CHAPTER IV

RESULTS

The results of this study were organized into two parts as follow:

Part 1: To determine the effects of chronic restraint stress on the sweet food intake and dopaminergic neurotransmission

Part 2: To examine type of opioid receptors mediates chronic restraint stress-induced sweet food intake

Part 1: The effects of chronic restraint stress on sweet food intake and dopaminergic neurotransmission

In this part, rats were divided into four groups including control (CON), stress (ST), control with Froot Loops® (CON+FL) and stress with Froot Loops® (ST+FL). To determine the stress effects on the general physiological profiles, body weight and food intake were recorded daily. After behavioral test, rats were sacrificed, the additional classical stress markers which are adrenal gland weight, serum corticosterone levels and numbers and types of white blood cells were evaluated.

1.1 Body weight and food intake level

The results of physiological changes by stress with or without sweet food intake compared to control were summarized in Table 4-1 which included daily weight gain (ADG), percent change of the body weight and daily feed intake (DFI) from all groups. Student-Newman-Keuls test showed an effect of exposure to chronic restraint stress on ADG and percent change of body weight, since stressed rats had ADG [$F(1, 27) = 14.50$; $P<0.001$] and percent change of body weight [$F(1, 26) = 18.15$; $P<0.001$] lower than non-stress rats. In addition, there was an effect of sweet food intake because sweet food intake rats shown significant higher of ADG [$F(1, 27) = 8.11$; $P<0.05$] and percent change of the body weight [$F(1, 26) = 17.91$; $P<0.001$] than non sweet food intake rats. There was no interaction between chronic stress and sweet food intake on ADG [$F(1, 27) = 0.00$; $P>0.05$] or percent change of body weight [$F(1, 26) = 0.00$; $P>0.05$]. Analyzing standard chow intake (DFI) showed no effect of either chronic stress [$F(1, 26) = 1.23$; $P>0.05$] or sweet food intake [$F(1, 26) = 0.54$, $P>0.05$].

1.2 Adrenal gland weight, serum corticosterone and CBC analysis

Adrenal gland weight and serum corticosterone were measured as a classical indices of chronic stress via HPA axis. Results are shown in Table 4-1 and Figure 4-3 A. Student Newman-Keuls test indicated that the adrenal gland mass index which is the percent of adrenal gland weight to body weight was increased in all stressed rats [F(1,

26) = 6.69; $P<0.05$]. Two ways ANOVA revealed no interaction between chronic stress and sweet food intake [F(1, 26) = 1.31; $P>0.05$] on the adrenal gland mass index.

Consistent to adrenal gland mass index, serum corticosterone, stress hormone, significantly increased in chronic stress rats when compared to control rats [F(1, 17) = 6.26; $P<0.05$]. Moreover, Student Neman-Keuls test showed an effect of sweet food intake on serum corticosterone levels [F(1, 17) = 51.65; $P<0.001$], in which rats given sweet food had higher serum corticosterone than those not given sweet food (Table 4-1 and Figure 4-3). There was also a significant interaction between chronic stress and sweet food intake [F(1, 17) = 9.10; $P<0.001$], which means that the chronic stress rats submitted to sweet food intake presented the highest of serum corticosterone than other groups.

Complete blood counted analysis which determined total numbers of red blood cells (RBC), white blood cells (WBC) and differential counted numbered of white blood cells as an index of chronic stress (Table 4-2). Student Neman-Keuls test showed RBC significantly increased in chronic stress group when compared to control group [F(1, 15) = 9.67; $P<0.05$]. WBC did not different between groups [F(1, 15) = 2.79; $P>0.05$], whereas neutrophil tended to be increased in chronic stress rats [F(1, 15) = 4.22; $P = 0.0579$]. Moreover, we found that lymphocyte significantly decreased in chronic stress rats [F(1, 15) = 5.10; $P< 0.05$] when compared to control group. There was no interaction between chronic stress or sweet food intake on RBC [F(1, 15) = 1.07; $P>0.05$] and WBC [F(1, 15) = 1.87; $P>0.05$].

Table 4-1 Summary of the mean ± S.E.M. of physiological changes of average of daily weight gain (ADG), % change of body weight, average daily feed intake (DFI), adrenal gland weight ratio of control (CON), stress (ST), and sweet food training rats (CON+FL or ST+FL).

	CON	ST	CON+FL	ST+FL
ADG (g/day)	4.42 ± 0.26	2.82 ± 0.35	5.28 ± 0.34	4.00 ± 0.46
% Change of BW	31.31 ± 2.47	15.71 ± 2.29	38.96 ± 3.08	28.14 ± 3.94
DFI (g/rat/day)	18.42 ± 1.85	18.82 ± 0.48	20.41 ± 0.51	17.98 ± 0.75
Adrenal gland weight index (%)	0.018 ± 0.001	0.025 ± 0.002	0.023 ± 0.002	0.026 ± 0.002
Serum Corticosterone (ng/ml)	46.29 ± 14.90	271.50 ± 70.19	414.60 ± 35.95	423.1 ± 18.95
Number of rats	6	8	8	9

Data presented as mean ± S.E.M. There was a significant interaction between chronic stress and sweet food intake (two-way ANOVA followed by Student- Newnan- Keuls; $P<0.05$).

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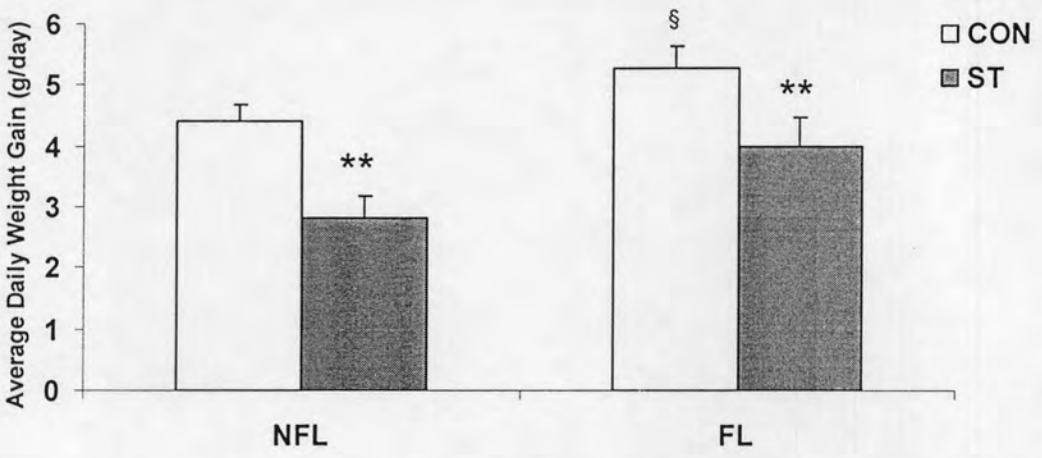
A.



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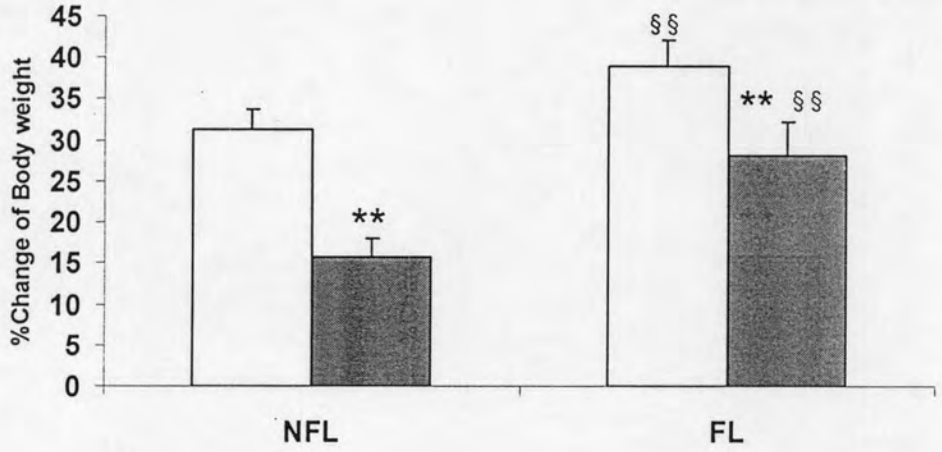

Figure 4-1 Histograms illustrate (A) mean ± S.E.M. of average of daily weight gain (ADG), (B) % of body weight change of control (CON), stress (ST), control+FL (CON+FL) and stress+FL (ST+FL) group. Data presented as mean ± S.E.M. Significant different at $P < 0.05$, two-way ANOVA followed by Student- Newnan Keuls test. Number of animals = 6-9 per group. * P<0.05, ** P<0.001 compared to control group (CON). § P<0.05, §§ P<0.001 compared to non sweet food intake (NFL) group.

