CHAPTER III

RESULTS

The results were divided into 3 parts on following :

1. Effects of 5-HT_1-agonist and 5-HT_2-agonist on thermal nociception by measuring tail flick latency.
2. Effects of 5-HT_1-agonist and 5-HT_2-agonist on chemical stimulation-induced nociceptive behaviors.
3. Effects of 5-HT_1-agonist and 5-HT_2-agonist on noxious stimulation-evoked Fos immunoreactive neurons in dorsal horn

1. TAIL FLICK TEST TO HEAT STIMULATION

There were significant difference in the tail flick latency between with and without administration of the agonists, whereas no different in the control group. The 8-OH-DPAT attenuated the nociceptive action as indicated by increasing of the time of tail flick latency While DOI enhanced it as indicated by the reducing tail-flick latency (Table 4, Figure 7).

Table 4. Comparison of pre-and post-test in tail flick latency among NSS, 8-OH-DPAT and DOI group.

Groups of treatment	Tail flick latency (sec)	
	Before administration	After administration
NSS	7.02±0.54	7.18±0.56
8-OH-DPAT	5.91±0.42	12.11±0.49 ж
DOI	7.35±0.81	1.29±0.13 Ψ

Data are compared as mean ± SEM

ж significant difference from the before 8-OH-DPAT administrative group with $p < 0.001$

Ψ significant difference from the before DOI administrative group with $p<0.001$

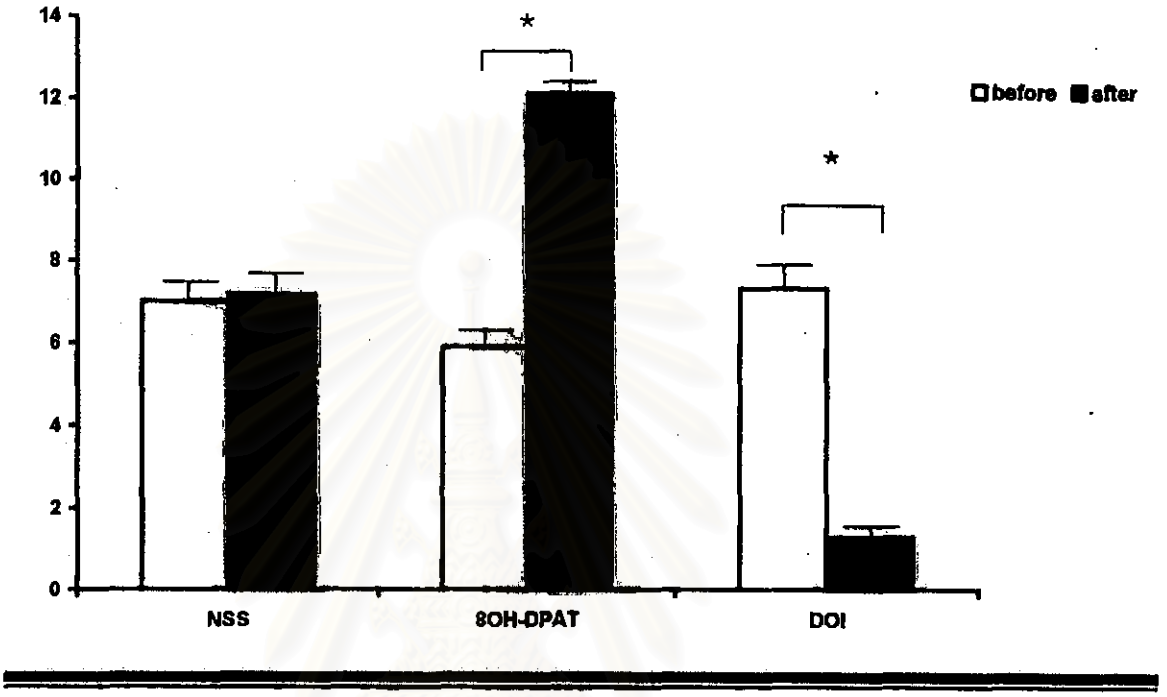


Figure 7. Effect of 8-OH-DPAT, DOI and NSS on tail flick latency, comparing between pre-and post-drug administration

Data are mean ± SEM

* significant difference with $p<0.001$

EFFECTS OF 5-HT AGONIST ON CHEMICAL STIMULATION INDUCED NOCICEPTIVE BEHAVIOR

Lifting behavior responses

The lifting time was significantly decreased by 8-OH-DPAT treatment. (Table 5, Figure 8).

Table 5. Comparison of time course of lifting behavior response

Time course of lifting behavior response (sec)			
Phase	NSS	8-OH-DPAT	DOI
I (1-10)	7.1±1.85	1.17±0.30 §	4.08±1.11
II (10-60)	34.95±8.76	4.3±2.60 §	24.04±3.22

