CHAPTER IV

RESULTS

The results of this study were organized into two parts as follows

Part I: Results from all experiments performed for studying the effect of N/OFQ on development of CSD-evoke depolarization shift.

Part II: Results from all experiment performed for studying the effect of N/OFQ on trigeminal nociceptive system in CSD model.

Part I: Results from all experiments performed for staying the effect of N/OFQ on cortical activity in CSD model.

CSD was induced by topical application of KCl 3 mg on surface of parietal cortex for 2 hours.CSD induced by KCl can induce cortical activity indicated by the DC shift of surface cortex. In contrast, application of solid NaCl, it did not effect on cortical activity because cannot induce DC shift as compared to KCl-vehicle group.

1.) The electrophysiological variables related to CSD-evoke DC shift

The DC shift change was studied in the all group in order to evaluate the effect of CSD on number of peak, peak amplitude, interpeak latency, duration, averaged and summed AUC of DC shift for 1 hour. Application of KCl induced a repeated pattern of cortical depolarization (Figure 4.1). In contrast, application of NaCl not effect on cortical activity (Figure 4.3). In KCl-vehicle group and KCl-N/OFQ, the mean ± SD of amplitude was 26.86±0.99 and 32.35 ± .54 mV, respectively (Table 4.1), the mean ± SD of numbers within 1 hour was 12.25 ± 0.31 and 14.88 ± 0.295 respectively (Table 4.2), the mean ± SD of interpeak latency was 165.05±72.32 and 104.95±38.65 seconds respectively (Table 4.3), the mean ± SD of duration was 64.92 ± 8.62 and 61.02 ± 7.29 seconds, respectively (Table 4.4), the mean ± SD of AUC was 20.02 ± .758 and 24.60 ± .92 mV-seconds, respectively (Table 4.5), and mean ± SD of sum of AUC was 181.56 ± 5.77 and 291.16 ± 9.54 mV-sec. respectively (Table 4.6).

Comparing KCl-vehicle and KCl-N/OFQ group, we found that the mean ± SD of amplitude were increase significantly (Figure 4.4) (26.86±0.99 and 32.35 ± .54 mV respectively) (Table 4.1). The numbers of peak were increase significantly (Figure 4.5) (12.25 ± 0.31 and 14.88 ± 0.29 waves respectively) (Table 4.2). The mean ± SD of AUC was increase significantly (Figure 4.6) (20.02 ± .75 and 24.60 ± .92 mV-seconds) (Table 4.3). The mean ± SD of sums AUC was increase significantly (Figure 4.4) (181.56 ± 5.77 and 291.16 ± 9.54 mV-seconds) (Table 4.7). In the contrary, no significantly difference was observed in the interpeak latency (Figure 4.8) and duration of DC shift (Figure 4.9).

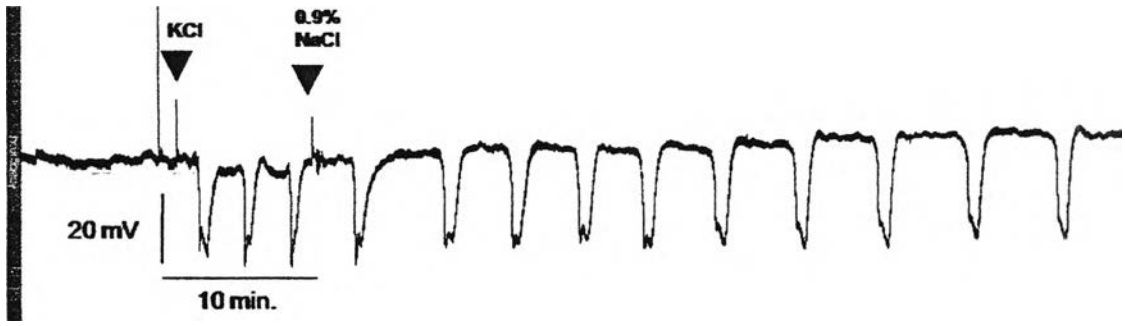


Figure 4.1 The tracing shows the KCl application induced repeated pattern of cortical depolarization characterizing the CSD.

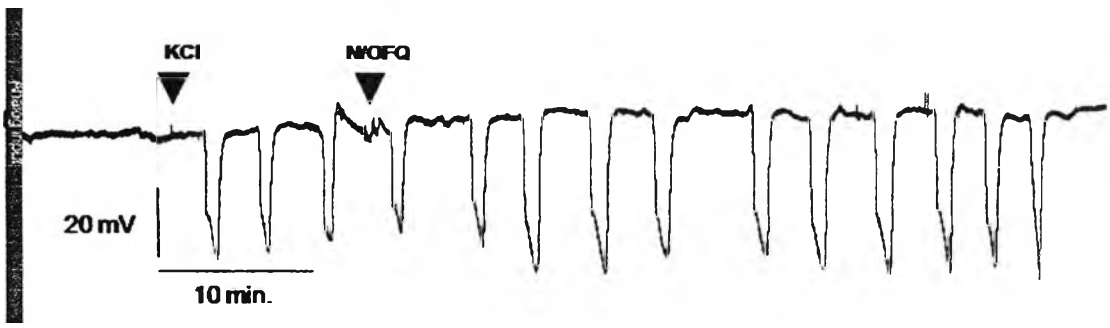


Figure 4.2 The tracing shows the KCl application and effect of N/OFQ on cortical activity.

Figure 4.3 The tracing shows the NaCl application had no effect on cortical activity.

Table 4.1 The mean ± SD of amplitude of DC shift obtained from NaCl-vehicle, KCl-vehicle and KCl-N/OFQ group.