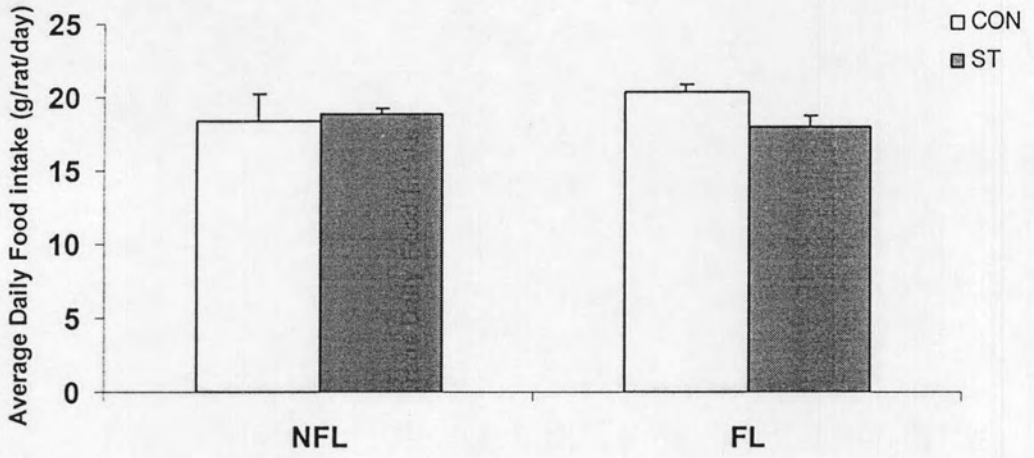

Figure 4-2 Histogram illustrates mean ± S.E.M. of average daily feed intake (ADI) of control (CON), stress (ST), control+FL (CON+FL) and stress+FL (ST+FL) group.

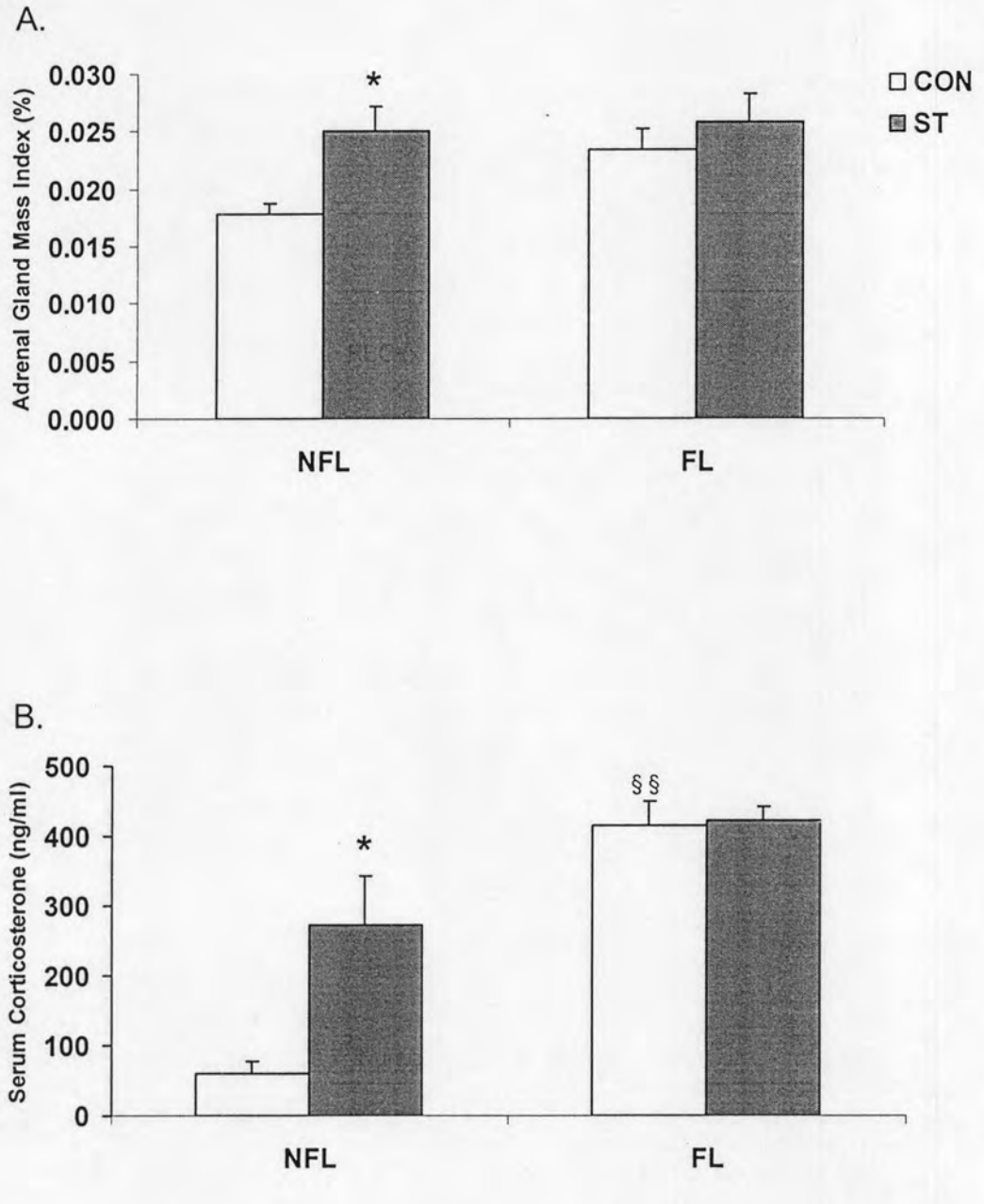

Figure 4-3 Histograms illustrate (A) percent adrenal mass index and (B) serum corticosterone in ng/ml of control (CON), stress (ST), control+FL (CON+FL) and stress+FL (ST+FL) rats. Data presented as mean ± S.E.M. Significant different at $P < 0.05$, two-way ANOVA followed by Student- Newnan Keuls test. Number of animals = 6-9 per group. * P<0.05 compared to control group (CON). §§.P<0.001 compared to non sweet food intake (NFL)

Table 4-2 Summary of of the mean values ± S.E.M of hematology of control (CON), stress (ST), control+FL (CON+FL) and stress+FL (ST+FL) rat group.

	CON	ST	CON+FL	ST+FL
RBC ($x10^6$ /µl)	5.44 ± 0.55	8.08 ± 0.88	6.98 ± 0.59	8.62 ± 0.51
WBC ($x10^3$ /µl)	2.79 ± 0.86	2.95 ± 0.29	3.39 ± 0.85	5.22 ± 0.0.62
-Band (%)	0	0	0	0
-Neutrophil (%)	8.00 ±1.58	13.80 ± 2.01	11.80 ± 2.99	15.60 ± 1.81
-Lymphocyte (%)	92.00 ± 1.58	85.60 ± 2.01	88.00 ± 2.97	84.00 ± 1.70
-Monocyte (%)	0	0	0.20 ± 0.89	0.20 ± 0.89
-Eosinophil (%)	0	0.60 ± 0 .40	0	0.20 ± 0.89
-Basophil (%)	0	0	0	0
Number of rats	4	5	5	5

Data presented as mean ± S.E.M. There was a significant interaction between chronic stress and sweet food intake (two-way ANOVA followed by Student- Newnan- Keuls; $P<0.05$).

1.3 The effect of chronic restraint stress on sweet food intake

After 14 days of stress, the sweet food intake test was studied in CON+FL and ST+FL group in order to evaluate the effect of chronic restraint stress on behavioral changes which is sweet food preference. As shown in Table 4-3 and Fig. 4-4, the chronically stressed group presented a higher ingestion of sweet pellets than control group (Student unpaired *t*- test, $P< 0.001$,).

1.4 The effect of chronic restraint stress on the dopaminergic neurotransmission

After sweet feeding behavior test, the rat's brains were rapidly removed for measurement of DA, DOPAC, and HVA levels by HPLC technique. The effect of chronic stress on DA, DOPAC, and HVA in mesocorticolimbic system, as well as the DOPAC/DA, and HVA/DA ratios, was shown in Table 4-4. In nucleus accumbens, chronic stress induced an increased of DA levels, whether or not the animals were submitted to sweet food intake [$F(1, 16) = 5.26$; $P< 0.0$]. In addition, DA levels tended to be increased in rats consumption of sweet food [$F(1, 16) = 3.13$; $P = 0.0960$]. Two ways ANOVA revealed a significant interaction between chronic stress and sweet food intake on HVA levels in nucleus accumbens [$F(1, 16) = 8.77$; $P<0.05$]. The chronic stress with sweet food consumption presented a higher HVA levels compared to other groups. In amygdala, chronic stress caused a decreased of dopaminergic activity, since DOPAC/DA [$F(1, 16) = 6.95$; $P<0.05$] and HVA/DA ratios [$F(1, 16) = 5.20$; $P<0.05$] was lower than control group. There was no interaction between chronic stress and sweet food intake on DOPAC/DA [$F(1, 16) = 0.54$; $P>0.05$] and HVA/DA ratios [$F(1, 16) = 0.04$; $P>0.05$]. In hippocampus, HVA levels of chronic stress rats was lower than control rats [$F(1, 16) = 5.43$; $P<0.05$]. Moreover, DOPAC/DA ratio was significantly increased in consumption sweet food rats, whether or not exposed to chronic stress [$F(1, 16) = 12.41$; $P<0.05$].

Table 4-3 Table represents mean ± S.E.M. of number of froot loops intake after chronic restraint stress.