Data are mean ± SEM

§ significantly different from control group with $p<0.05$

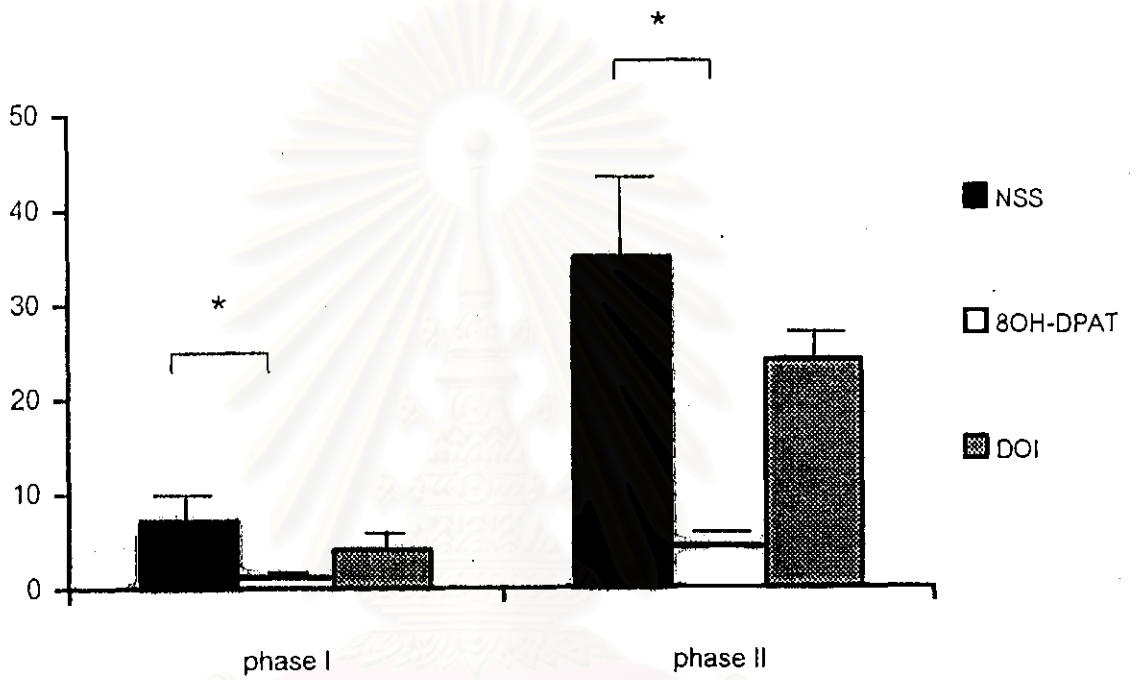


Figure 8. Bar graphs represented the time course of lifting behavior response among NSS, 8-OH-DPAT, and DOI group.

Data are mean ± SEM

* significant difference with $p<0.05$

Licking behavior response

Phase I, the licking behavior of the control and treated groups were not different. However, 8-OH-DPAT reduced licking behavior response significantly at phase II (Table 6, Figure 9).

Table 6. Comparison of time course of licking behavior responses

Time course of licking behavior response (sec)			
Phase	NSS	8-OH-DPAT	DOI
I (1-10)	1.08±0.63	0.22±0.13	0.14±0.07
II (10-60)	3.39±1.09	0.75±0.04 §	2.07±0.38

Data are mean ± SEM

§ significantly different from control group with $p<0.05$

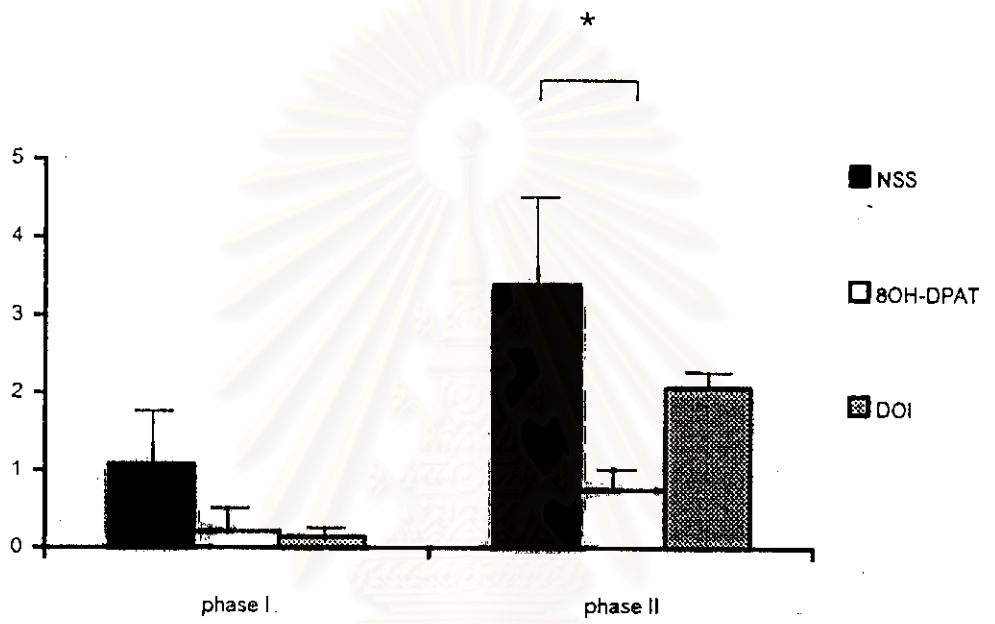


Figure 9. Bar graphs represented the time course of licking behavior response among NSS, 8-OH-DPAT, and DOI group.

Data are mean ± SEM

* significant difference with $p<0.05$

Scratching behavior response

The 8-OH-DPAT reduced the scratching behavior significantly in both phase I and II while those in DOI treated groups remained unchanged (Table 7, Figure 10).

Table 7. Comparison of time course of scratching behavior responses

Time course of scratching behavior response (sec)			
Phase	NSS	8-OH-DPAT	DOI
I (1-10)	0.2±1.85	0.05±0.36 §	1.00±0.02
II (10-60)	2.63±8.78	0.24±2.44 §	0.18±3.22

