control group compared with the PCPA-induced 5-HT depleted group

Still but Alert Behaviors (sec)				
Group	Control	PCPA-induced 5-HT depletion	Mean difference (95% CI)	p-value of control & PCPA
Day 0	325.4 ±134.7	112.4 ±71.7	213.0 (55.7 to 370.3)	0.050
Day 1	217.5 ±86.9	246.5 ±170.1	-29.0 (-398.4 to 340.4)	0.838
Day 3	161.8 ±252.6	116.3 ±124.1	-4.5 (-348.8 to 339.8)	0.976
Day 5	168.7 ±146.9	83.5 ±70.4	82.3 (-126.4 to 296.7)	0.348
Day 7	71.8 ±82.5	86.8 ±31.7	-15.0 (-123.1 to 93.1)	0.746

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

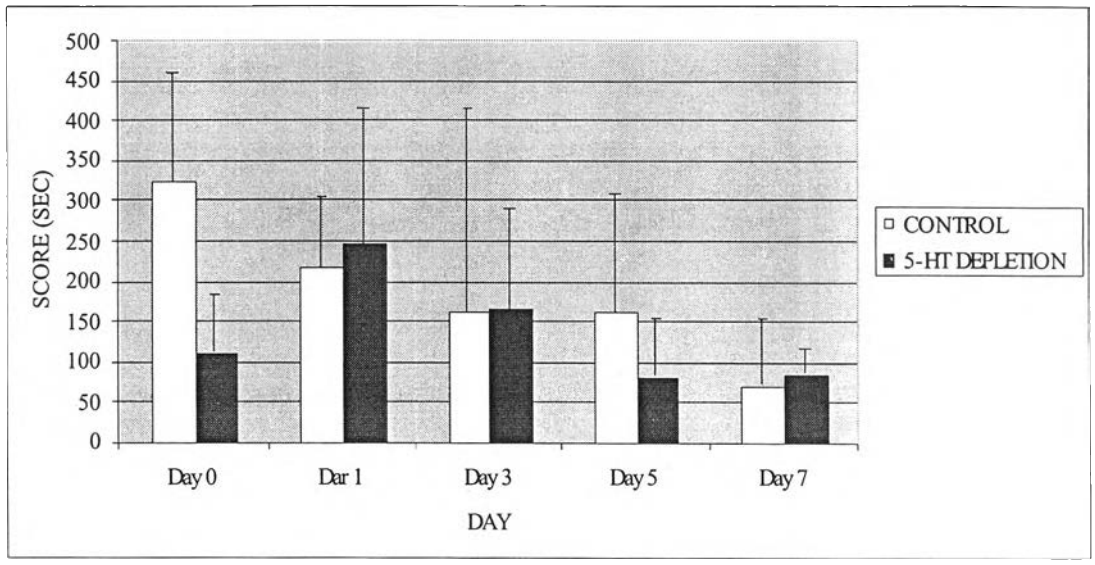

Figure [4-22] Bar graph showing the mean value ± SD of still but alert behaviors of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-17] The rest or sleep behaviors of the rats in the control group compared with the PCPA-induced 5-HT depleted group

Rest or Sleep Behaviors (sec)				
Group	Control	PCPA-induced 5-HT depletion	Mean difference (95% CI)	p-value of control & PCPA
Day 0	188.2 ±278.4	1.2 ± 2.7 [a]	186.8 (-100.4 to 473.9)	0.172
Day 1	47.0 ±66.5	395.5 ±340.0 [b]	-348.5 (-1061.0 to 364.0)	0.246
Day 3	0.0 ±0.0	153.8 ±151.1 [ab]	-153.8 (-338.6 to 38.1)	0.088
Day 5	0.0 ±0.0	14.3 ±26.5 [a]	-14.3 (-54.6 to 26.1)	0.406
Day 7	0.0 ±0.0	147.8 ±110.7 [ab]	-147.8 (-283.2 to -12.3)	0.037