	CON+FL	ST+FL
Numbers of froot loop intake (pellets)	3.81 ± 0.46	7.02 ± 0.68**
Number of rats	8	9

Significant difference was assessed with unpaired t-test.

**$P<0.001$ compared to control +FL group.

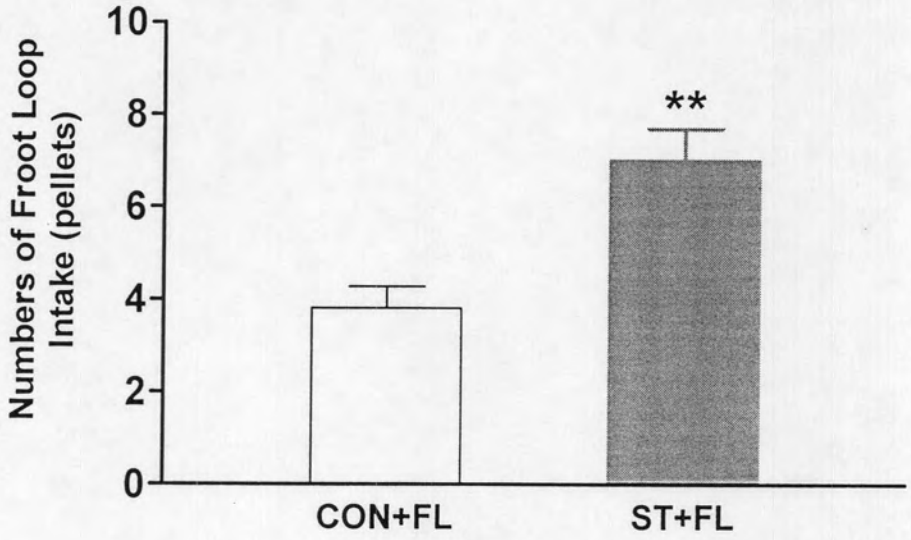

Figure 4-4 Histograms illustrate mean ± S.E.M. of number of froot loops intake after chronic restraint stress. Significant difference was assessed with unpaired t-test. $^{**}P<0.001$ compared to control +FL group. Number of animals = 8-9 per group.

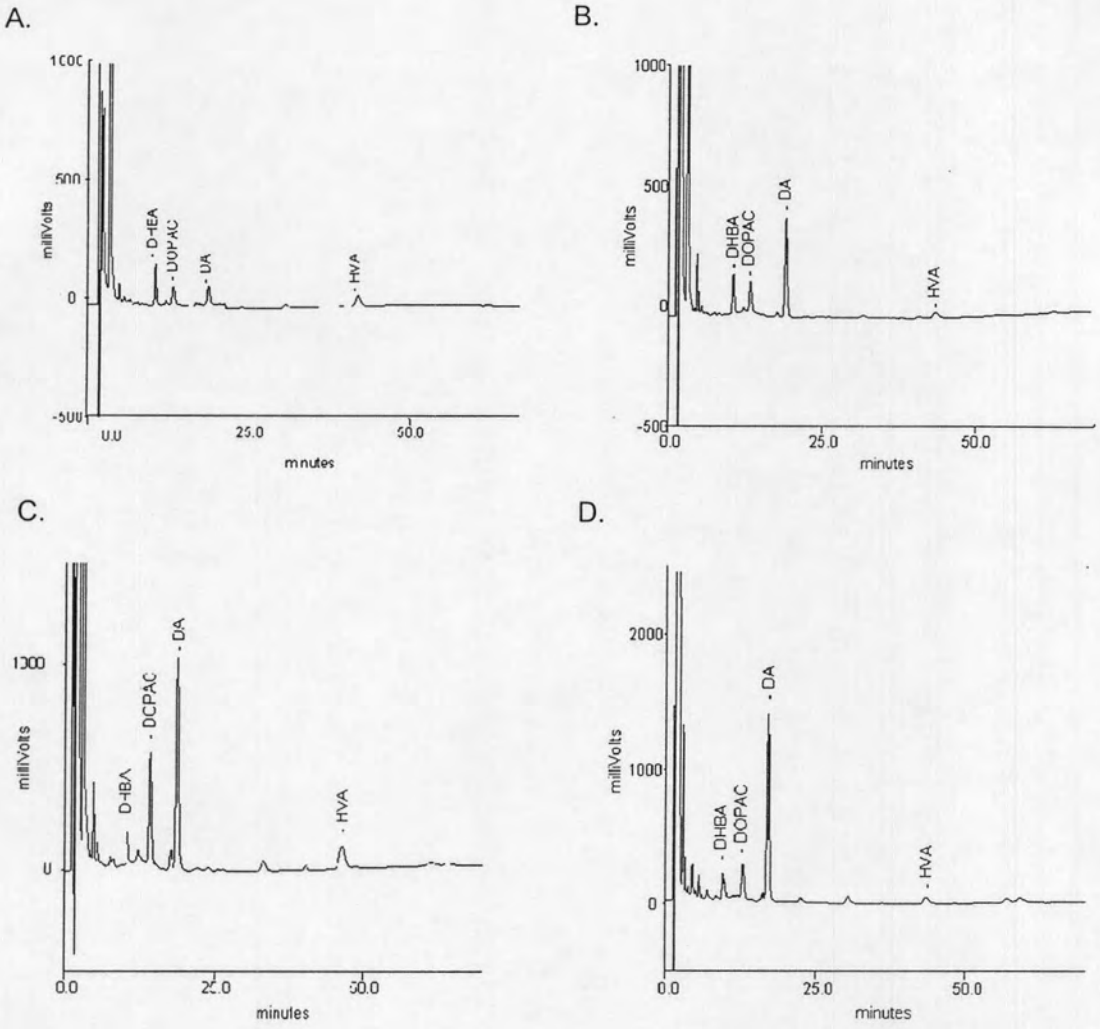

Figure 4-5 The chromatograms represent DA, DOPAC and HVA levels in nucleus accumbens of (A) control (CON), (B) stress (ST), (C) control +FL (CON+FL) and (D) stress + FL (ST+FL) rats measured by HPLC-EC. The retention times of DOPAC, DA and HVA were approximately 13.29, 18.17 and 42.26 minutes, respectively.

Table 4-4 DA, DOPAC, HVA, DOPAC/DA and HVA/DA in amygdala (AMYG), frontal cortex (FC), hippocampus (HIP) and nucleus accumbens (NAc) of control (CON), stress (ST), control+FL(CON+FL) and stress+FL (ST+FL) rats.

Structure	CON (n=6)	ST (n=6)	CON+FL (n=4)	ST+FL (n=4)
AMY				
DA	7.87 ± 2.57	5.57 ± 1.10	3.23 ± 1.30	6.87 ± 2.45
DOPAC	9.74 ± 5.99	2.40 ± 0.59	1.75 ± 0.31	2.83 ± 1.04
HVA	8.05 ± 3.97	2.12 ± 0.62	2.66 ± 0.59	3.37 ± 0.92
DOPAC/DA	1.09 ± 0.27	0.44 ± 0.08	0.79 ± 0.25	0.44 ± 0.08
HVA/DA	1.18 ± 0.35	0.49 ± 0.19	1.36 ± 0.58	0.52 ± 0.05
FC				
DA	1.03 ± 0.15	5.25 ± 2.22	1.75 ± 0.47	0.88 ± 0.14
DOPAC	087 ± 0.22	1.75 ± 0.69	1.25 ± 0.17	1.03 ± 0.30
HVA	2.34 ± 0.44	3.05 ± 1.00	2.28 ± 0.69	2.42 ± 0.49
DOPAC/DA	0.84 ± 0.17	0.42 ± 0.12	0.93 ± 0.29	1.19 ± 0.37
HVA/DA	2.66 ± 0.83	1.10 ± 0.64	1.84 ± 0.89	2.68 ± 0.15
HIP				
DA	1.29 ± 0.27	1.44 ± 0.35	0.89 ± 0.21	0.82 ± 0.13
DOPAC	0.88 ± 0.25	0.65 ± 0.15	0.85 ± 0.06	1.11 ± 0.14
HVA	3.18 ± 0.65	1.78 ± 0.37	2.35 ± 0.35	1.66 ± 0.15
DOPAC/DA	0.67 ± 0.12	0.55 ± 0.13	1.06 ± 0.16	1.50 ± 0.36
HVA/DA	3.07 ± 1.11	1.57 ± 0.34	2.76 ± 0.22	2.10 ± 0.24
NAc				
DA	19.52 ± 5.56	47.69 ± 9.42	37.07 ± 12.88	90.12 ± 37.09
DOPAC	19.42 ± 4.63	26.17 ± 6.04	18.18 ± 4.76	37.79 ± 12.34
HVA	14.80 ± 4.85	12.59 ± 4.63	10.30 ± 0.72	37.97 ± 7.06
DOPAC/DA	1.28 ± 0.30	0.63 ± 0.13	0.67 ± 0.18	0.56 ± 0.12
HVA/DA	1.55 ± 0.77	0.38 ± 0.16	0.79 ± 0.54	0.87 ± 0.36

Data presented as mean ± S.E.M., there was a significant interaction between chronic stress and sweet food intake (two-way ANOVA followed by Student- Newnan- Keuls; $P<0.05$). Number of animals = 4-6 per group.