Group	n	Mean ± SD of amplitude (mV)
NaCl-vehicle	6	N/A
KCl-vehicle	8	26.86 ± 0.99
KCl-N/OFQ	8	32.35 ± 0.54*

Note: * significantly different compared to KCl-vehicle group ($p < .05$)
N/A= Not Available

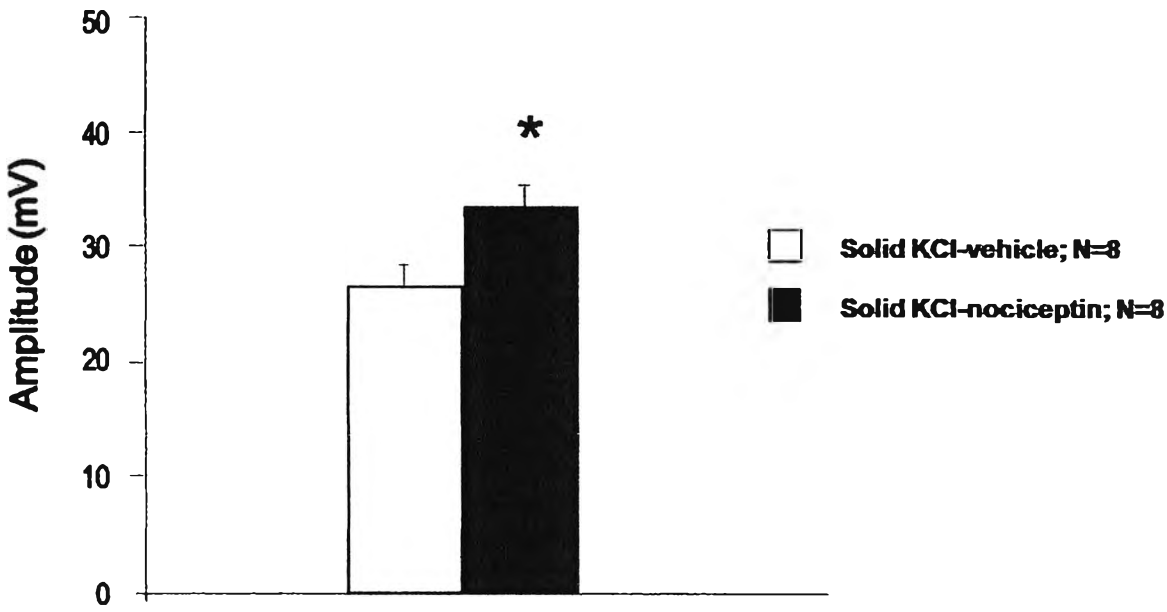


Figure 4.4 Bar graph shows the mean value ± SD of amplitude of peaks from KCl-vehicle and KCl-N/OFQ group. Significant difference was assessed with Student t-test. *$p<0.05$ compared with the KCl-vehicle group

Table 4.2 The mean ± SD of average numbers of DC shift obtained from KCl-vehicle and KCl-N/OFQ group.

Group	n	Mean ± SD of numbers of DC shift (waves)
NaCl-vehicle	6	N/A
KCl-vehicle	8	12.25 ± 0.31
KCl-N/OFQ	8	14.88 ± 0.29*

Note: * significantly different compared to NaCl-vehicle group ($p< .05$)
N/A= Not Available

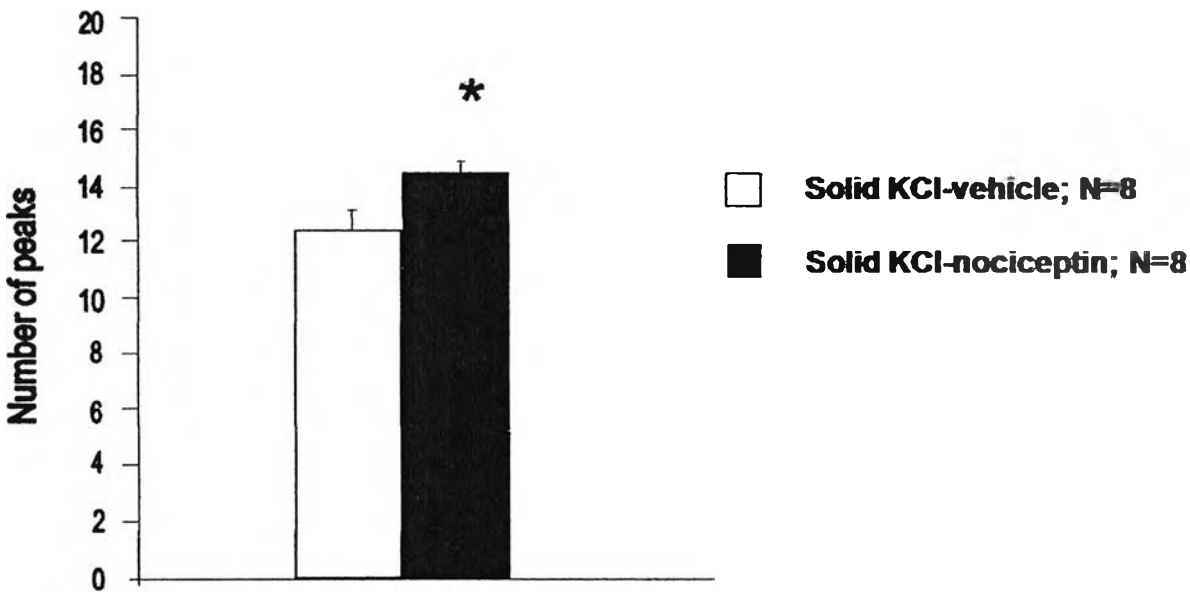


Figure 4.5 Bar graph shows the mean value ± SD of number of peaks from KCl-vehicle and KCl-N/OFQ group. Significant difference was assessed with Student t-test. *$p< 0.05$ compared with the KCl-vehicle group

Table 4.3 The mean ± SD of AUC of DC shift obtained from KCl-vehicle and KCl-N/OFQ group.

Group	n	Mean ± SD of AUC (mV-sec.)
NaCl-vehicle	6	N/A
KCl-vehicle	8	20.02 ± 0.75
KCl-N/OFQ	8	24.60 ± 0.92*

Note: * significantly different compared to NaCl-vehicle group ($p < .05$)
N/A= Not Available