Data are mean ± SEM

§ significantly different from the NSS group with $p<0.05$

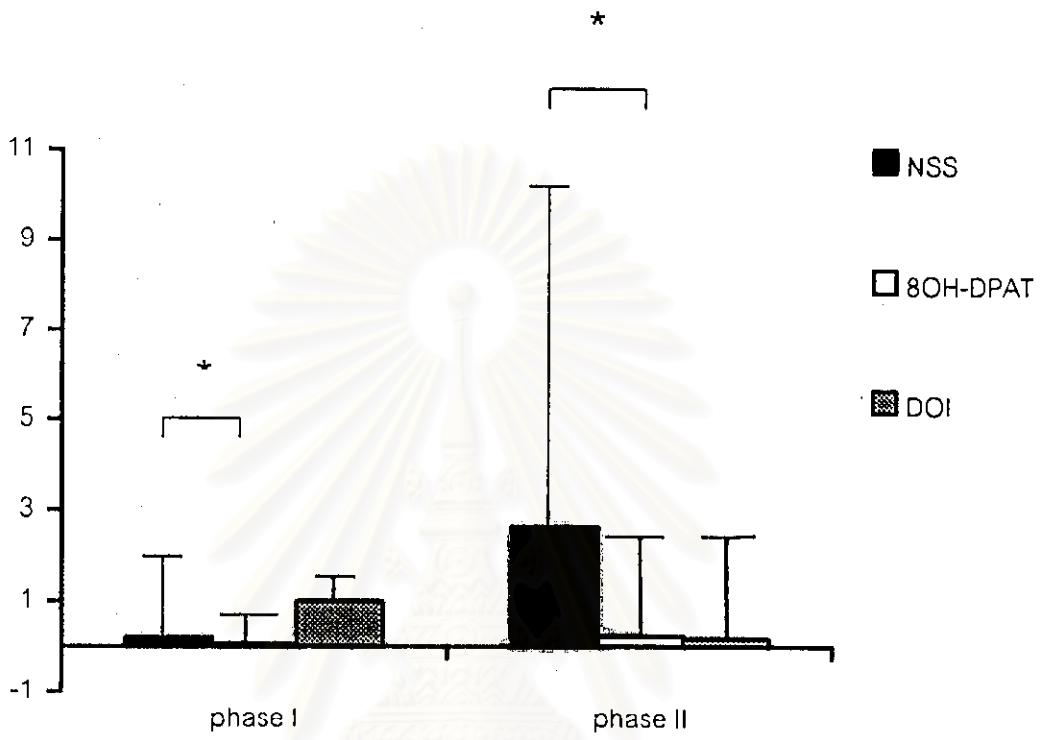


Figure 10. Bar graphs represented the time course of scratching behavior response among NSS, 8-OH-DPAT, and DOI group.

Data are mean ± SEM

* significant difference with $p<0.05$

3. FOS EXPRESSION IN THE FORMALIN TEST

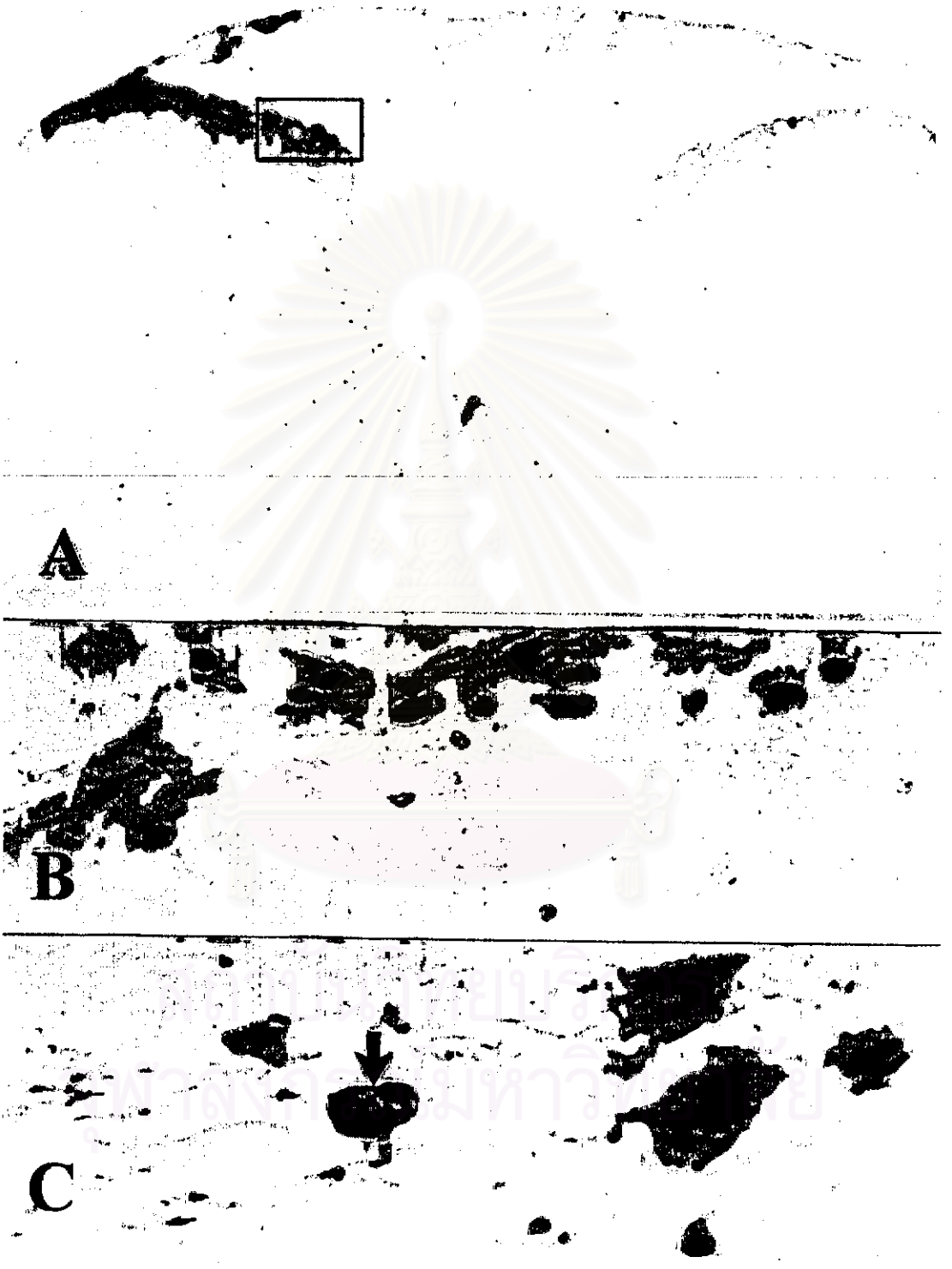
3.1 Fos-like immunoreactivity in the spinal level

The Fos-like immunoreactivity (FLI) in the spinal level was extremely low in the animals injected with only normal saline solution into the plantar forepaw. Less than five neurons from the C_7-T_1 cord segment were stained ipsilaterally (Table 8). In contrast, injection of formalin into the plantar forepaw evoked a great number of FLI in the gray matter of the cervical enlargement. The greatest number of labeled neurons were noted at the C_7-T_1 spinal level which corresponded to the segmental innervation of the rat plantar forepaw. Most of FLI was ipsilaterally confined ipsilaterally to the injured paw. Only few FLI neurons were seen at the contralateral side. In addition, the superficial dorsal horn (lamina I and II), nucleus proprius (lamina III/IV), and the neck of dorsal horn (lamina V) were the layers of the cord that presented a dense FLI (Table 12, Figure 18).

Therefore, in this experiment the FLI of the lamina I/II, lamina III/IV and lamina V at the spinal level of C_7-T_1 of the ipsilateral side were examined and counted for the data analysis.