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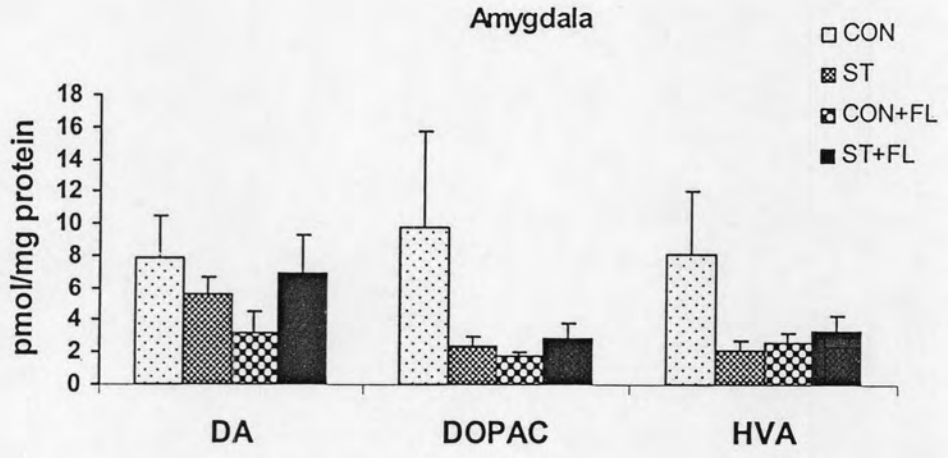

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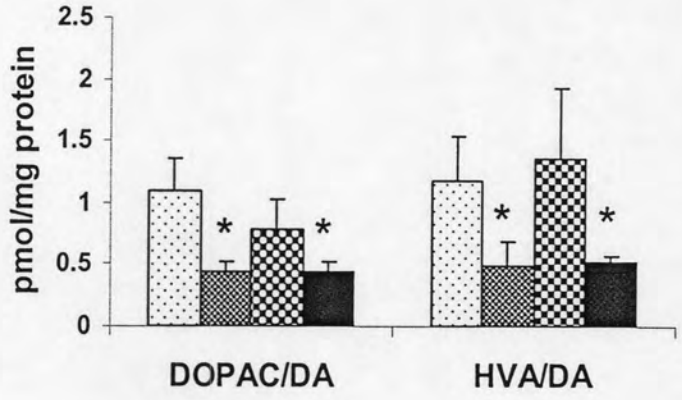

Figure 4-6 Histograms illustrate mean ± S.E.M. of (A) DA, DOPAC and HVA (B) DOPAC/DA and HVA/DA ratios in amygdala of control (CON), stress (ST), control with froot loops (CON+FL) and stress with froot loops (ST+FL) rats. There was a significant interaction between chronic stress and sweet food intake (two-way ANOVA followed by Student- Newnan-Keuls; * $P<0.05$). Number of animals = 4-6 per group.

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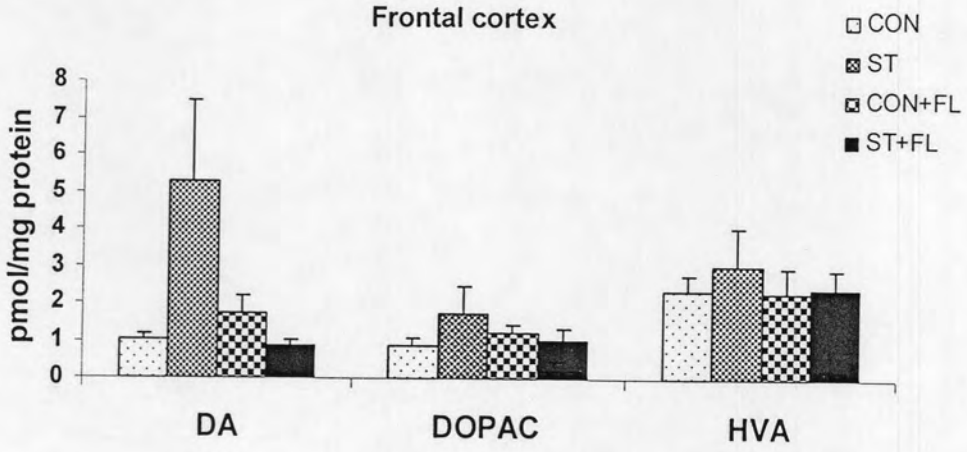

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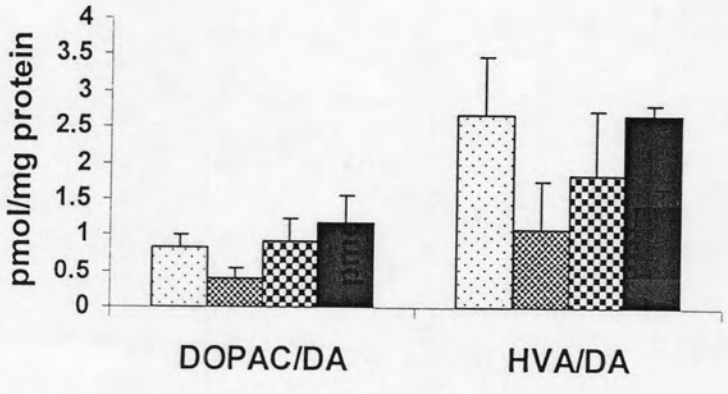

Figure 4-7 Histograms illustrate mean ± S.E.M. of (A) DA, DOPAC and HVA (B) DOPAC/DA and HVA/DA ratios in frontal cortex of control (CON), stress (ST), control with froot loops (CON+FL) and stress with froot loops (ST+FL) rats. Number of animals = 4-6 per group.

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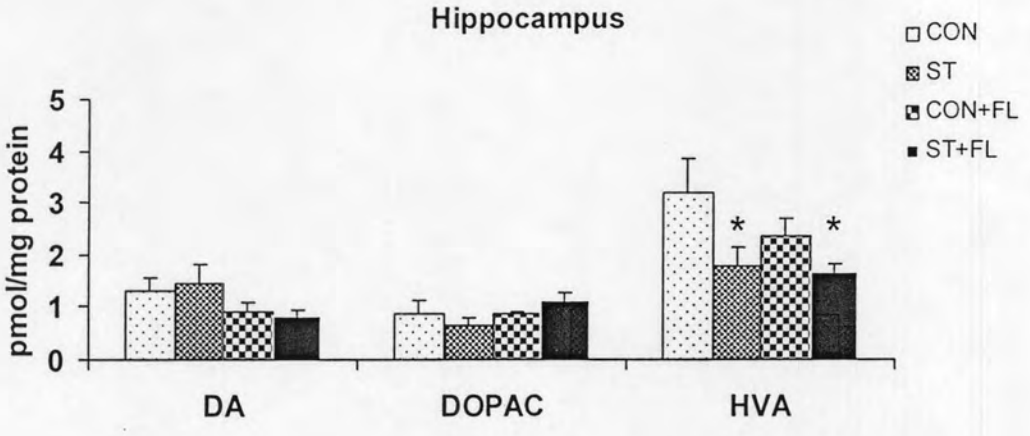

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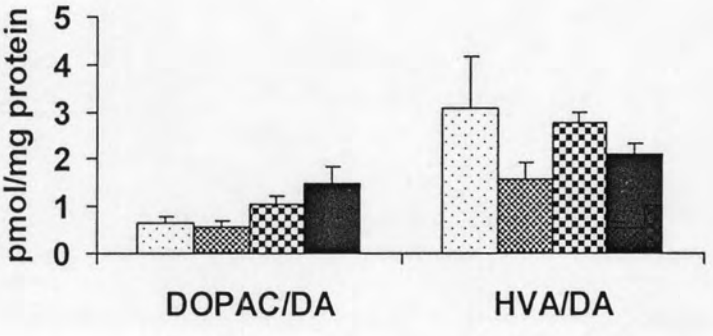
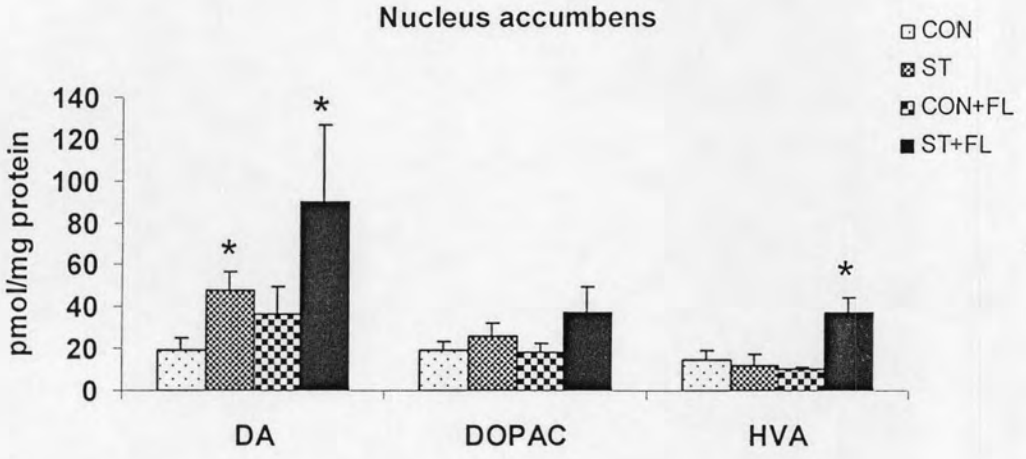


Figure 4-8 Histograms illustrate mean ± S.E.M. of (A) DA, DOPAC and HVA (B) DOPAC/DA and HVA/DA ratios in hippocampus of control (CON), stress (ST), control with froot loops (CON+FL) and stress with froot loops (ST+FL) rats. There was a significant interaction between chronic stress and sweet food intake (two-way ANOVA followed by Student- Newnan-Keuls;* $P<0.05$). Number of animals = 4-6 per group.