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

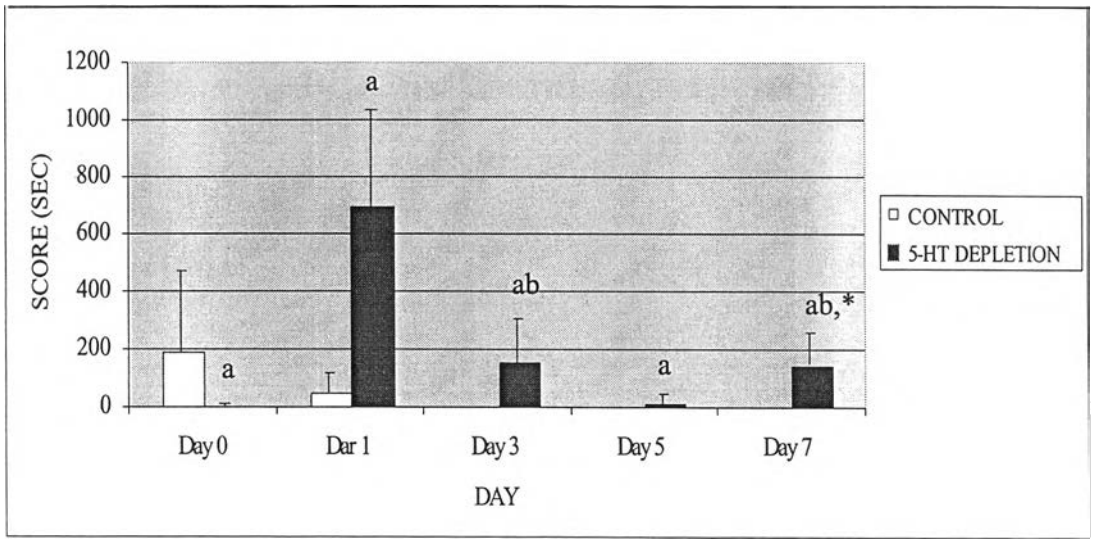

Figure [4-23] Bar graph showing the mean value ± SD of the rest or sleep behaviors of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-18] The paw withdrawal latency in left side of the PCPA-induced 5-HT depletion compared with the control groups

Group	Latency (Sec)		Mean difference (95% CI)	p-value of control & PCPA
	Control	PCPA-induced 5-HT depletion		
Day 0	9.9 ±2.9	7.9 ±0.7 [bc]	2.04 (-2.845 to 0.85)	0.233
Day 1	10.0 ±1.9	9.2 ±2.7 [cd]	0.74 (-2.58 to 4.06)	0.605
Day 3	9.2 ±1.4	10.9 ±1.7 [ad]	-1.74 (-3.99 to 0.51)	0.450
Day 5	9.3 ±0.6	9.3 ±1.2 [cd]	0.10 (-1.28 to 1.48)	0.904
Day 7	10.9 ±0.8	7.4 ±1.3 [bc],*	3.42 (0.79 to 6.05)	0.014

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

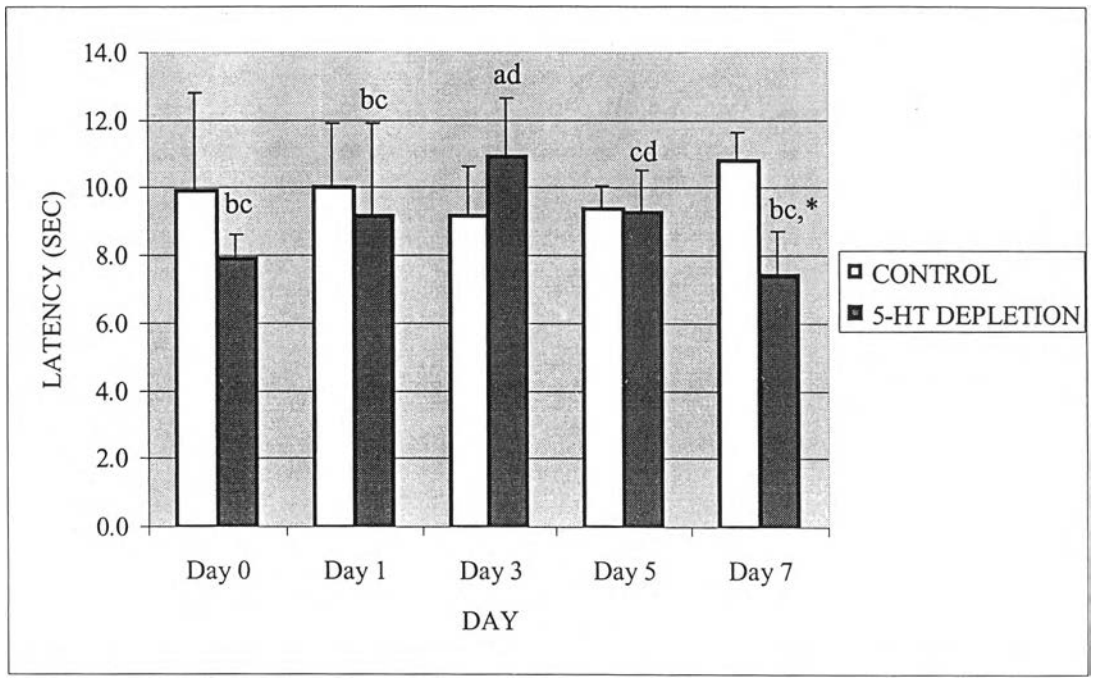

Figure [4-24] Bar graph showing the mean value ± SD of the paw withdrawal latency in left side of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-19] The paw withdrawal latency in right side of the PCPA-induced 5-HT depletion compared with the control groups