Table 8. Comparison of pattern and number of Fos-immunoreactive neurons in formalin-injected and NSS-injected left forepaw

Level	NSS-injected forepaw (n=3)	Formalin-injected forepaw (n=8)
Ipsilateral side		
C_5 lamina I/II	0	0
Lamina III/IV	0	0
Lamina V	0	0
C_6 lamina I/II	0	0
Lamina III/IV	0	0
Lamina V	0	0
C_7 lamina I/II	1.25±0.09	12.93±0.96
Lamina III/IV	0	8.09±0.98
Lamina V	2.5±1.03	17.15±1.66
C_8 lamina I/II	1.67±0.02	17.69±0.83
Lamina III/IV	0	8.69±0.76
Lamina V	3±0.36	17.50±1.21
T_1 lamina I/II	1.00±0.09	10.14±0.59
Lamina III/IV	0	5.09±0.53
Lamina V	2.34±1.01	12.80±1.11
Contralateral side		
C_5 lamina I/II	0	0
Lamina III/IV	0	0
Lamina V	0	0
C_6 lamina I/II	0	0
Lamina III/IV	0	0
Lamina V	0	0
C_7 lamina I/II	0	2.3±0.22
Lamina III/IV	0	0
Lamina V	0	2.75±0.18
C_8 lamina I/II	0	3.18±0.13
Lamina III/IV	0	1.5±0.07
Lamina V	0	1.21±0.04
T_1 lamina I/II	0	1.59±0.28
Lamina III/IV	0	0.75±0.01
Lamina V	0	1.12±0.14


A
B
C

3.2 Effects of 5-HT-agonists on noxious stimulation evoked Fos-immunoreactive cells in dorsal horn neurons

At the C_7 spinal level, the number of FLI neurons in both lamina I/II and V were decreased non significantly by 8-OH-DPAT treatment, whereas those in DOI group were increased. However there were the opposite results in the lamina III/IV. There were significant increase of the number of FLI neurons in DOI treated-agonist group in lamina I/II (Table 9, Figure 12-13).

Table 9. The number of Fos-immunoreactive neurons at C_7 spinal level in control and treated groups

Number of Fos-immunoreactive neurons at C_7level (cells / section)			
	NSS	8-OH-DPAT	DOI
Lamina I/II	12.93±0.96	12.57±0.66 †	16.78±0.72 §
Lamina III/IV	8.09±0.98	10.32±1.46	7.21±0.43
Lamina V	17.15±1.66	12.87±1.16	20.31±1.12 £

Data are the mean ± SEM

§ significant difference from the control group with $p<0.05$

£ significant difference from the 8-OH-DPAT group with $p<0.001$

† significant difference from the DOI group with $p<0.05$

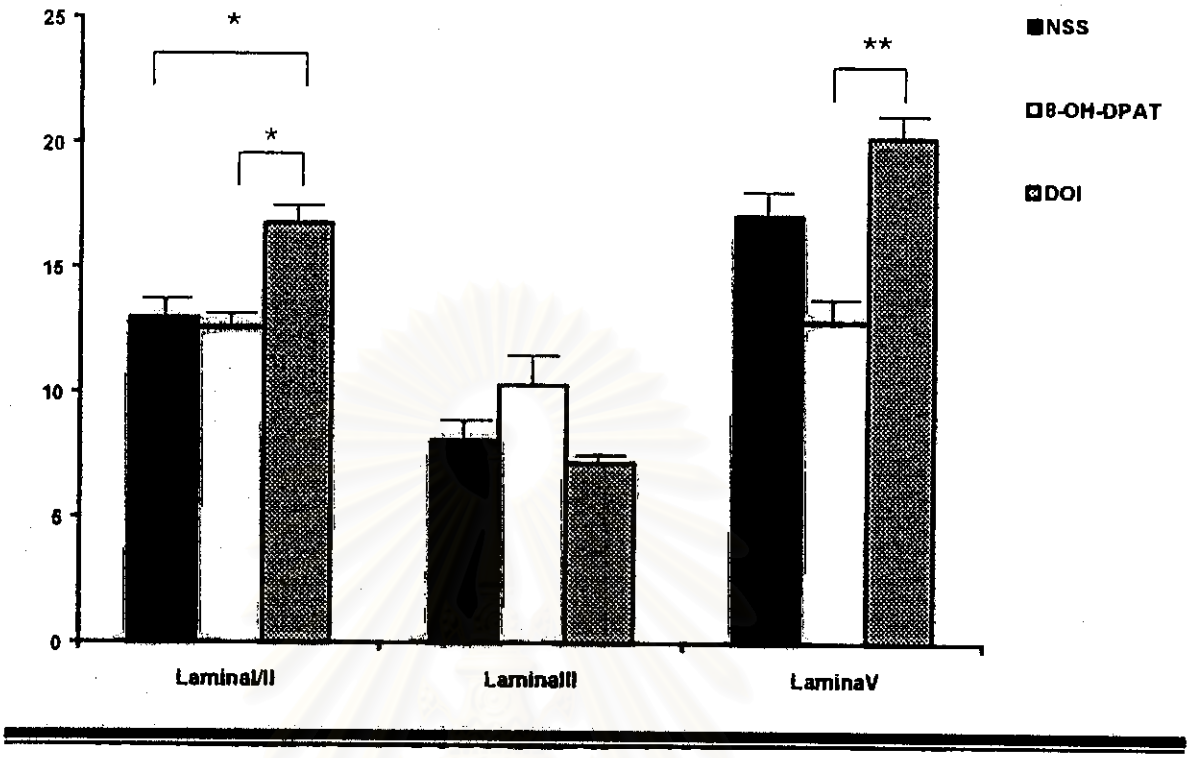


Figure 12. Bar graphs represented the number of Fos-immunoreactive neurons at C_7 spinal level