A.



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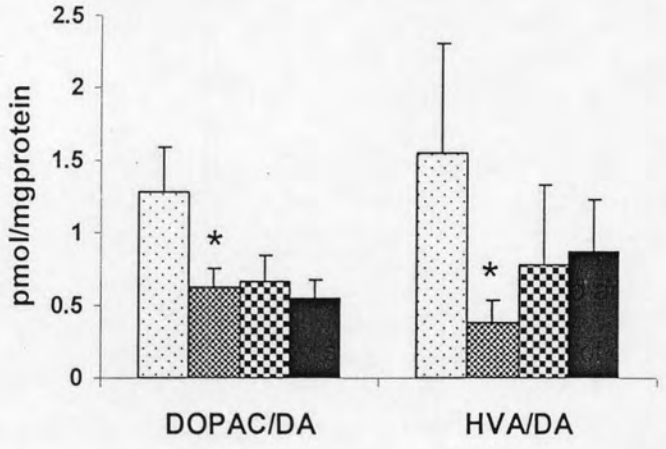


Figure 4-9 Histograms illustrate mean ± S.E.M. of (A) DA, DOPAC and HVA (B) DOPAC/DA and HVA/DA ratios in nucleus accumbens of control (CON), stress (ST), control with froot loops (CON+FL) and stress with froot loops (ST+FL) rats. There was a significant interaction between chronic stress and sweet food intake (two-way ANOVA followed by Student- Newnan-Keuls; * $P<0.05$). Number of animals = 4-6 per group.

Part 2: To examine the types of selective opioid receptor antagonists to inhibit sweet food intake in rats exposed to chronic restraint stress

In this experiment, rats were divided into 2 main groups including CON+FL and ST+FL group. On the test day, rats were random injected with selective opioid antagonist or equivalent volume of vehicle before sweet food intake test. After sweet food intake test, the open field test was performed in order to measure locomotor activity. After that, the protocol was similar to experiment 1.

2.1 Effect of selective opioid receptor antagonists on chronic restraint stress-induced sweet food intake.

As shown in Table 4-5 and Figure 4-10, naloxone, non selective opioid receptor antagonist, at a dosage of 0.5 mg/kg significantly attenuated chronic restraint stress-induced sweet food intake [$F(3, 23) = 7.150$; $P<0.05$]. In contrast, selective opioid receptor antagonists (naltridole, δ-opioid receptor antagonist and nor-binaltorphimine, κ-opioid receptor antagonist) at a dose of 1mg/kg did not affect to sweet food intake in chronic restraint stress rats. However, naloxone, naltridole and nor-binaltorphimine did not affect to sweet food intake in control rats. For μ-opioid receptor antagonist (CTOP) at a dosage of 1 mg/kg al so did not affect to chronic stress and non stress rats.

All of opioid antagonists did not affect to locomotor activity, as indicated by the same total numbers of crosses in all groups (Table 4-5 and Figure 4-11).

2.2 Effect of opioid antagonist on dopaminergic neurotransmission

Effect of naloxone (NX), naltridole (NT) and nor-binaltorphimine (nor-BNI) on DA, DOPAC, and HVA in mesocorticolimbic system in chronic restraint stress-induced sweet food intake rats, as well as the DOPAC/DA, and HVA/DA ratios, were shown in Table 4-7. NT at a dosage of 1 mg/kg significantly decreased DA and DOPAC levels in nucleus accumbens as compared to Vehicle group [$F(3, 11) = 2.718$; $P<0.05$ for DA; $F(4, 13) = 3.885$; $P<0.05$ for DOPAC] (Figure 4-18), which were increased DOPAC/DA and HVA/DA ratio [$F(4, 13) = 5.529$; $P<0.05$ for DOPAC/DA ratio; $F(4,13) = 10.13$; $P<0.05$ for HVA/DA ratio] (Figure 4-19). No effect of opioid antagonists was observed in

other brain areas (P>0.05) in chronic stress rats. However, naloxone, naltridole and nor-binaltorphimine did not affect to DA and its metabolites in control rats (P> 0.05 in all cases; as shown in Table 4-7).

Table 4-5 Table represents mean ± S.E.M. of numbers of froot loops intake after applied opioid receptor antagonist (A) vehicle (EtOH, ethanol), naloxone (NX), naltridole (NT), and nor- binaltorphimine (nor-BNI) groups, (B) vehicle (H_2O) or CTOP groups .

A.

	CON+FL	ST+FL
Veh (EtOH)	6.50 ± 1.40 (n=4)	6.57 ± 0.89 (n=5)
NX	6.00 ± 0.82 (n=6)	2.96 ± 0.59* (n=6)
NT	7.50 ± 0.56 (n=6)	7.00 ± 0.85 (n=9)
Nor-BNI	8.25 ± 0.63 (n=4)	7.94 ± 0.67 (n=7)

B.

	CON+FL	ST+FL
CTOP	7.00 ± 0.33 (n=3)	6.67 ± 1.11 (n=4)

Table 4-6 Table represents mean ± S.E.M. of locomotor activity after applied opioid receptor antagonist (A) vehicle (EtOH, ethanol), naloxone (NX), naltridole (NT), and nor- binaltorphimine (nor-BNI) groups, (B) vehicle (H_2O) or CTOP groups .

A.

	CON+FL	ST+FL
Veh (EtOH)	134.25 ± 14.18 (n=4)	149.50 ± 9.64 (n=5)
NX	124.50 ± 10.84 (n=6)	129.00 ± 9.10 (n=6)
NT	126.83 ± 11.45 (n=6)	145.50 ±10.92 (n=9)
Nor-BNI	100.50 ±16.50 (n=4)	156.33 ± 24.03 (n=7)

B.

	CON+FL	ST+FL
CTOP	99.67 ± 13.53 (n=3)	132.25 ± 5.54 (n=4)