Group	Latency (Sec)			
	Control	PCPA-induced 5-HT depletion	Mean difference (95% CI)	p value
Day 0	9.9±1.7	7.0±1.2 [abc, *]	2.88 (0.71 to 5.05)	0.002
Day 1	9.5±2.4	8.8±1.9 [b]	0.77 (-2.42 to 4.13)	0.598
Day 3	8.3±0.6	8.7±1.4 [b]	-0.42 (-2.05 to 1.18)	0.589
Day 5	9.3±0.8	8.2±0.6 [abc]	1.10 (-0.02 to 2.22)	0.089
Day 7	9.3 ±0.7	6.8 ±1.8 [c,*]	2.62 (0.58 to 4.64)	0.011

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

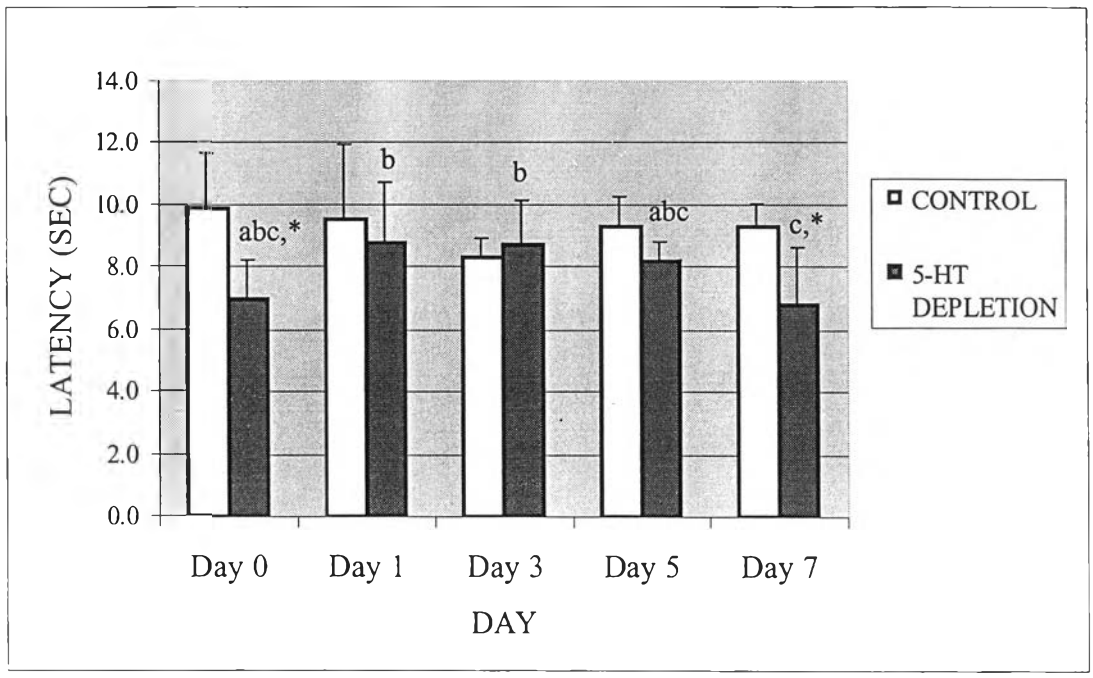

Figure [4-25] Bar graph showing the mean value ± SD of paw withdrawal latency in right side of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-20] The number of Fos positive cells in left side of the PCPA-induced 5-HT depletion in somatosensory cortex compared with the control group

Group	Fos positive cells		Mean difference (95% CI)	p-value of control & PCPA
	Control	PCPA-induced 5-HT depletion		
Day 0	7 ±3	8 ± 5	-1.75 (-13.22to 9.72)	0.711
Day 1	5 ±1	6 ± 2	-1.83 (-6.91 to 3.25)	0.334
Day 3	6 ±2	7 ± 0	-0.88 (-3.72 to 1.11)	0.205
Day 5	7 ±3	8 ±1	-1.33 (-5.96 to 3.29)	0.469
Day 7	7 ±2	8 ±3	-1.33 (-5.96 to 3.29)	0.469