Data are mean ± SEM

* significant difference with $p<0.05$

** significant difference with $p<0.001$

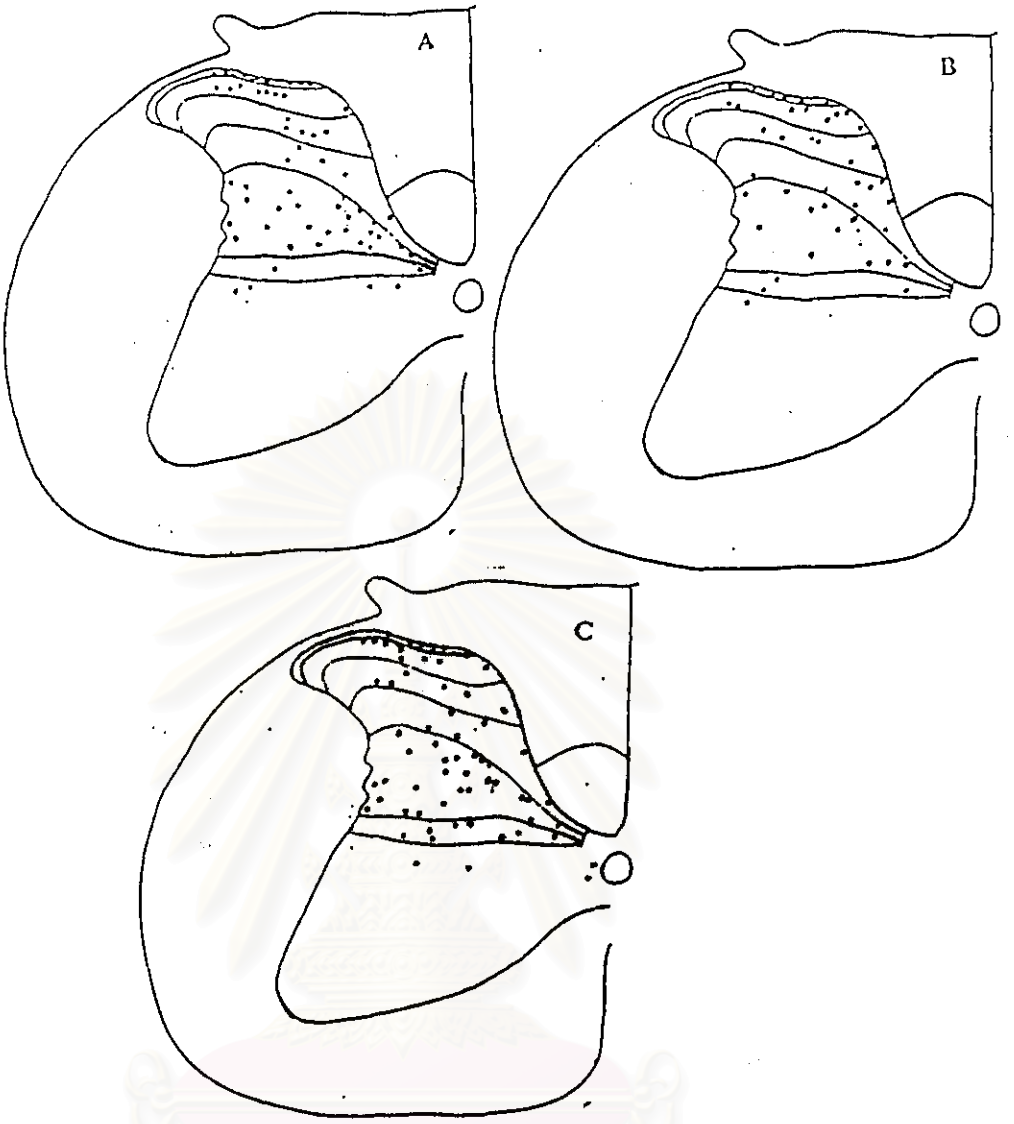


FIGURE 13. Diagram showing the distribution of Fos-immunoreactive neurons (dot) at C_7 spinal level 2 hours after formalin injection. There is labeling in the ipsilateral to noxious stimuli. The densest labeling is in the lamina I,II and V. (A.) NSS-treated (B.) 8-OH-DPAT-treated and (C.) DOI-treated rats

Regarding the C_8 spinal level, the number of FLI neurons were significantly increased in 8-OH-DPAT treated-agonist group in lamina III/IV while DOI decreased non significantly (Table 10, Figure 14-15, 19).

Table 10. The number of Fos-immunoreactive neurons at C_8 spinal level in control and treated groups

Number of Fos-immunoreactive neurons at C_8 level (cells/ section)			
	NSS	8-OH-DPAT	DOI
Lamina I/II	17.69±0.83	16.98±1.12	15.80±0.68
Lamina III/IV	8.69±0.76	12.13±1.49 §	5.45±0.36 £
Lamina V	17.50±1.21	15.49±0.89	14.88±0.69