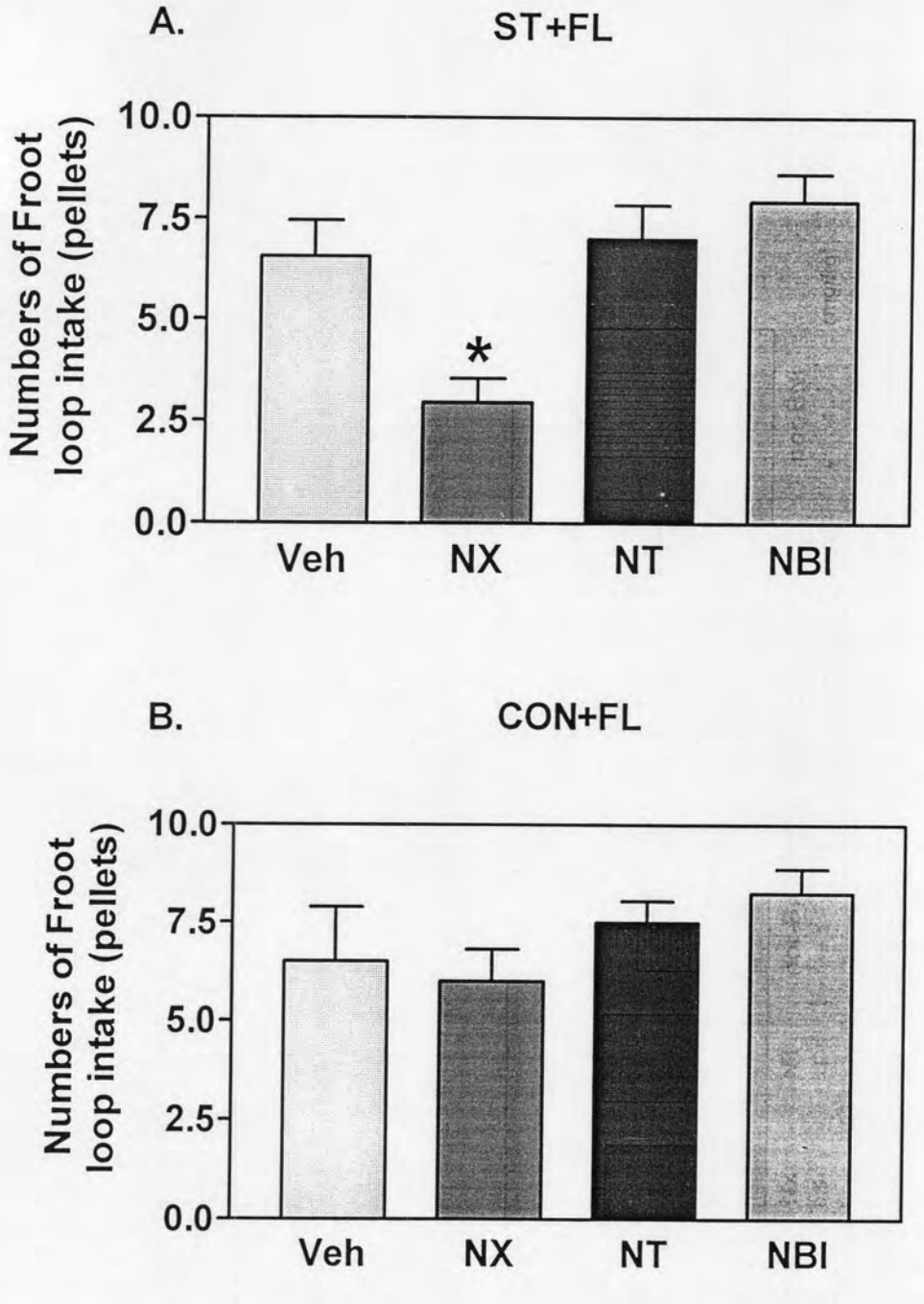


Figure 4-10 Effect of opioid receptor antagonists (NX, naloxone; NT, natridole; nor-BNI, nor-binaltorphimine) on sweet food intake in (A) chronic restraint stress rats (B) control rats. Data expressed as mean ± S.E.M. of number of froot loops intake. Significant difference was assessed with Dunnett t-test. *$P<0.05$ compared to vehicle treated group. Number of rats 4-7 per group.

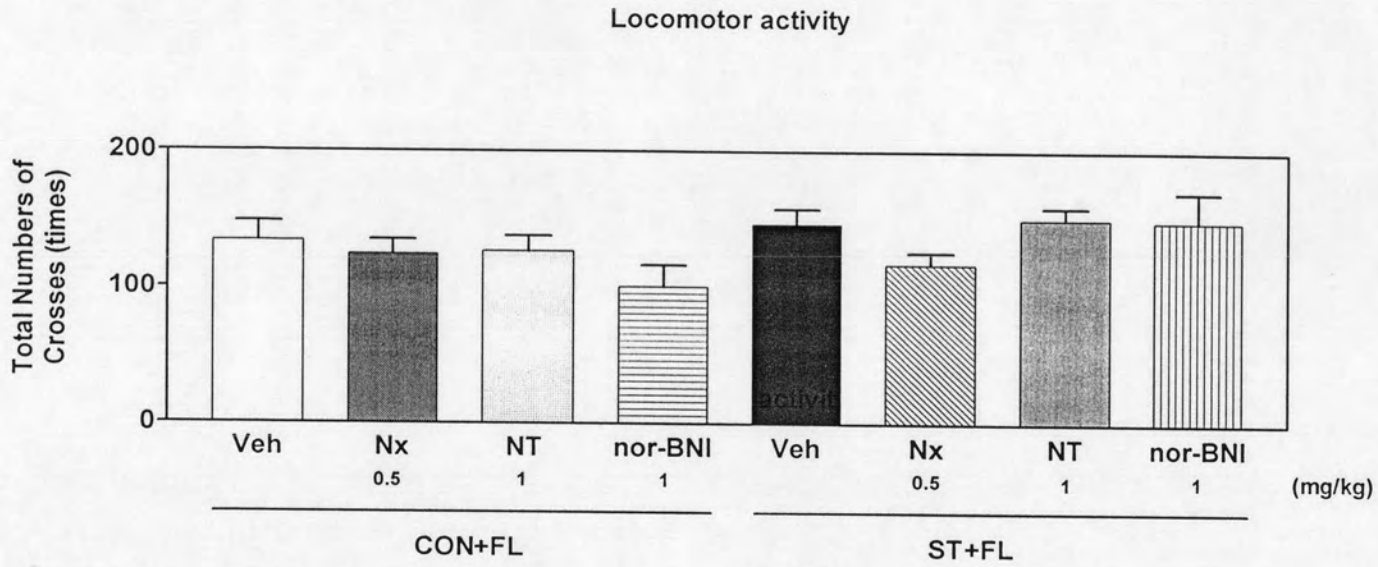


Figure 4-11 Effect of opioid receptor antagonists (NX, naloxone; NT, natridole; nor-BNI, nor-binaltorphimine) on locomotor activity in the open field. Data expressed as mean ± S.E.M. of total numbers of crosses.

Table 4-7 Effect of naloxone (Nx), naltridole (NT) and nor- binaltorphimine (nor-BNI) on DA and its metabolites in mesolimbic system in chronic restraint stress-induced sweet food intake rats.

Structure	ST+FL			
	Veh (n=3)	Nx 0.5 mg/kg (n=5)	NT 1 mg/kg (n=3)	Nor-BNI 1 mg/kg (n=3)
AMY				
DA	8.29 ± 2.04	11.53 ± 1.53	19.76 ± 11.16	11.70 ± 4.61
DOPAC	3.94 ± 0.57	4.19 ± 0.50	5.87 ± 1.11	3.66 ± 1.64
HVA	4.27 ± 0.96	2.58 ± 0.62	5.62 ± 1.59	5.38 ± 3.15
DOPAC/DA	0.50 ± 0.05	0.46 ± 0.12	0.45 ± 0.16	0.29 ± 0.03
HVA/DA	0.65 ± 0.31	0.28 ± 0.08	0.41 ± 0.17	0.39 ± 0.09
FC				
DA	3.04 ± 1.21	1.92 ± 0.40	1.16 ± 0.08	0.65 ± 0.07
DOPAC	1.63 ± 0.16	1.49 ± 0.73	0.73 ± 0.10	0.70 ± 0.09
HVA	2.39 ± 0.20	2.36 ± 0.93	1.62 ± 0.46	1.20 ± 0.12
DOPAC/DA	0.78 ± 0.35	0.70 ± 0.20	0.63 ± 0.09	1.08 ± 0.15
HVA/DA	1.21 ± 0.45	1.17 ± 0.21	1.39 ± 0.37	1.88 ± 0.25
HIP				
DA	0.65 ± 0.14	0.89 ± 0.09	0.65 ± 0.09	0.49 ± 0.11
DOPAC	1.02 ± 0.32	0.84 ± 0.31	0.62 ± 0.23	0.24 ± 0.07
HVA	2.13 ± 0.40	1.65 ± 0.31	1.14 ± 0.32	0.61 ± 0.18
DOPAC/DA	1.49 ± 0.21	0.85 ± 0.22	0.92 ± 0.23	0.55 ± 0.13
HVA/DA	3.35 ± 0.17	1.85 ± 0.29	1.70 ± 0.36	1.54 ± 0.64
NAc				
DA	142.73±8.45	97.60±34.47	3.16±1.53 *	89.51 ± 39.27
DOPAC	58.28±5.28	32.47±6.83	5.81±1.90 *	37.65 ± 14.81
HVA	26.02±4.34	16.78±4.75	5.37±1.11	25.54 ± 10.72
DOPAC/DA	0.42±0.06	0.44±0.11	2.25±0.81 *	0.46 ± 0.06
HVA/DA	0.19±0.04	0.24±0.08	0.34±0.71	0.30 ± 0.04

Data presented as mean±S.E.M., significant difference was assessed with ANOVA followed by multiple-comparison test of Dunnett. * $P<0.05$ compared to ST+FL+Veh group.

Table 4-8 Effect of naloxone (Nx), CTOP, naltridole (NT) and nor- binaltorphimine (nor-BNI) on DA and its metabolites in mesolimbic system in control rats.

Structure	CON+FL			
	Veh	Nx	NT	Nor-BNI
	(n=5)	0.5 mg/kg (n=3)	1 mg/kg (n=3)	1 mg/kg (n=3)
AMYG				
DA	26.13 ± 14.69	18.48 ± 7.97	11.34 ± 5.82	9.42 ± 0.84
DOPAC	8.94 ± 4.43	4.69 ± 2.02	4.16 ± 2.41	3.23 ± 0.38
HVA	5.85 ± 2.27	3.06 ± 1.56	3.57 ± 1.75	3.06 ± 0.43
DOPAC/DA	0.46 ± 0.13	0.26 ± 0.04	0.35 ± 0.06	0.35 ± 0.05
HVA/DA	0.39 ± 0.16	0.16 ± 0.03	0.34 ± 0.07	0.34 ± 0.08
FC				
DA	1.59 ± 0.30	4.30 ± 2.10	1.78 ± 0.25	0.78 ± 0.08
DOPAC	1.60 ± 0.34	1.20 ± 0.33	1.16 ± 0.18	0.70 ± 0.06
HVA	3.00 ± 0.66	1.83 ± 0.13	2.65 ± 0.91	1.22 ± 0.06
DOPAC/DA	0.99 ± 0.11	0.36 ± 0.12	0.67 ± 0.12	0.90 ± 0.04
HVA/DA	1.85 ± 0.15	0.62 ± 0.21	1.41 ± 0.32	1.58 ± 0.10
HIP				
DA	1.53 ± 0.43	0.85 ± 0.20	1.06 ± 0.29	0.69 ± 0.40
DOPAC	0.55 ± 0.12	0.42 ± 0.04	1.00 ± 0.26	0.32 ± 0.09
HVA	1.56 ± 0.32	0.95 ± 0.25	1.45 ± 0.33	0.83 ± 0.19
DOPAC/DA	0.47 ± 0.13	0.56 ± 0.10	0.96 ± 0.11	0.81 ± 0.43
HVA/DA	1.25 ± .36	1.26 ± 0.43	1.40 ± 0.09	2.19 ± 1.10
NAc				
DA	124.94 ± 34.56	100.27 ± 12.72	132.00 ± 30.78	46.38 ± 29.32
DOPAC	45.45 ± 11.43	56.12 ± 21.89	42.38 ± 9.87	15.55 ± 5.86
HVA	23.40 ± 5.76	17.68 ± 6.96	28.65 ± 5.81	10.96 ± 3.37
DOPAC/DA	0.37 ± 0.02	0.54 ± 0.16	0.33 ± 0.03	0.47 ± 0.11
HVA/DA	0.19 ± 0.02	0.17 ± 0.05	0.22 ± 0.01	0.36 ± 0.10