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

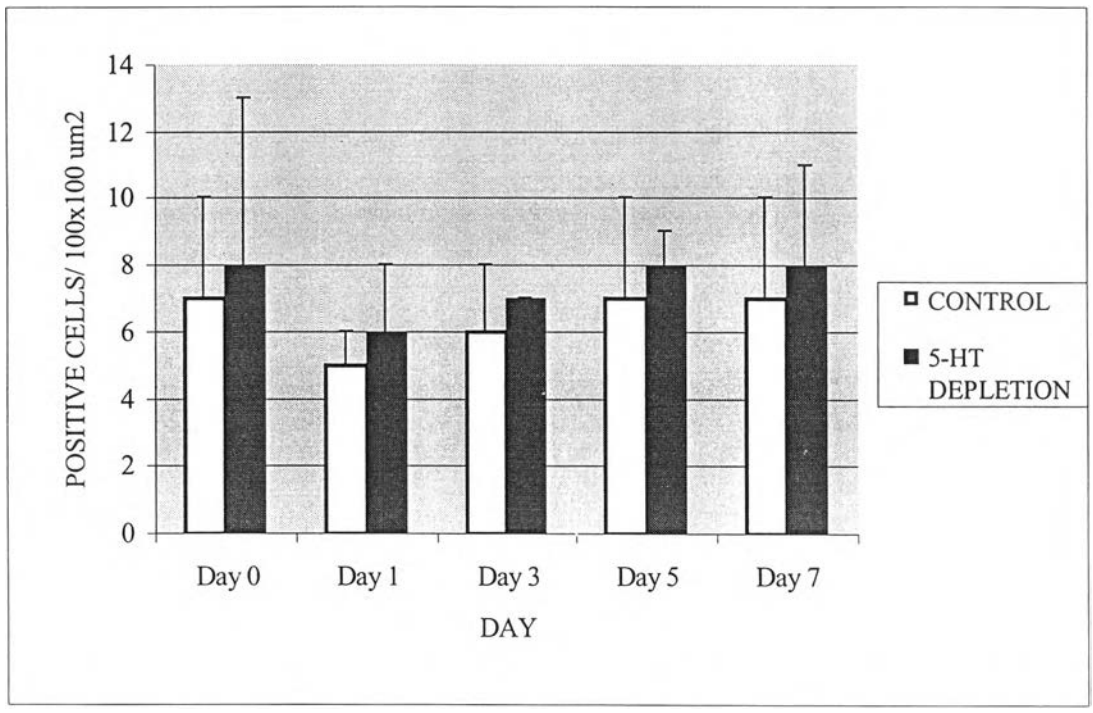

Figure [4-26] Bar graph showing the mean value ± SD of the number of Fos-IR neurons in left side of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

Table [4-21] The number of Fos positive cells in right side of the PCPA-induced 5-HT depletion in somatosensory cortex compared with the control group

Group	Fos positive cells				Mean difference (95% CI)	p value
	Control		PCPA-induced 5-HT depletion			
Day 0	7	±7	9	± 4 [a]	-1.75 (-13.22to 9.72)	0.711
Day 1	4	±1	5	± 3 [ab]	-1.83 (-9.55to 5.88)	0.505
Day 3	4	±2	5	± 1 [b]	-1.00 (-4.07 to -2.07)	0.417
Day 5	9	±5	5	±1 [b]	-4.00 (-3.8 to 11.8)	0.228
Day 7	9	±5	5	±1 [b]	-4.00 (-3.8 to 11.8)	0.228

- In the same column, the different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level.