Data are the mean ± SEM

§ significant difference from the control group with $p<0.05$

£ significant difference from the 8-OH-DPAT group with $p<0.001$

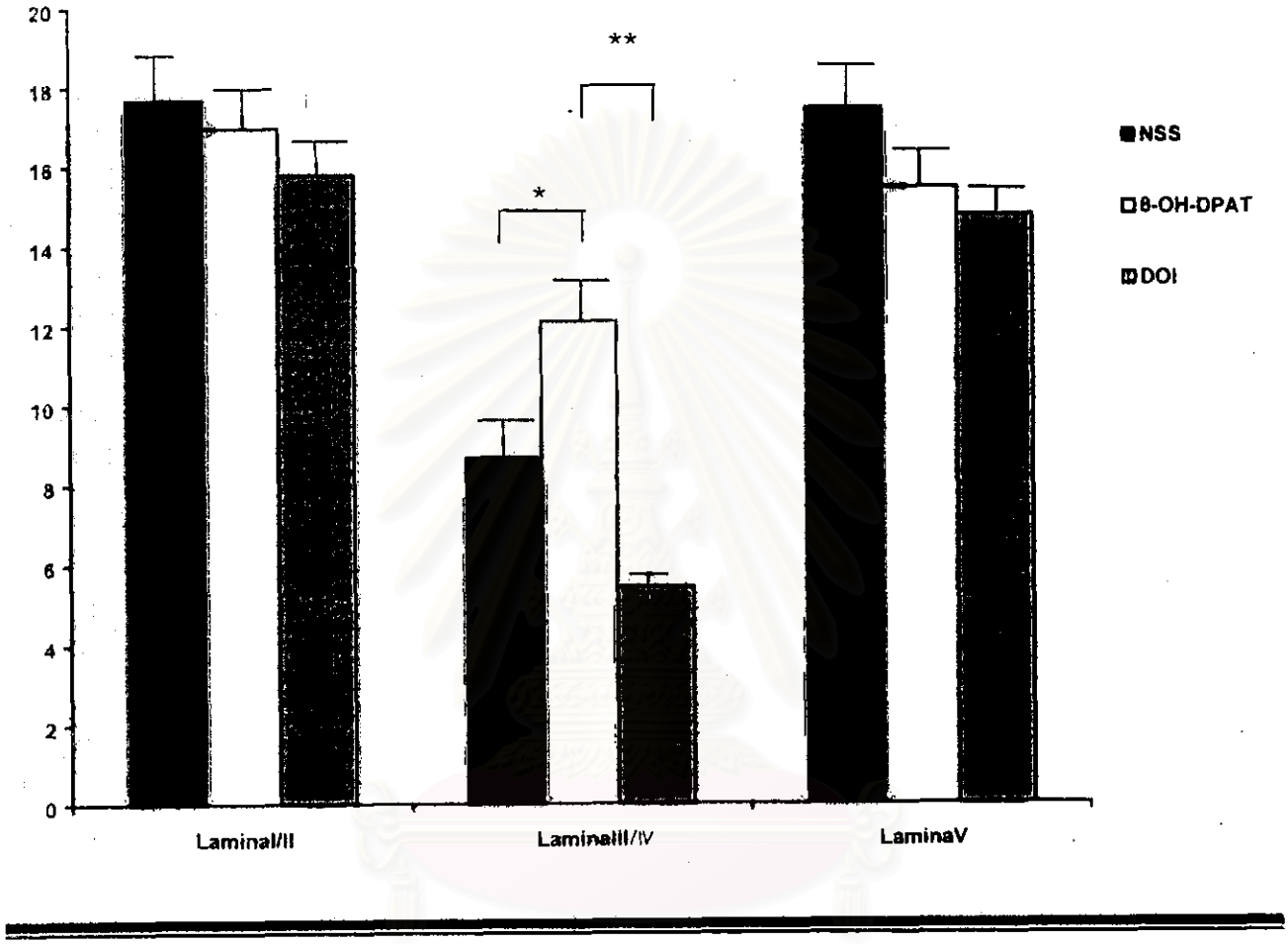


Figure 14. Bar graphs represented Fos-immunoreactive neurons at C_8 spinal level

Data are mean ± SEM

* significant difference with $p<0.05$

** significant difference with $p<0.001$

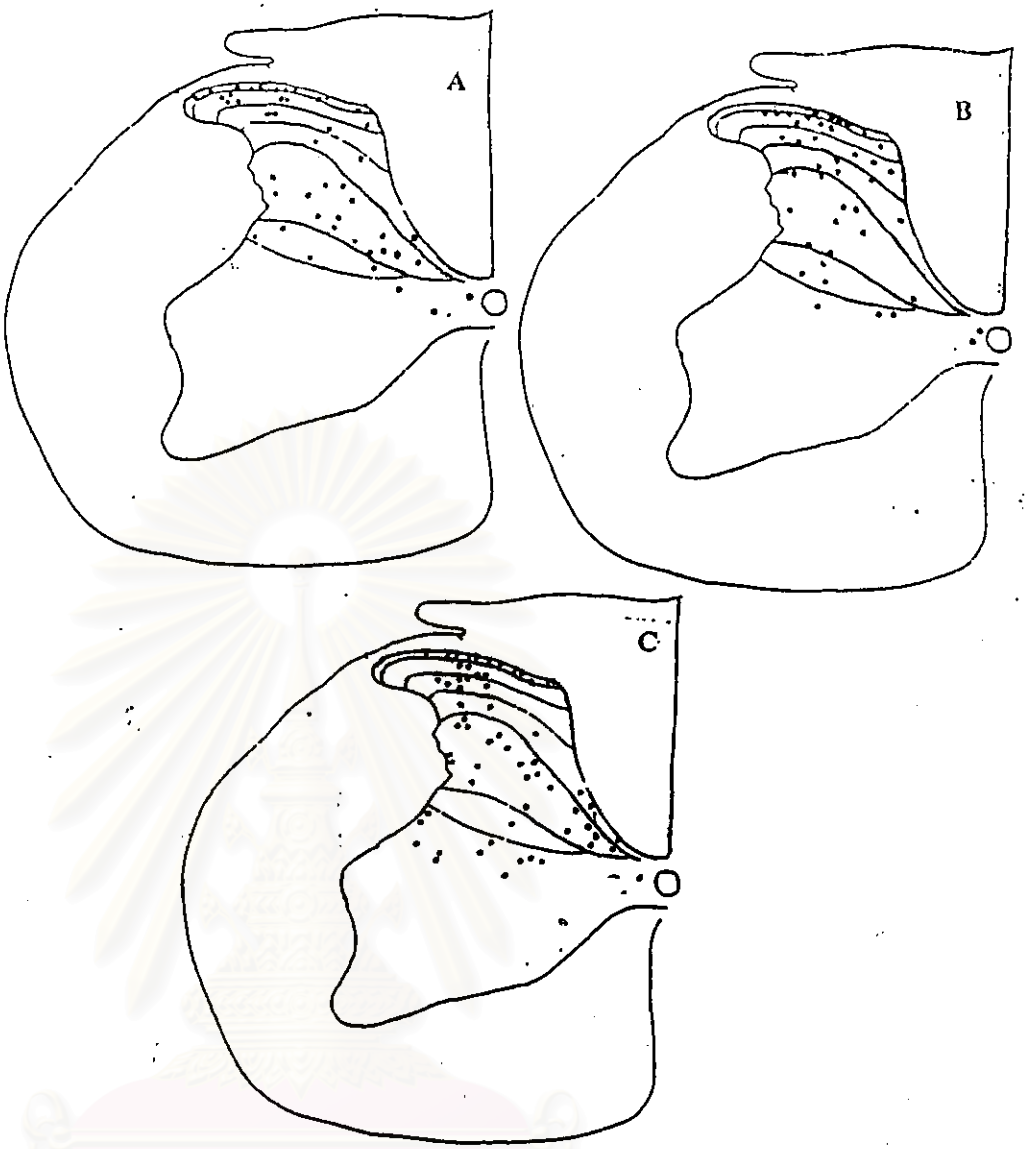


FIGURE 15. Diagram showing the distribution of Fos-immunoreactive neurons (dot) at C_8 spinal level 2 hours after formalin injection. There is labeling in the ipsilateral to noxious stimuli. The densest labeling is in the lamina I,II and V. **(A.)** NSS-treated **(B.)** 8-OH-DPAT-treated and **(C.)** DOI-treated rats

At the T_1 spinal level, DOI increased the number of FLI neurons in lamina I/II and decreased them in lamina III/IV when it compared to 8-OH-DPAT. In contrast 8-OH-DPAT increased the number of FLI neurons in lamina III/IV form control (Table 11, Figure 16-17).