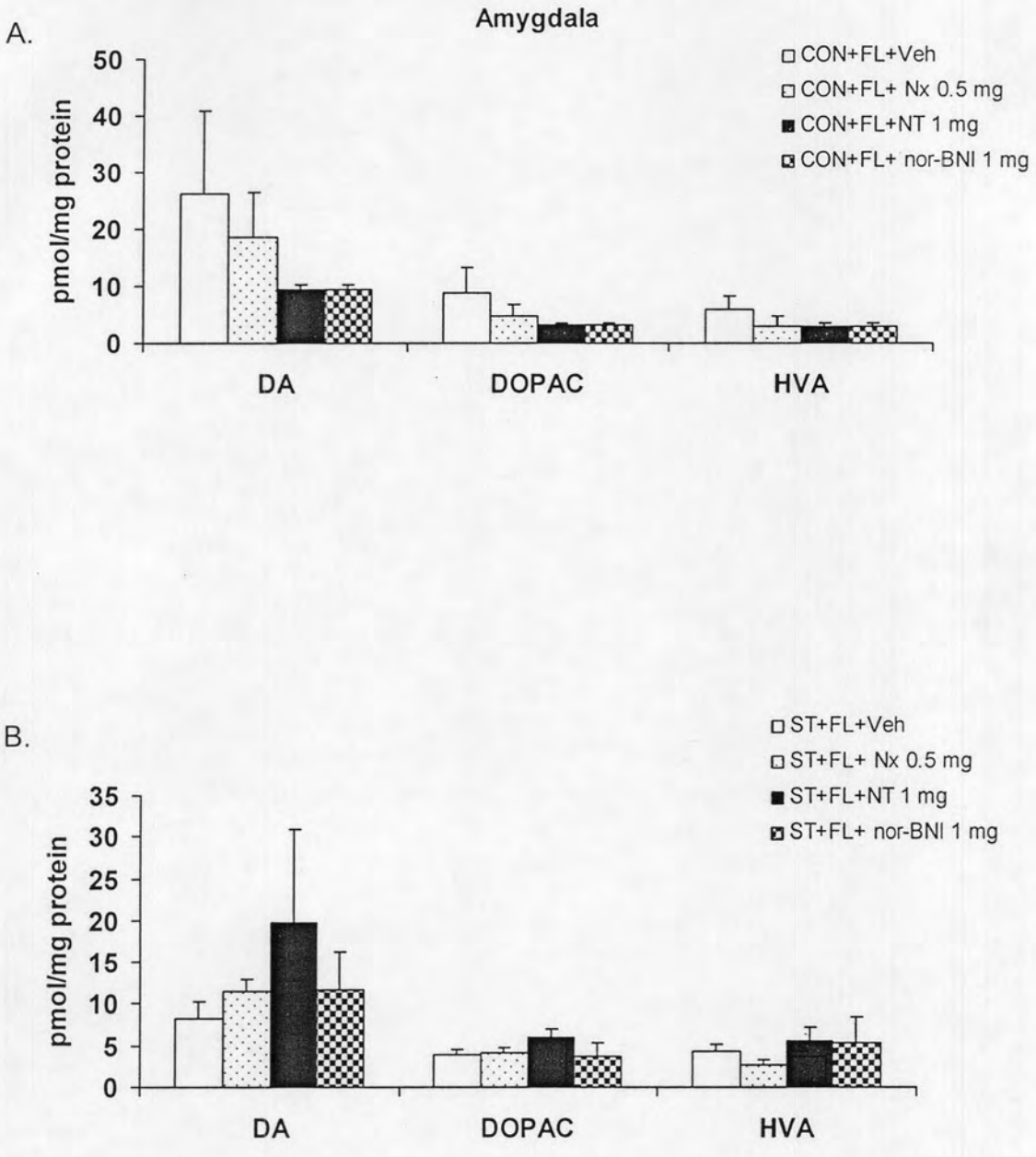


Figure 4-12 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DA and its metabolite in amygdala (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M.

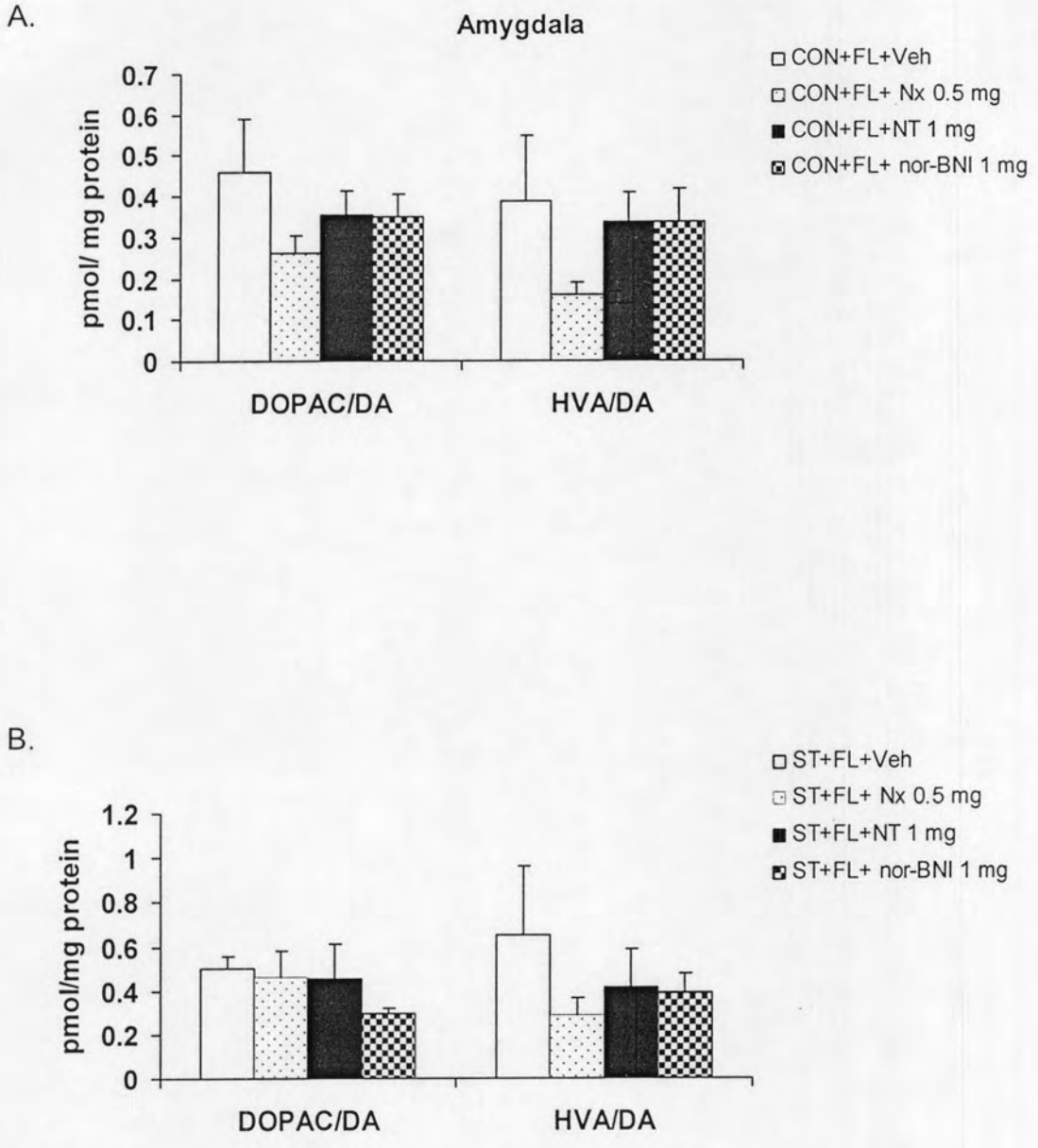
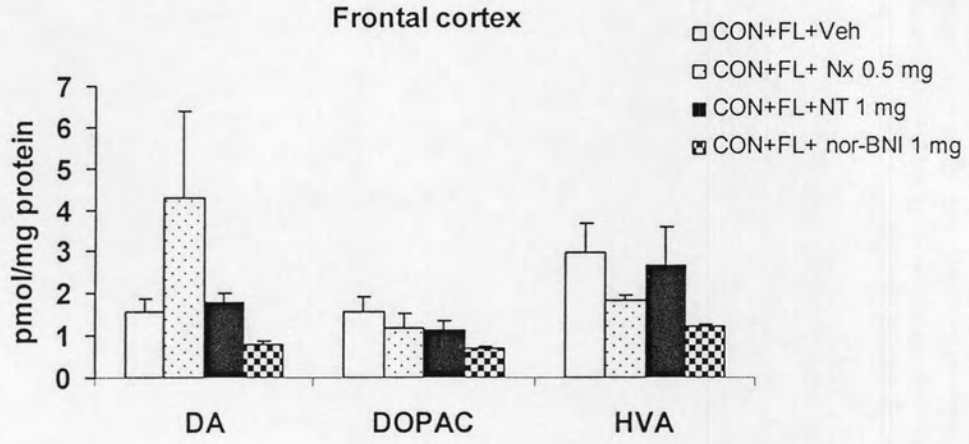


Figure 4-13 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DOPAC/DA and HVA/DA ratios in amygdala (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M.