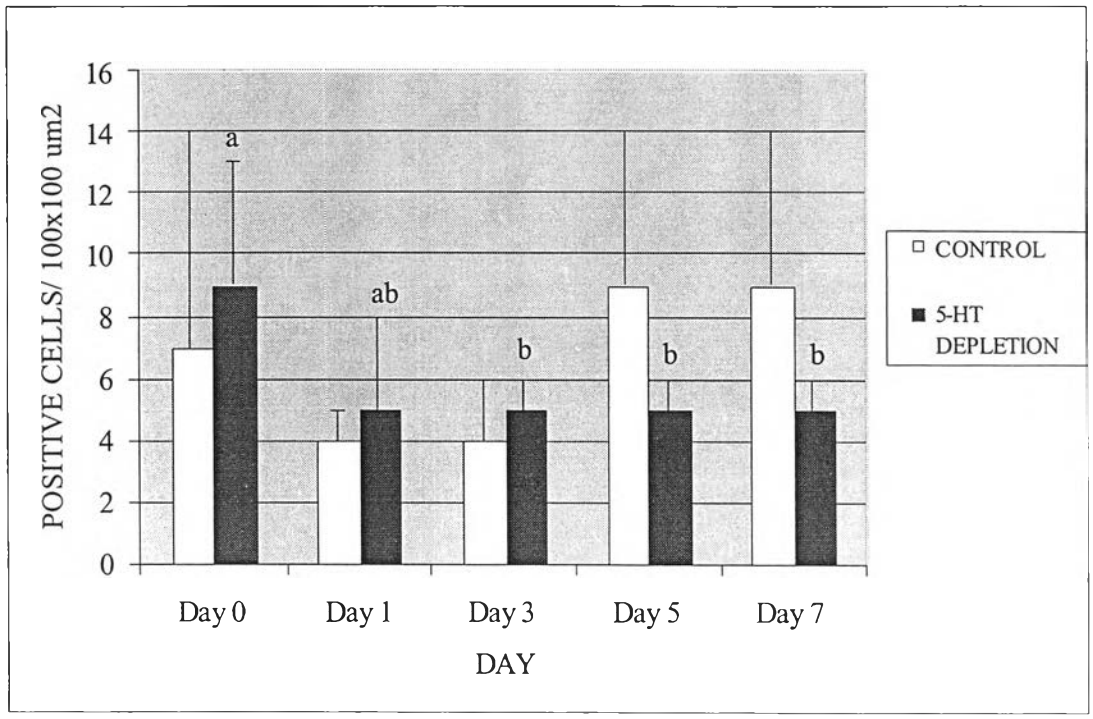

Figure [4-27] Bar graph showing the mean value ± SD of the number of Fos-IR neurons in right side of the rats in the control group compared with the PCPA-induced 5-HT depletion group. Significant difference of time series in the same treatment was assessed with ANOVA with the LSD test. Significant difference of different treatment was assessed with independent sample t-test. The different alphabet indicates the significantly difference between/among time series. The same alphabet indicates the non-significant difference level. * indicated the significantly difference between groups.