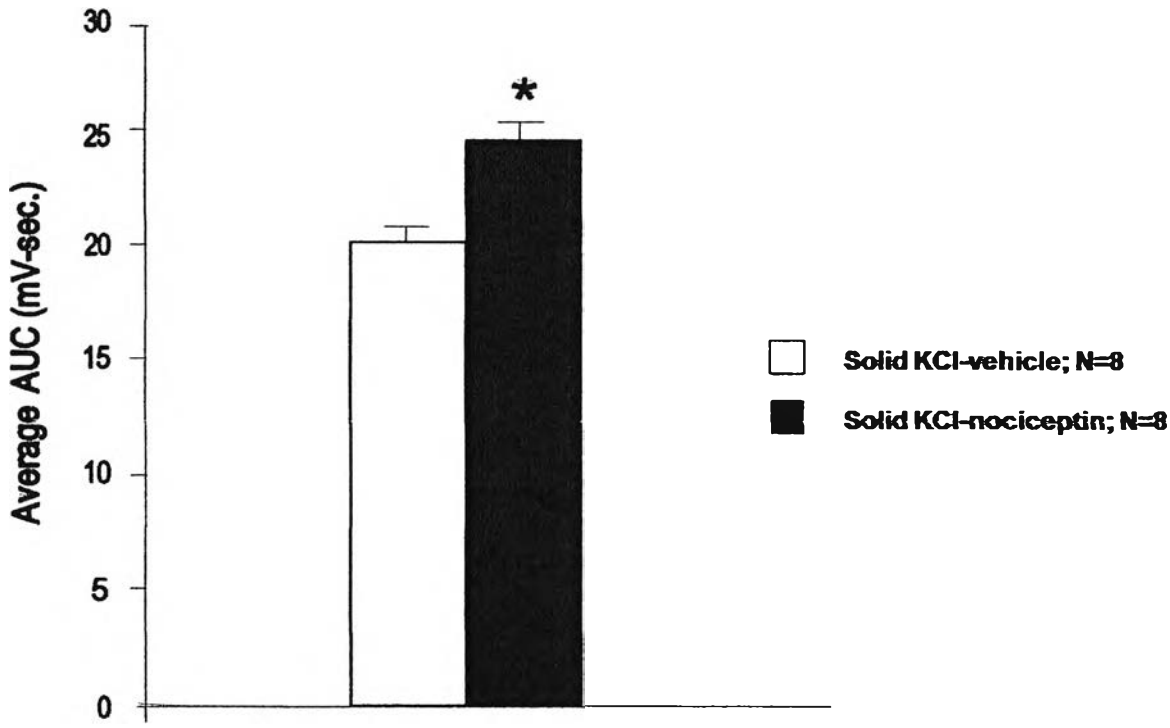


Figure 4.6 Bar graphs shows the mean value ± SD of average AUC from KCl-vehicle and KCl-N/OFQ group. Significant difference was assessed with Student t-test. *p< 0.05 compared with the KCl-vehicle group

Table 4.4 The mean ± SD of sum of AUC of DC shift obtained from KCl-vehicle and KCl-N/OFQ group.

Group	n	Mean ± SD of sum of AUC (mV-sec.)
NaCl-vehicle	6	N/A
KCl-vehicle	8	181.56 ± 5.77
KCl-N/OFQ	8	291.16 ± 9.54*

Note: * significantly different compared to NaCl-vehicle group ($p< .05$)
N/A= Not Available

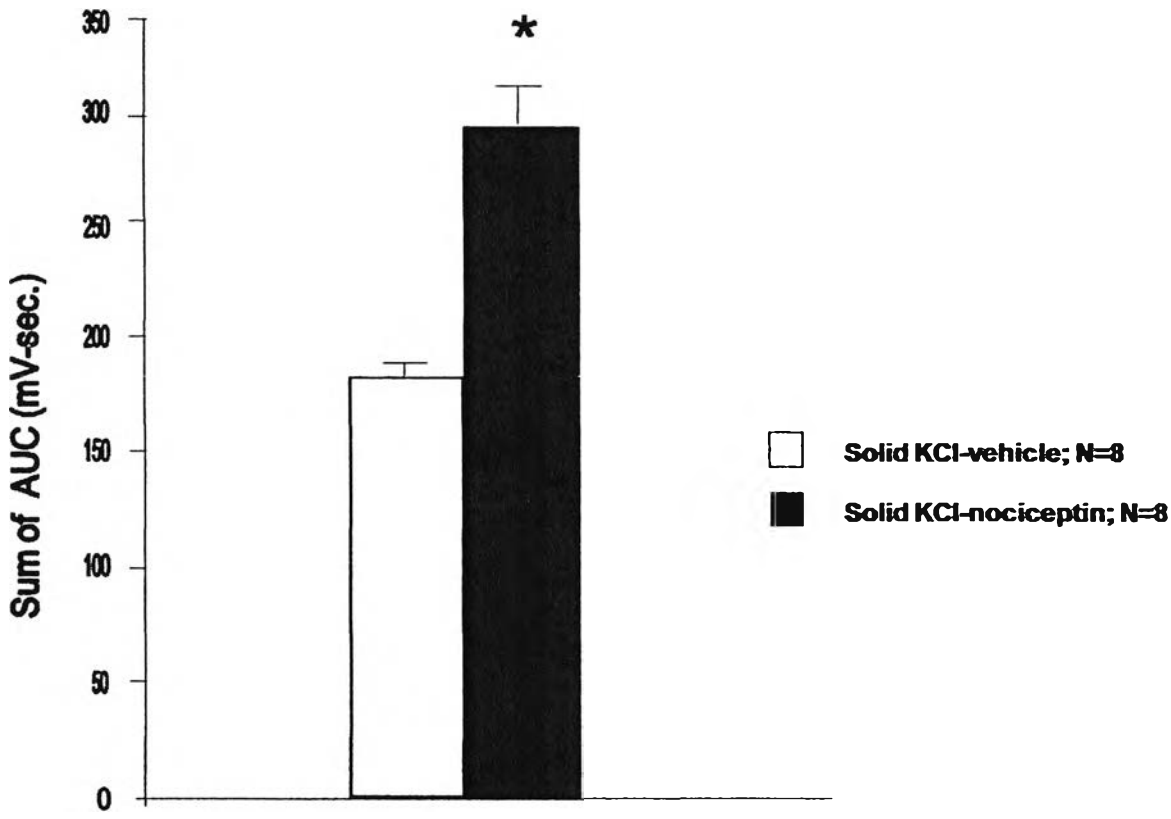


Figure 4.7 Bar graph shows the mean value ± SD of sum of AUC from KCl-vehicle and KCl-N/OFQ group. Significant difference was assessed with Student t-test. *$p<$ 0.05 compared with the KCl-vehicle group

Table 4.5 The mean value ± SD of interpeak latency of DC shift obtained from KCl-vehicle and KCl-N/OFQ group.