Table 11. The number of Fos-immunoreactive neurons at T_1 spinal level in control and treated groups

Number of Fos-immunoreactive neurons at T_1 level (cells / section)			
	NSS	8-OH-DPAT	DOI
Lamina I/II	10.14±0.59	9.53±0.57	12.02±0.68 ✯
Lamina III/IV	5.09±0.53	7.6±0.94 §	5.18±0.43 ✯
Lamina V	12.80±1.11	13.71±1.06	16.18±0.94

Data are the mean ± SEM

§ significant difference from the control group with $p<0.05$

✯ significant difference from the 8-OH-DPAT group with $p<0.05$

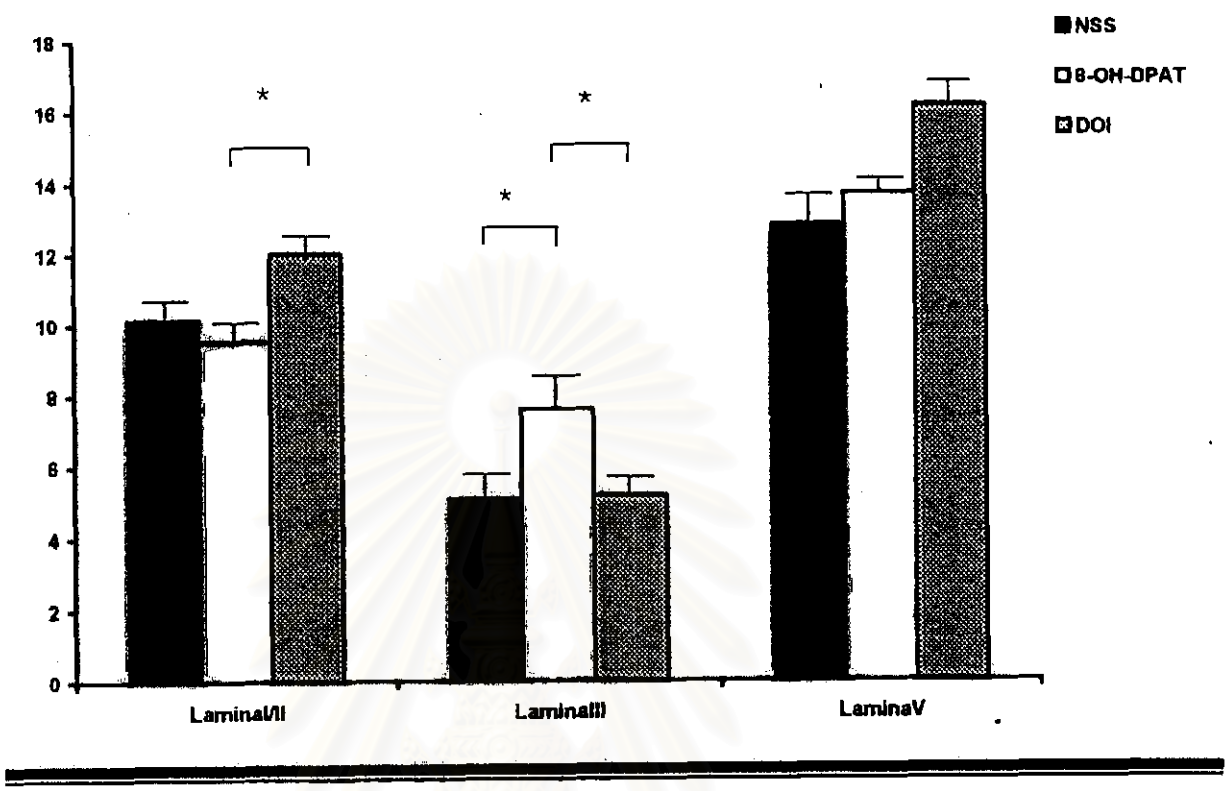


Figure 16. Bar graphs represented Fos-immunoreactive neurons at T_1 spinal level

Data are mean ± SEM

* significant difference with $p<0.05$

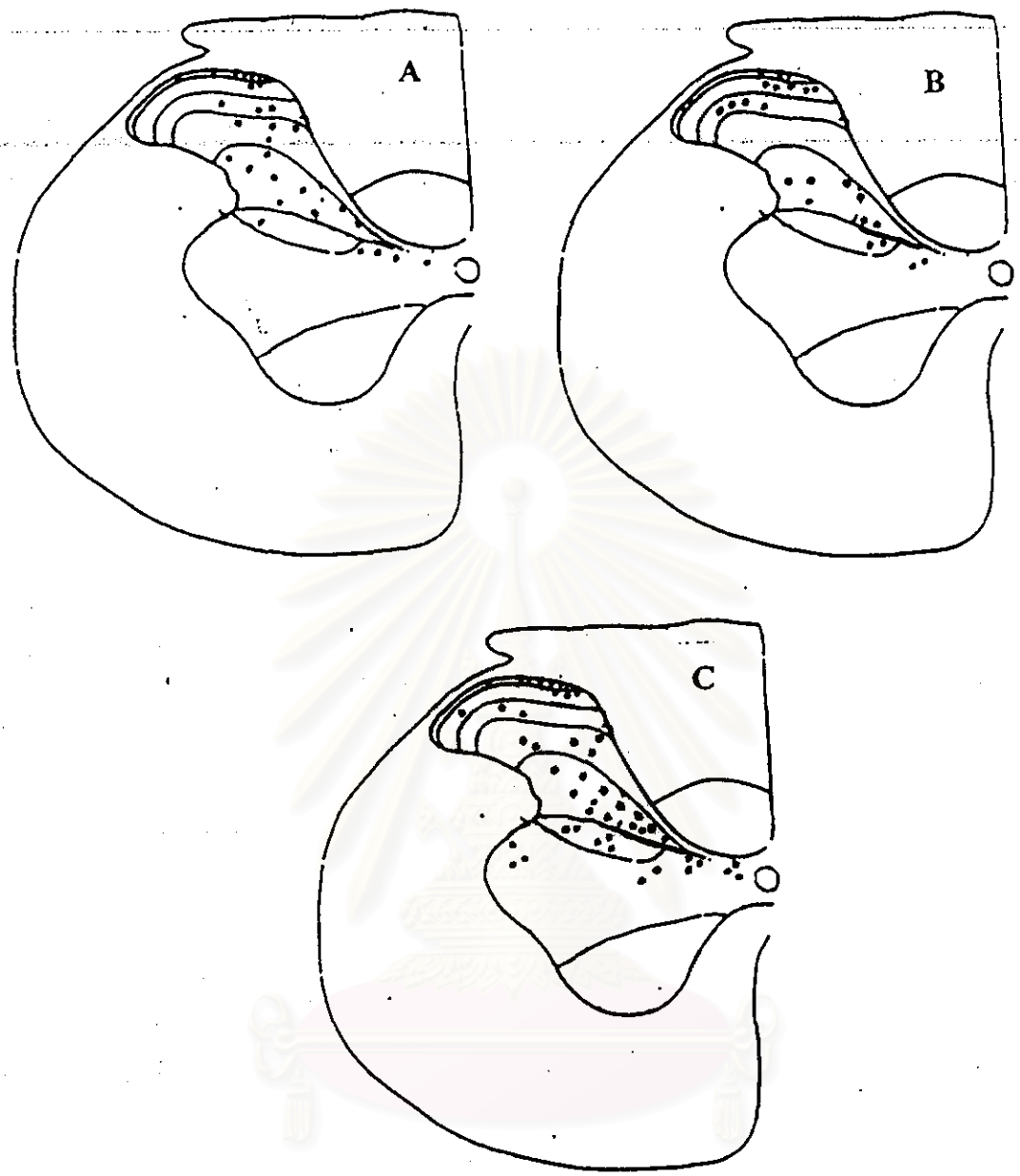


FIGURE 17. Diagram showing the distribution of Fos-immunoreactive neurons (dot) at T_1 spinal level 2 hours after formalin injection. There is labeling in the ipsilateral to noxious stimuli. The densest labeling is in the lamina I,II and V. (A.) NSS-treated (B.) 8-OH-DPAT-treated and (C.) DOI-treated rats

The number of FLI neurons counted from the C_7-T_1 spinal level all together were demonstrated in the Table 12. The 8-OH-DPAT increased significantly the number of FLI in lamina III/IV as compared to control group (Table 12, Figure 18).