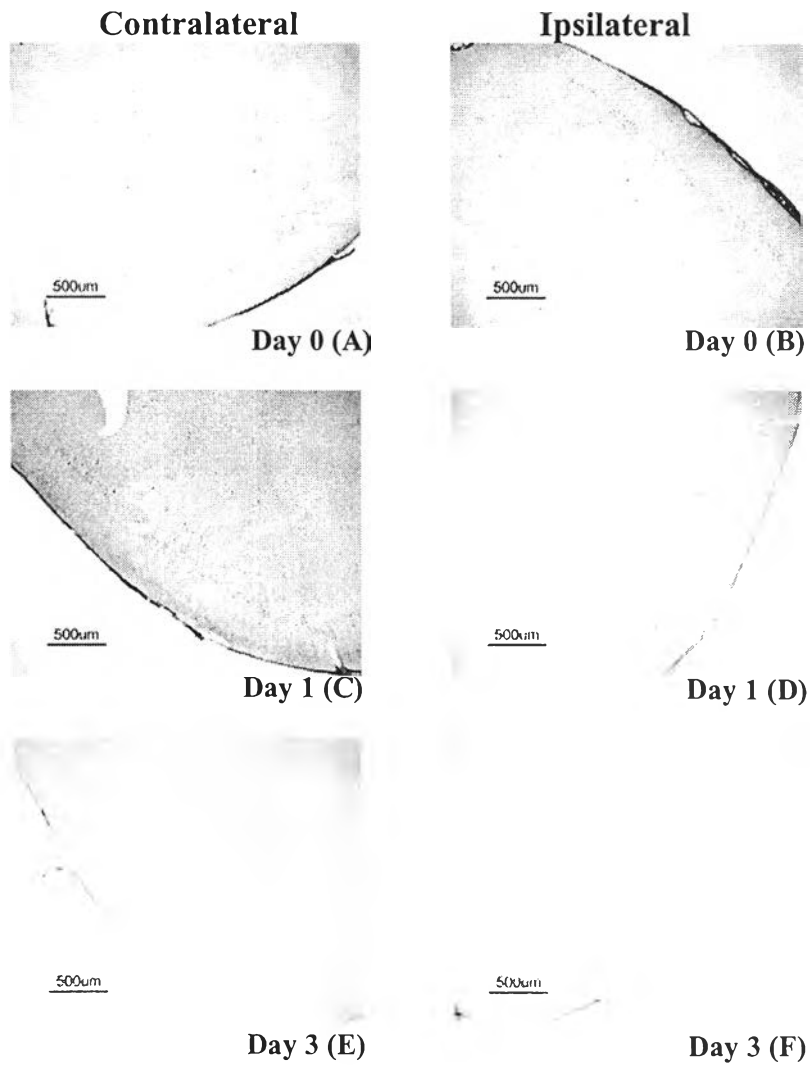

Figure [4-28] The pictures showing

(A)the expression of Fos-IR neurons in left side of PCPA group, Day 0

(B)the expression of Fos-IR neurons in right side of the PCPA group, Day 0

(C)the expression of Fos-IR neurons in left side of the PCPA group, Day 1

(D)the expression of Fos-IR neurons in right side of the PCPA group, Day 1

(E) the expression of Fos-IR neurons in left side of the PCPA group, Day 3

(F) the expression of Fos-IR neurons in right side of the PCPA group, Day 3

Bar = 500 μm

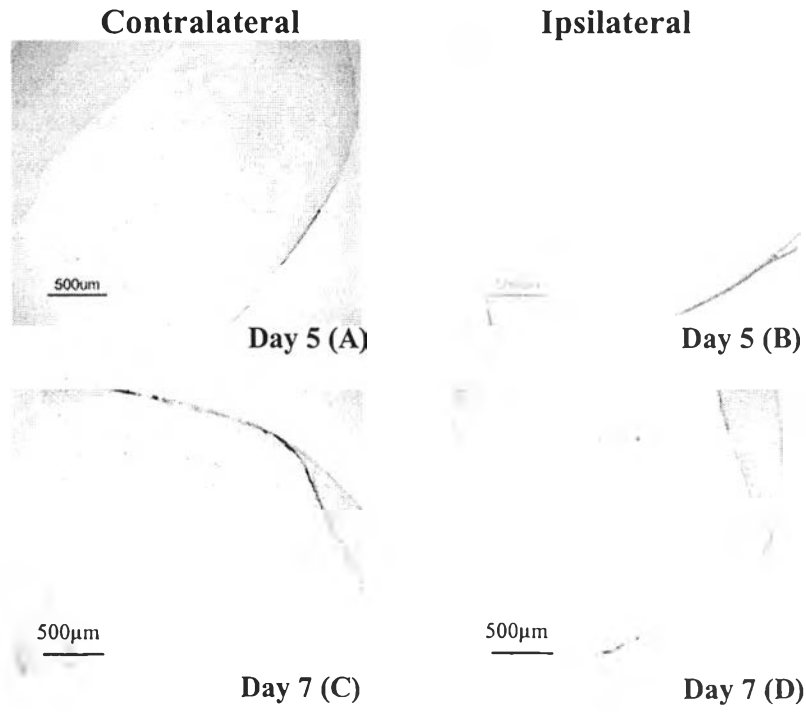

Figure [4-29] The pictures showing

(A)the expression of Fos-IR neurons in left side of the PCPA group, Day 5

(A)the expression of Fos-IR neurons in right side of the PCPA group, Day 5

(B)the expression of Fos-IR neurons in left side of the PCPA group, Day 7

(C)the expression of Fos-IR neurons in right side of the PCPA group, Day 7

Bar = 500 μm

2.1 The role of the 5-HT_{2A} receptor in 5-HT depleted state by using behavioral assessment and Fos protein expression

It was found similar results as the control group. Ketanserin reduced non-nociceptive behavior significantly compared with the PCPA administered and the control which treated with ketanserin (977.2259.4, 1108.0±383.3 seconds, respectively, $p<0.05$) Table [4-22]. The still but alert behaviors increased significantly compared with the PCPA administered group too (447.60±239.2, 170.1±166.3 seconds, respectively, $p<0.05$). For resting and sleeping time, there was no difference found between the two groups Figure [4-30].

For the paw withdrawal test, ketanserin did not alter the paw withdrawal latency of 5-HT depletion group (8.9±1.7, 8.7±1.4 seconds, respectively). By using the pair t-test no difference was detected between the latency of the ipsilateral and contralateral hind paws Table [4-24], Figure [4-31].

With the immunohistochemical study, it was found that ketanserin did not alter the expression of Fos protein in both hemispheres of the somatosensory cortex Table [4-25], Figure [4-32], [4-33].

Table [4-22] The effect of ketanserin on behaviors of the rats in the control group compared with the PCPA-induced 5-HT depletion group

Behaviors (Sec)	Group: PCPA-induced 5-HT depletion	Group: PCPA with ketanserin	Mean difference (95% CI)	p-value of PCPA & PCPA +ketanserin
Spontaneous nociceptive	0.0 ±0.0	0.0 ±0.0	0.0 (0.0 to 0.0)	1.000
Non-nociceptive	1108.0±383.3	997.2 ±259.4	501.30 (97.1 to 905.5)	0.022
Still but Alert	692.0±383.3	447.6 ±237.2	-281.4 (-593.5 to 30.8)	0.071
Rest or Sleep	0.0 ±0.0	375.2 ±308.4	-221.5 (-623.2 to 180.3)	0.234