Group	n	Mean ± SD of interpeak latency (seconds)
NaCl-vehicle	6	N/A
KCl-vehicle	8	165.05 ± 72.32
KCl-N/OFQ	8	104.95 ± 38.65

Note: N/A= Not Available

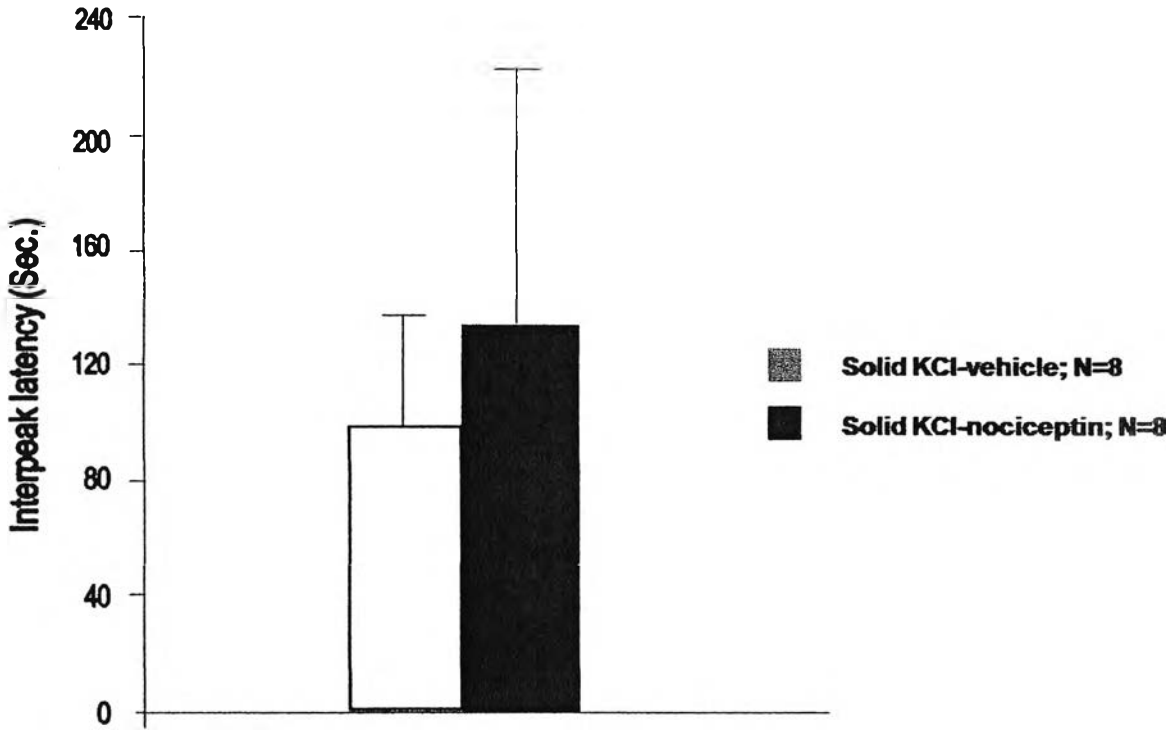


Figure 4.8 Bar graph shows the mean value ± SD of interpeak latency from KCl-vehicle and KCl-N/OFQ group. No significant difference was assessed with Student t-test.

Table 4.6 The mean value±SD of duration of DC shift obtained from KCl-vehicle and KCl-N/OFQ group.

Group	n	Mean ± SD of duration (seconds)
NaCl-vehicle	6	N/A
KCl-vehicle	8	64.92 ± 8.62
KCl-N/OFQ	8	61.02 ± 7.29

Note: N/A= Not Available

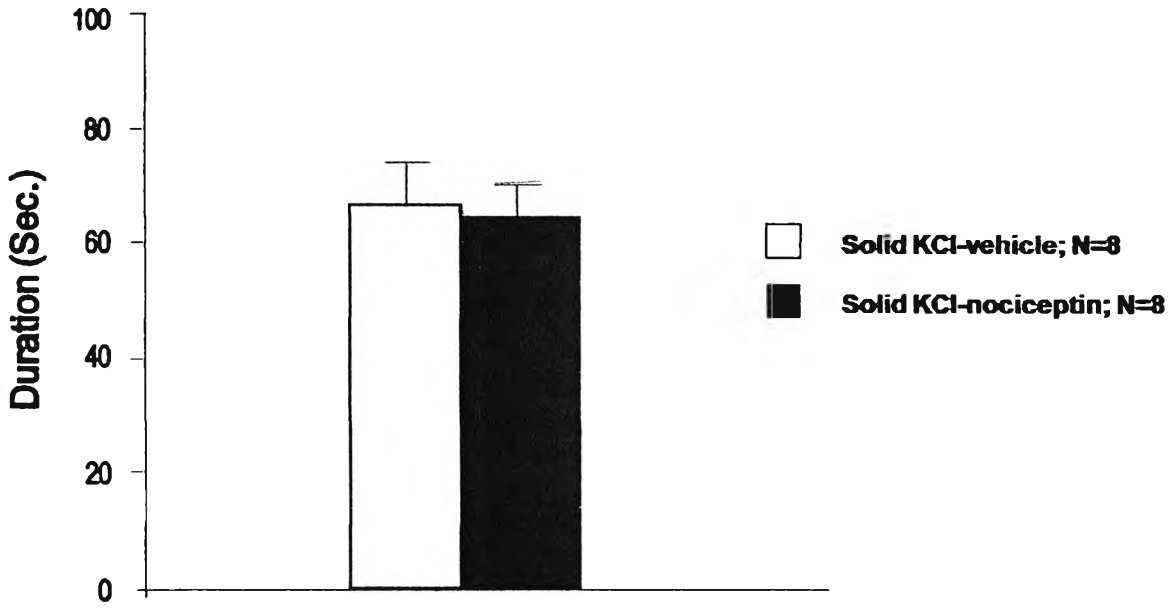


Figure 4.9 Bar graph shows the mean value ± SD of duration from KCl-vehicle and KCl-N/OFQ group. No significant difference was assessed with Student t-test.

Table 4.7 comparing the electrophysiology variables related to CSD between the KCl-vehicle and KCl-N/OFQ group.