Table 12. The numbers of Fos-immunoreactive neurons at C_7-T_1 spinal level in the NSS, 8-OH-DPAT, and DOI group.

Number of Fos-immunoreactive neurons at C_7-T_1 level (cells / section)			
	NSS	8-OH-DPAT	DOI
Lamina I/II	12.93±0.96	12.57±0.66	16.79±0.73 ☆
Lamina III/IV	8.08±0.99	10.32±1.46 §	6.06±0.25 £
Lamina V	17.15±1.65	12.87±1.16	20.31±1.12 ☆

Data are the mean ± SEM

§ significant difference from the control group with $p<0.05$

☆ significant difference from the 8-OH-DPAT group with $p<0.05$

£ significant difference from the 8-OH-DPAT group with $p<0.001$

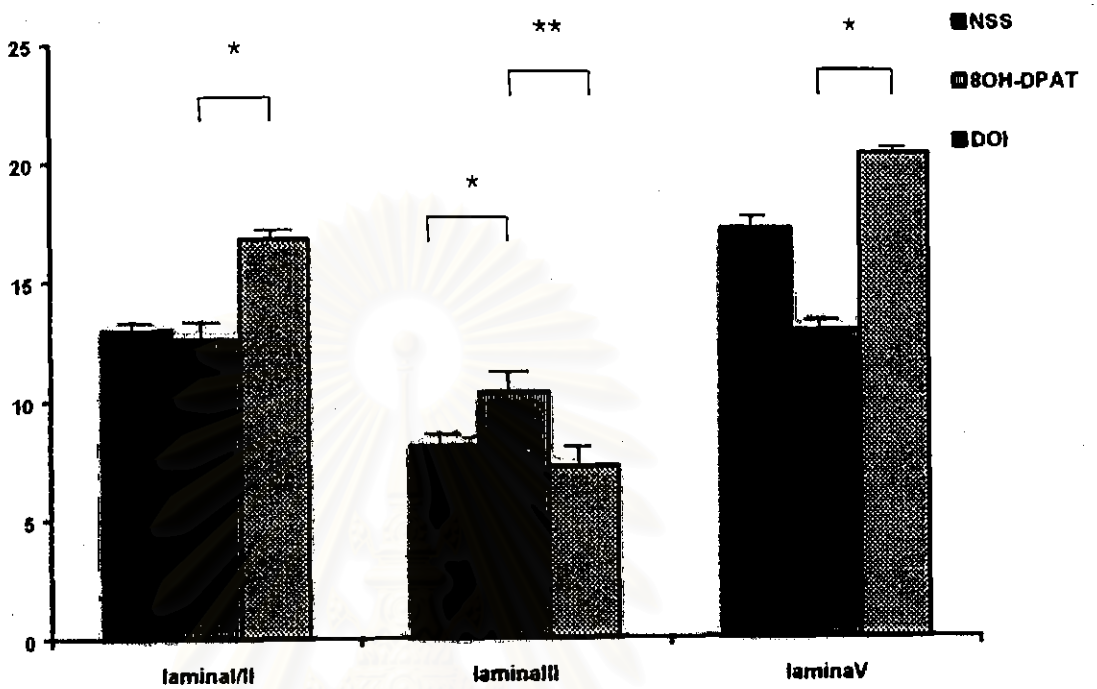


Figure 18. Bar graphs represented Fos-immunoreactive neurons at C_7-T_1 spinal level

Data are mean ± SEM

* significant difference with $p<0.05$

** significant difference with $p<0.001$

FIGURE 19. The photomicrograph illustrated the pattern of formalin-evoked Fos immunoreactive neurons from sections of the C_8 spinal cord of **A)** NSS-treated **B)** 8-OH-DPAT treated and **C)** DOI-treated rats. Pretreatment with 8-OH-DPAT reduced the number of labeled cells in the lamina I,II and V and while pretreatment with DOI enhanced the number of labeled cells in the lamina I,II and V.

C