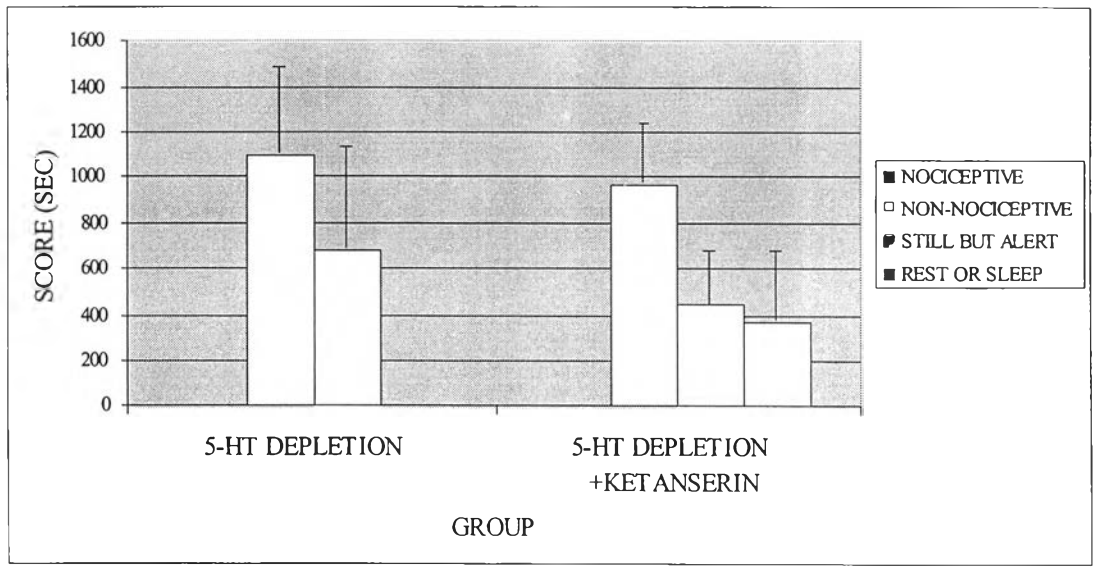

Figure [4-30] Bar graph showing the mean value ± SD of the observed behaviors of the rats in the 5-HT depletion group compare, with ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. * indicated the significantly difference between groups.

Table [4-23] The effect of ketanserin on paw withdrawal latency of the PCPA-induced 5-HT depletion compared with the control group

Latency (Sec)	Group		Mean difference (95% CI)	p-value of PCPA & PCPA +ketanserin
	PCPA-induced 5-HT depletion	PCPA with ketanserin		
Left side	11.2 ±2.6	9.7±0.9	1.28 (-0.68 to 3.24)	0.171
Right side	9.3±1.0	8.8±1.7	-0.04 (-2.35 to 2.27)	0.969

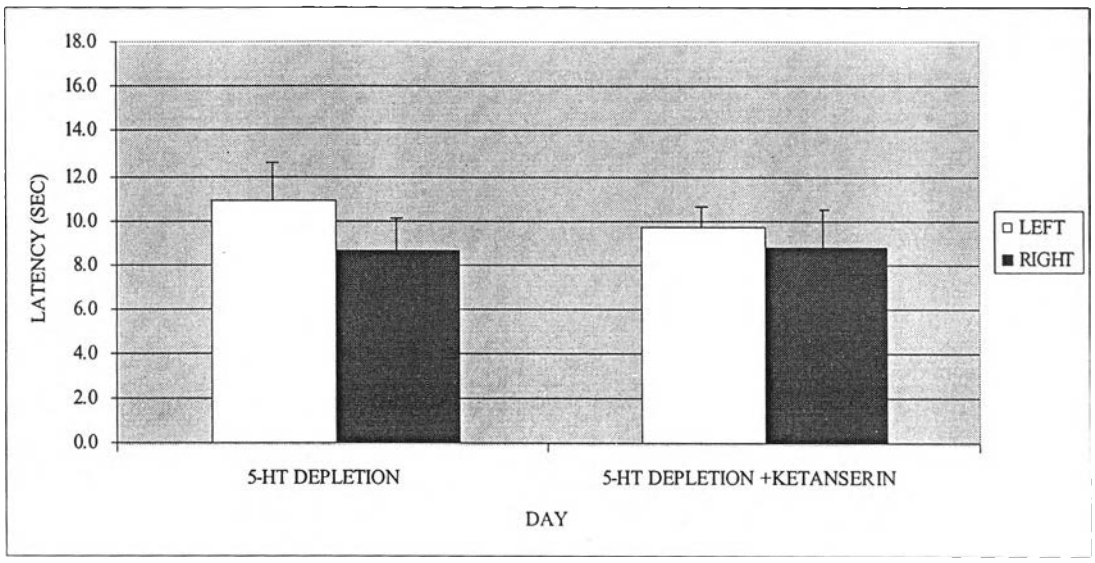

Figure [4-31] Bar graph showing the mean value ± SD of the paw withdrawal latency of the rats in the 5-HT depletion group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. * indicated the significantly difference between groups.