	KCl-vehicle (N=8)	KCl-N/OFQ (N=8)	*p*-value
Amplitude (mV)	26.8 ± 60.33	32.35 ± 0.54*	0.012
Number of peak (waves)	12.25 ± 0.31	14.88 ± 0.29*	0.037
Interpeak latency (Seconds)	165.05 ± 72.32	104.95 ± 38.65	0.091
Duration (Seconds)	64.92 ± 8.62	61.02 ± 7.29	0.170
Average AUC (mV-second)	20.02 ± 0.75	24.60 ± 0.92*	0.013
Summed AUC (mV-seconds)	181.56 ± 5.77	291.16 ± 9.54*	0.007

Note: * significantly different compared to KCl-vehicle group ($p < .05$)

2. Effect of N/OFQ on development of CSD-evoked depolarization shift

From above data are showing that N/OFQ can alter electrophysiological variables after N/OFQ injection. Interestingly, amplitude of DC shift was successively decreased lower than 22 mV for 2 waves after N/OFQ injection (Figure 4.10) as compared with the KCl-vehicle group. However, after 10 minutes from injection time point, amplitude was rise over than 30 mV, upward amplitude across 1 hour. From these data found that amplitude of DC shift from KCl-N/OFQ group significantly decreased when compared with KCl-vehicle group during early-phase. In contrast, amplitude of DC shift from KCl-N/OFQ group significantly increase when compared with KCl-vehicle group as showed in table 4.8

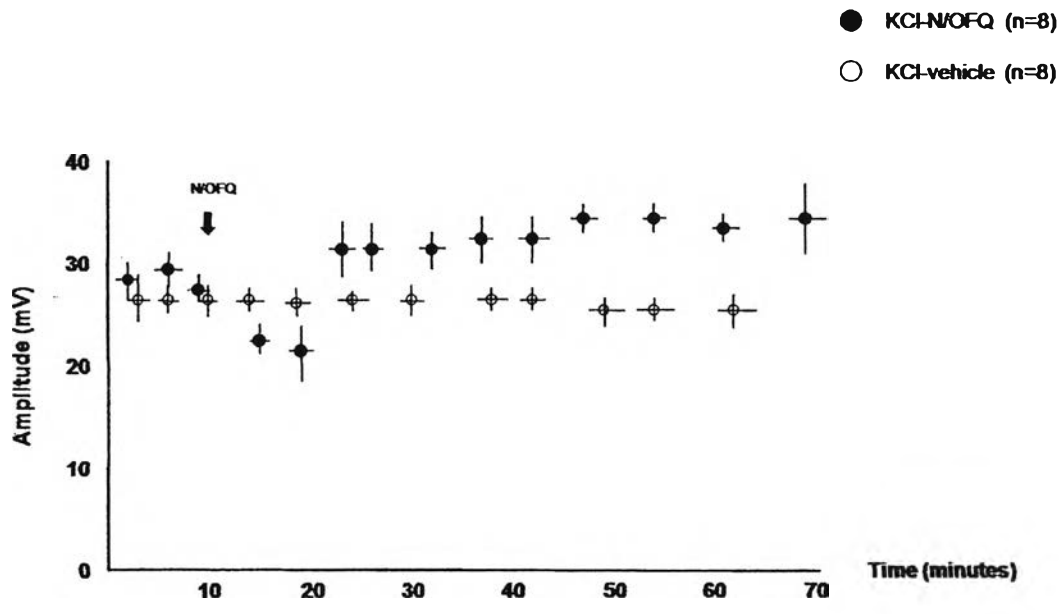


Figure 4.10 The mean value ± SD of time course amplitude of DC shift obtained from KCl-vehicle and KCl-N/OFQ group. Vertical lines represent the SD value of amplitude each DC shift. Horizontal lines represent the SD of the onset of DC shift each DC shift.

Table 4.8 The mean ± SD of amplitude sequence obtained from KCl-vehicle and KCl-N/OFQ group

	Mean±SD of amplitude (mV)		
Series of DC shift	**KCl-vehicle**	**KCl-N/OFQ**	***p*-value**
DC1	27.125±2.23	29.25±2.37	0.092
DC2	27.125±1.35	30.12±1.96	0.075
DC3	26.87±1.55	29.87±1.21	0.084
Vehicle or N/OFQ⟶			
DC4	27.00±1.19	21.25±1.15*	0.015
DC5	27.00±1.41	21.37±1.19*	0.026
DC6	27.00±0.92	30.14±2.70*	0.013
DC7	26.62±1.40	31.14±3.39*	0.016
DC8	27.12±1.12	31.87±2.85*	0.028
DC9	26.75±1.16	32.87±1.55*	0.011
DC10	26.87±1.45	33.75±1.75*	0.026
DC11	26.87±1.12	34.37±2.26*	0.017
DC12	25.80±1.64	34.50±2.20*	0.011

Note: * significantly different compared to KCl-vehicle group ($p < .05$)

Part II: Results from all experiment performed for studying the effect of N/OFQ on trigeminal nociceptive system in CSD model

1.) Effect of N/OFQ on TRPV1 expression in TG

Topical application of KCl on surface cortex for 2 hours was performed to induce CSD. TRPV1 expression was detected using IHC at 2 hours after the beginning of CSD induction.

In this experiment, 8 sections from ipsilateral side and 8 sections from contralateral side of TG were collected from each rat. The slides of the sections were studied under the light microscope and neurons were classified as immunoreactivity or non-reactivity based on the immunostaining feature. The TRPV1-IR neurons were defined as those with dark-brown stained in their cytoplasm. It was shown that TRPV1-IR neurons comprised small to medium sized neurons. The total TRPV1-IR of small to medium sized was counted from each slide. Data were expressed as mean and standard deviation of percent of TRPV1-IR neurons. Effect of CSD-evoke DC shift on TRPV1-IR expression was compared between NaCl-vehicle, KCl-vehicle group, and KCl-N/OFQ group (Figure 4.11)

TRPV1-IR cells were confined in TG and were more prevalent on the ipsilateral side. In NaCl-vehicle group, the numbers of TRPV1-IR cells in the ipsilateral side and contralateral side were 9.50 ± 1.38 and 8.50 ± 1.87 %/rat respectively. In KCl-vehicle group, TRPV1-IR from ipsilateral side and contralateral side were 20.17 ± 2.14 and 17 ± 3.03 %/rat respectively. Intrathecal injection of N/OFQ can enhances the response of trigeminal nociceptive system. The numbers of TRPV1-IR in CSD-N/OFQ group were 47.33 ± 5.47 and 40.17 ± 6.27 %/rat for ipsilateral side and contralateral side respectively. The data are shown in Table 4.8. The difference in the number of TRPV1-IR cells between negative control group, KCl-vehicle and KCl-N/OFQ were statistically significant (Figure 4.11)

Table 4.9 The mean value ± SD of numbers of TRPV1-IR cells in the TG section obtained from NaCl-vehicle and KCl-vehicle group.

		Percentage of TRPV1-IR (%/rat)			
Group	**n**	**Ipsilateral**	***p*-value**	**Contralateral**	***p*-value**
NaCl-vehicle	6	9.50 ± 1.38	N/A	8.50 ± 1.87	N/A
KCl-vehicle	8	20.17 ± 2.14*	0.001	17 ± 3.03*	0.001
KCl-N/OFQ	8	47.33 ± 5.47* #	0.001	40.17 ± 6.27* #	0.001

Note: * significantly different compared to NaCl-vehicle group ($p < .05$)

significantly different compared to KCl-vehicle group ($p < .05$)

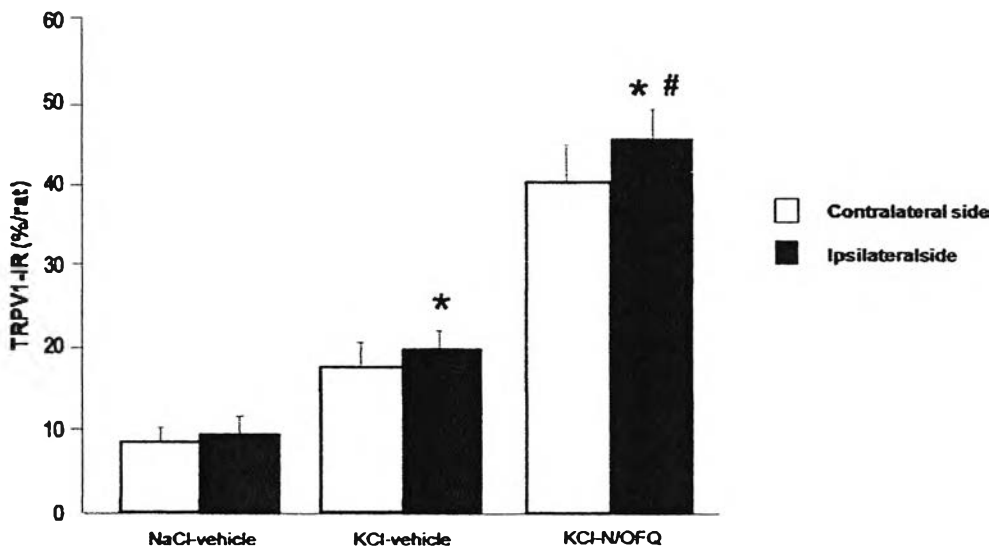
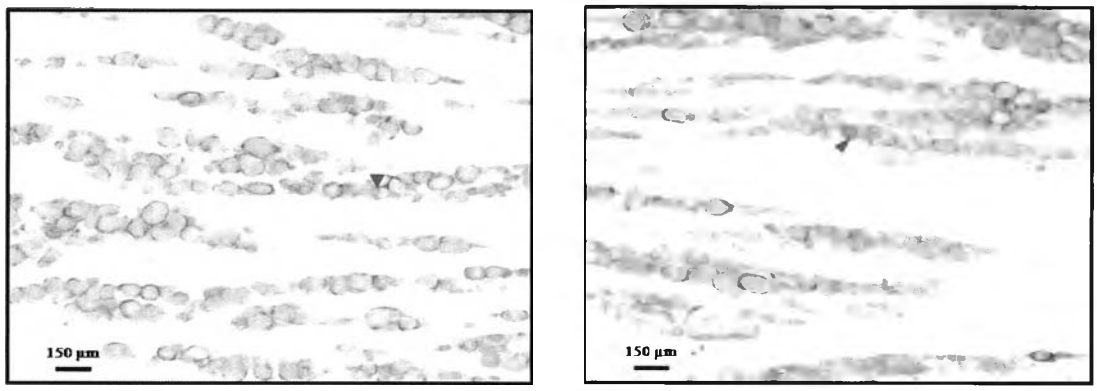


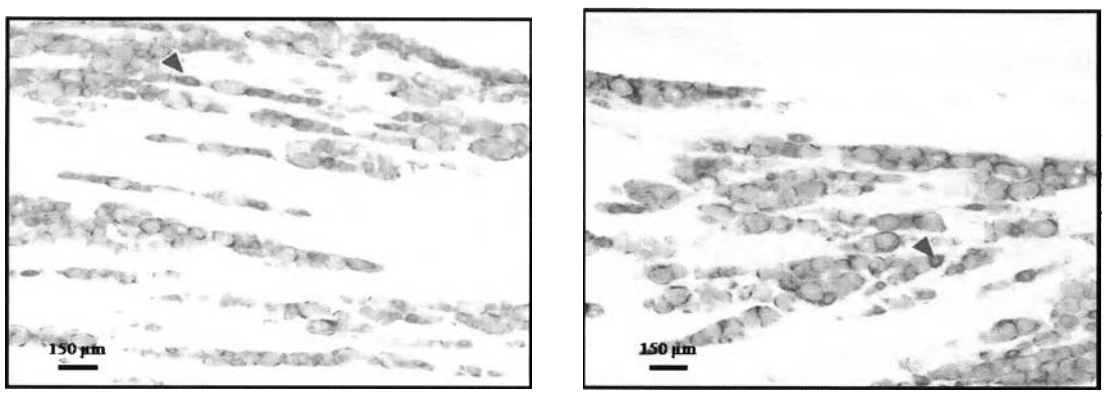
Figure 4.11 Bar graph shows the mean value ± SD of number of TRPV1-IR from NaCl-vehicle, KCl-vehicle and KCl-N/OFQ group. Significant difference was assessed with one-way ANOVA. * $p < 0.05$ when compared with the NaCl-vehicle group. # $p < 0.05$ when compared with the KCl-vehicle group.

Contralareal side **Ipsilateral side**

A. NaCl-vehicle group



B. KCl-vehicle group



C. KCl-N/OFQ group

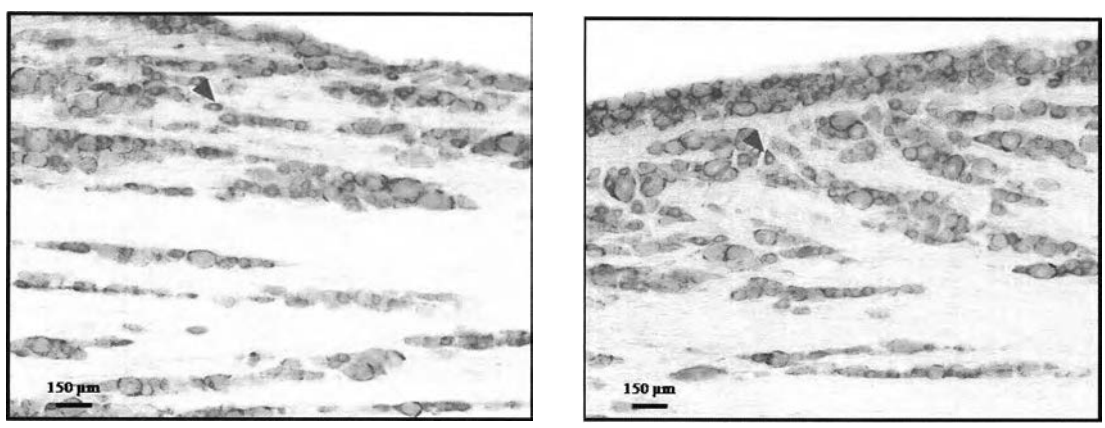


Figure 4.12 The photomicroscope shows the TRPV1-IR cells in the TG section obtained from A. NaCl-vehicle group B. KCl-vehicle group. C. KCl-N/OFQ group Bar = 150µm