Table [4-24] The effect of ketanserin on the number of Fos positive cells of the PCPA-induced 5-HT depletion compared with the control group

Fos positive cells	Group		Mean difference (95% CI)	p-value of PCPA & PCPA +ketanserin
	PCPA-induced 5-HT depletion	PCPA with ketanserin		
Left side	6 ±2	5 ±1	0.67 (-2.26 to 3.59)	0.561
Right side	4 ±2	5 ±1	-1.00 (-4.07 to 2.07)	0.417

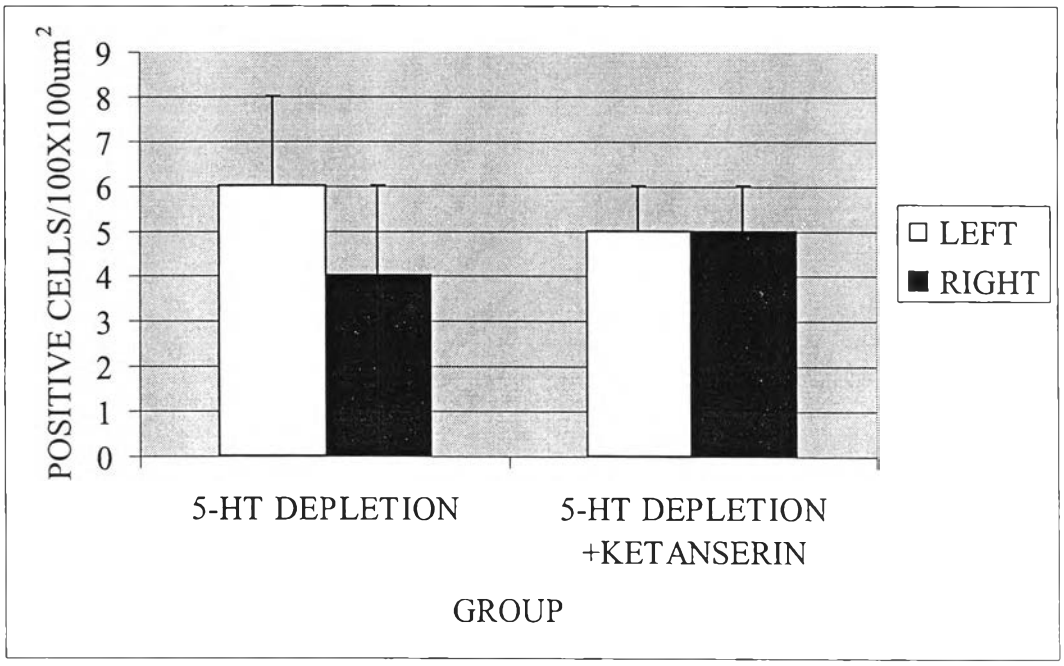

Figure [4-32] Bar graph showing the mean value ± SD of the number of Fos-IR neurons of the rats in the 5-HT depletion group compared with the ketanserin treated group. Significant difference of different treatment was assessed with independent sample t-test. * indicated the significantly difference between groups.

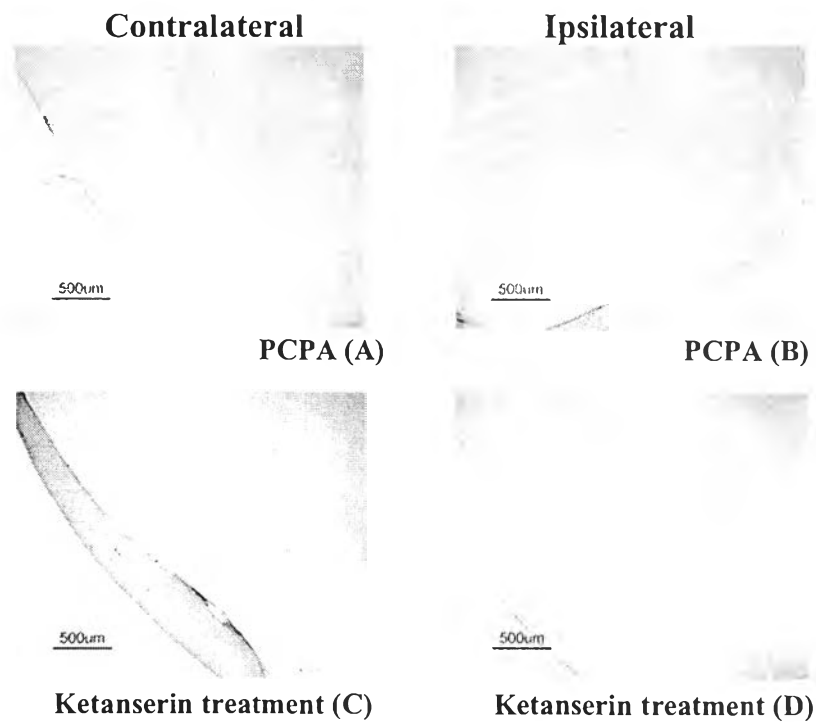

Figure [4-33] The pictures showing

(A)the expression of Fos-IR neurons in left side of the PCPA group

(B)the expression of Fos-IR neurons in right side of the PCPA group

(C)the expression of Fos-IR neurons in left side of the PCPA group with ketanserin treated

(D)the expression of Fos-IR neurons in right side of the PCPA group with ketanserin treated

Bar = 500 μm