2.) Effect of N/OFQ on the c-Fos expression in TNC

To investigated whether the second order neuron in trigeminal nociceptive system is activated by CSD. The expression of c-Fos, a surrogate marker for neuronal activation was examined. Ten sections were randomly selected from each rat and the Fos-IR cells were counted and reported as the number of immunoreactive cells in the dorsal horn contralateral and ipsilateral side in all groups. The value data are reported as the mean value and standard deviation.

The fos expression after CSD induction for 1 hour was mainly distributed in the lamina I and II of the cervical spinal cord sections on the ipsilateral side to the KCl application (Figure 4.13). In NaCl-vehicle group, the numbers of Fos-IR cells in ipsilateral and contralateral side were 6.37±0.57 and 5.02±0.58 cells/rat respectively. In KCl-vehicle group (positive control); the numbers of Fos-IR cells in ipsilateral and contralateral side were 24.61±2.04 and 12.71±2.52 cells/rat respectively. In KCl-N/OFQ group, the numbers of Fos-IR cells in ipsilateral and contralateral side were 34.68±3.26 and 17.58±2.08 cells/rat respectively. The data are show in table 4.9. The difference in the number of Fos-IR cells in all groups were statistically significant (Figure 4.15).

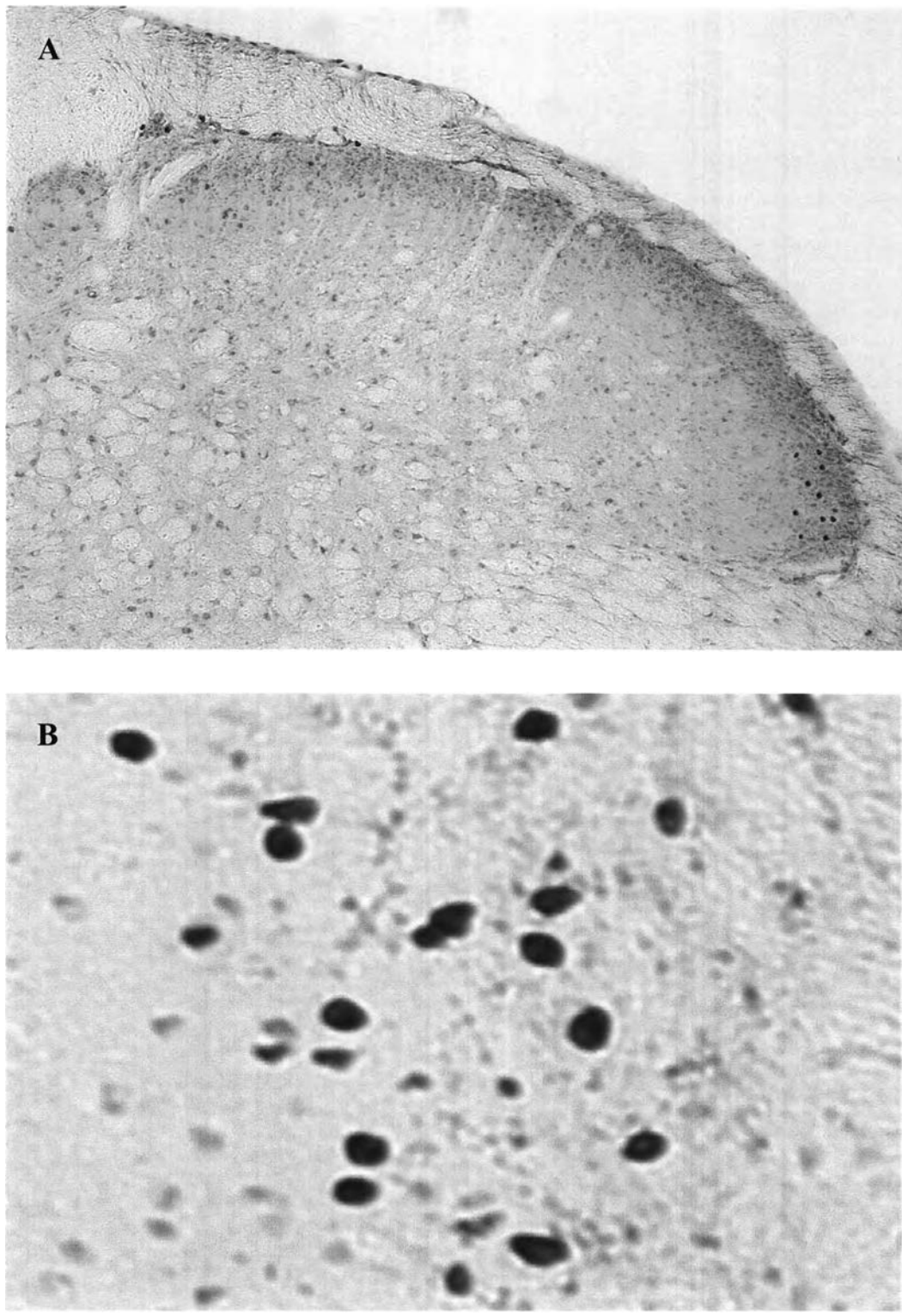


Figure 4.13 A. Distribution of Fos-IR cells in dorsal horn of TNC. The labeling was most dense in lamina I, and II (10x objective lens). B. Cellular patterns staining of Fos was restricted to the nucleus (40x objective lens)

Table 4.10 The mean value ± SD of number of Fos-IR cells in the TNC section obtained from NaCl-vehicle, KCl-vehicle, and KCl-N/OFQ groups.

		Numbers of Fos-IR cells (cells/rat)			
Group	**n**	**Ipsilateral**	***p*-value**	**Contralateral**	***p*-value**
NaCl-vehicle	6	6.37 ± 0.57	N/A	5.02 ± 0.58	N/A
KCl-vehicle	8	20.17 ± 2.14*	0.001	12.71 ± 2.52*	0.001
KCl-N/OFQ	8	34.68 ± 3.26* #	0.001	17.58 ± 2.08* #	0.001

Note: * significantly different compared to NaCl-vehicle group ($p < .05$)

significantly different compared to KCl-vehicle group ($p < .05$)

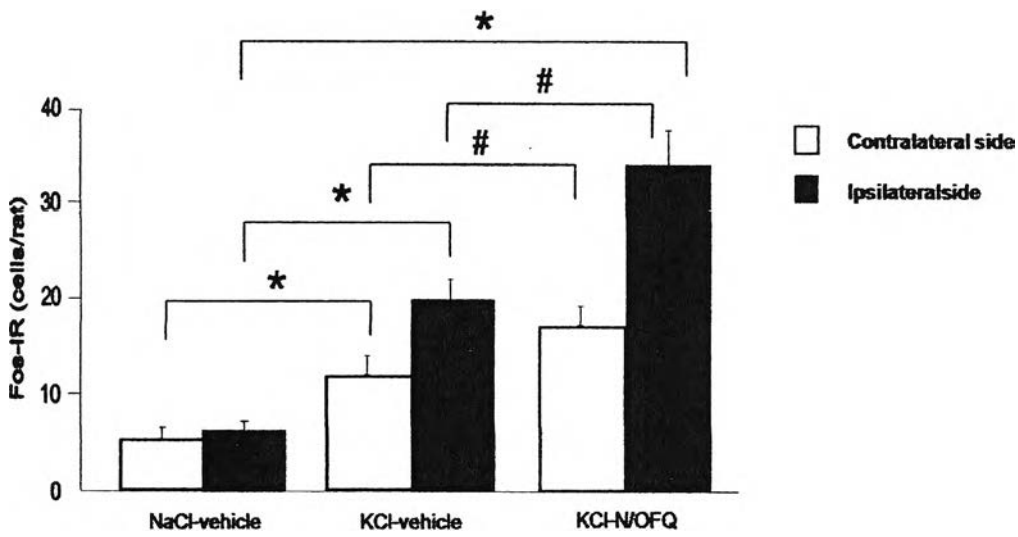


Figure 4.14 The bar graphs show the mean value ± SD of number of Fos-IR from NaCl-vehicle, KCl-vehicle and KCl-N/OFQ group. Significant difference was assessed with one-way ANOVA. * $p < 0.05$ when compared with the NaCl-vehicle group. # $p < 0.05$ when compared with the KCl-vehicle group.

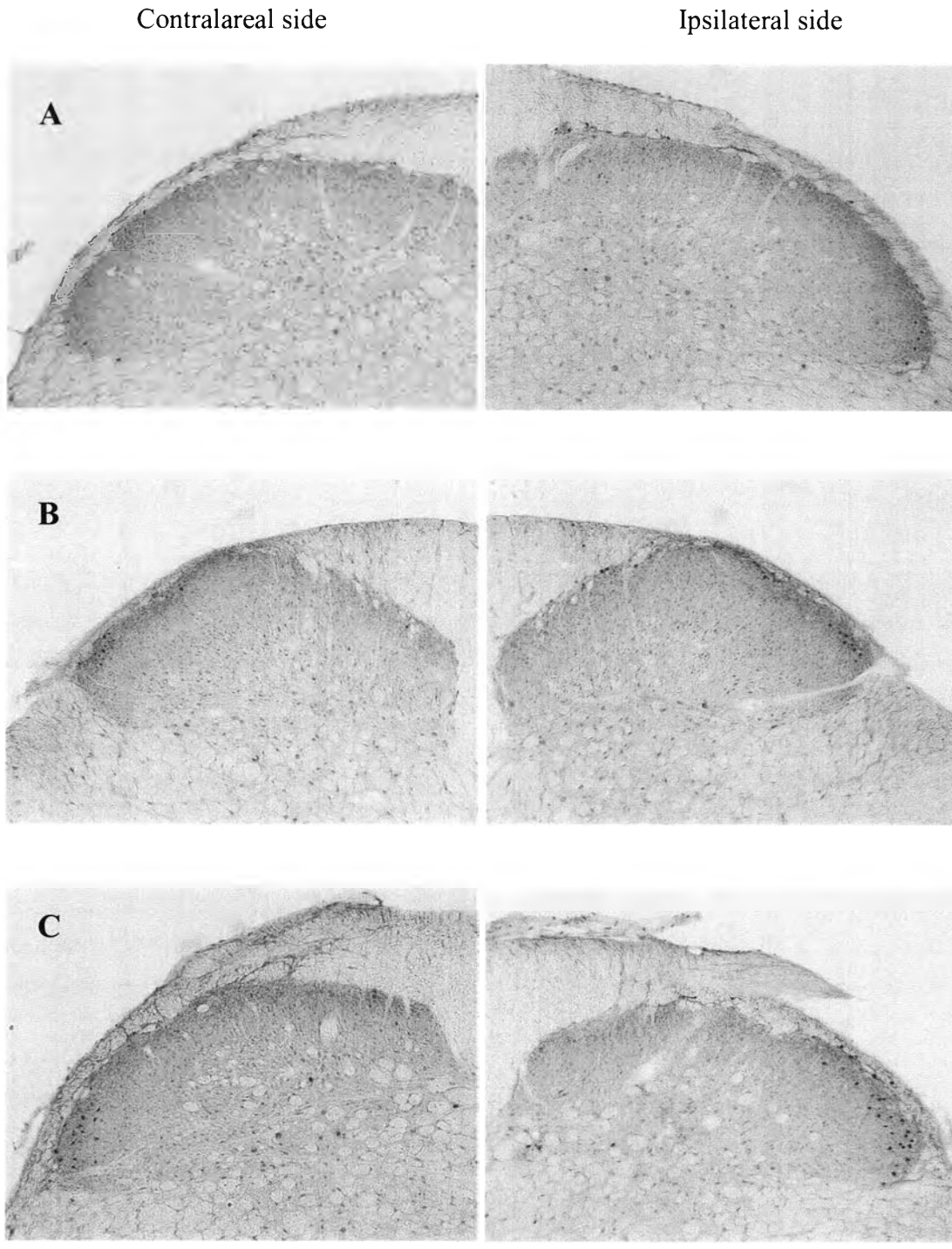


Figure 4.15 The photomicrograph shows A) The Fos-IR cells in the TNC section from NaCl-vehicle group. B) The Fos-IR cells in the TNC section from KCl-vehicle group. C) The Fos-IR cells in the TNC section from KCl-N/OFQ group.