A.



B.

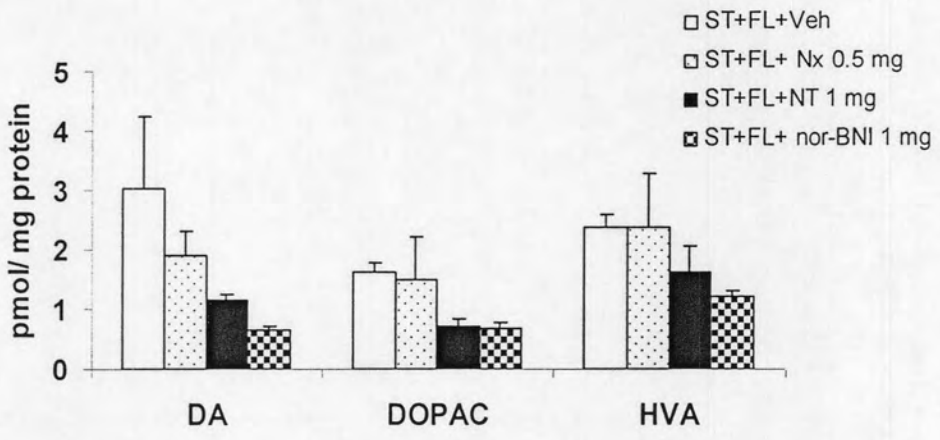


Figure 4-14 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DA and its metabolites in frontal cortex (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M.

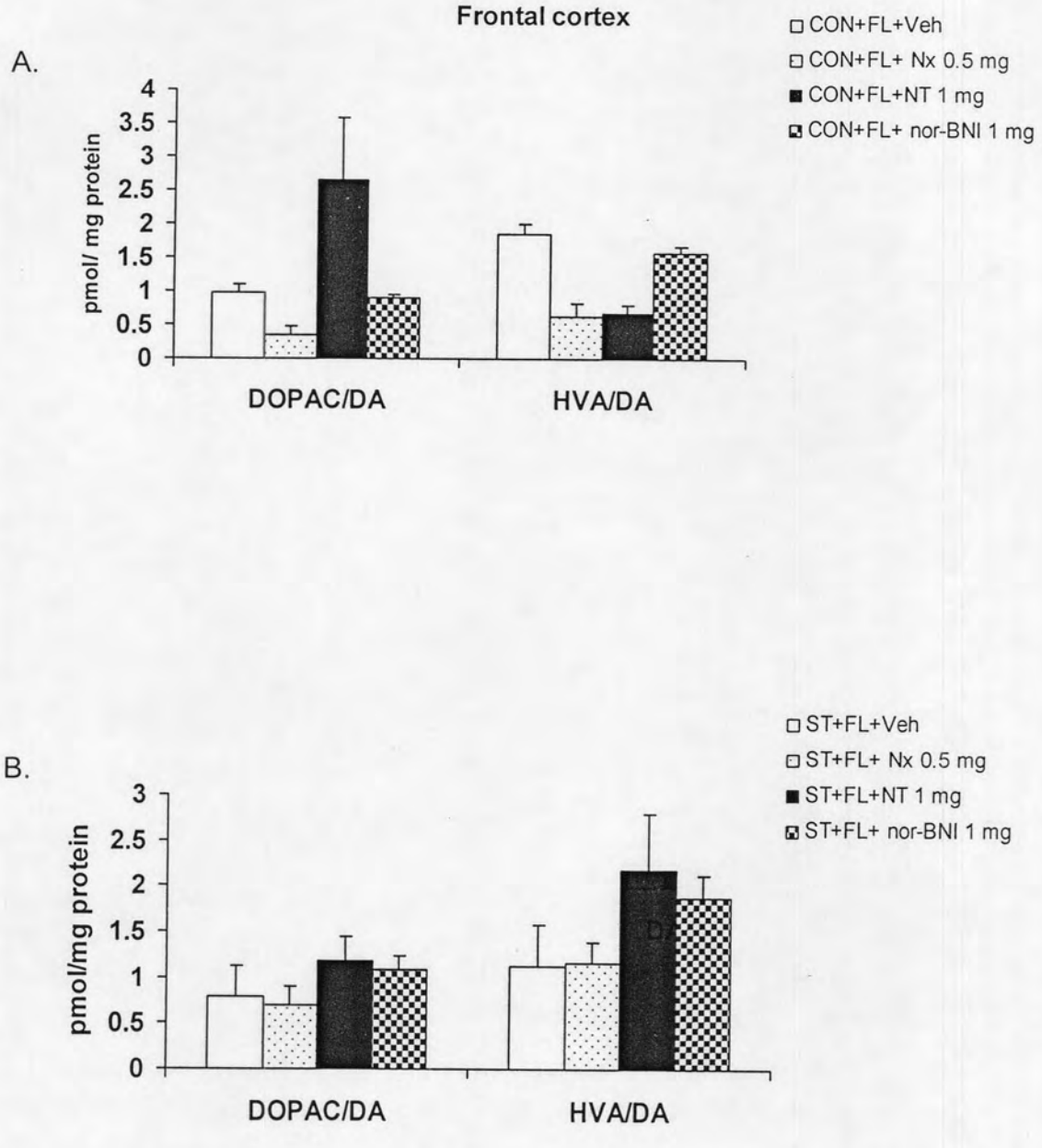
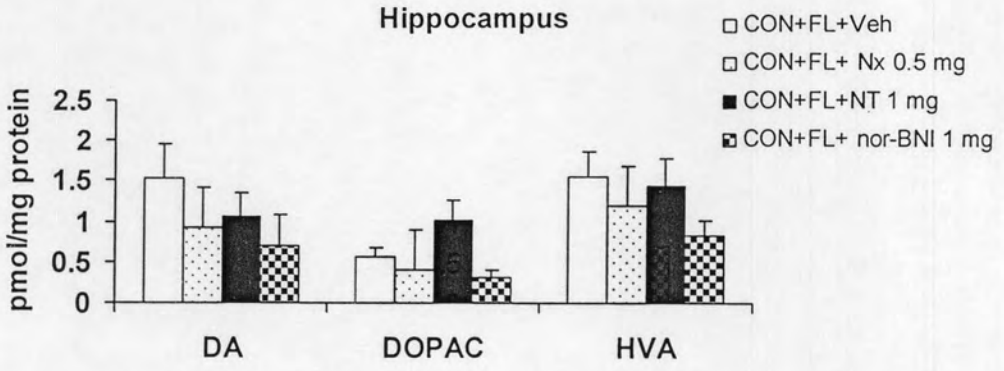


Figure 4-15 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DOPAC/DA and HVA/DA ratios in frontal cortex (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M.

A.



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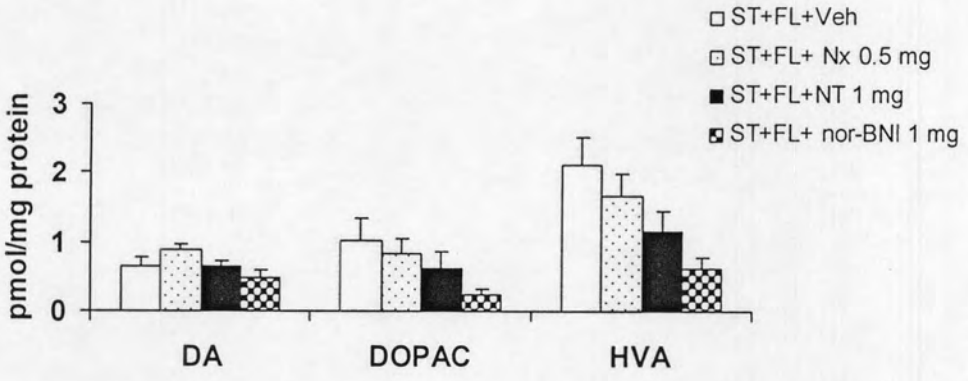


Figure 4-16 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DA and its metabolites in hippocampus (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M.

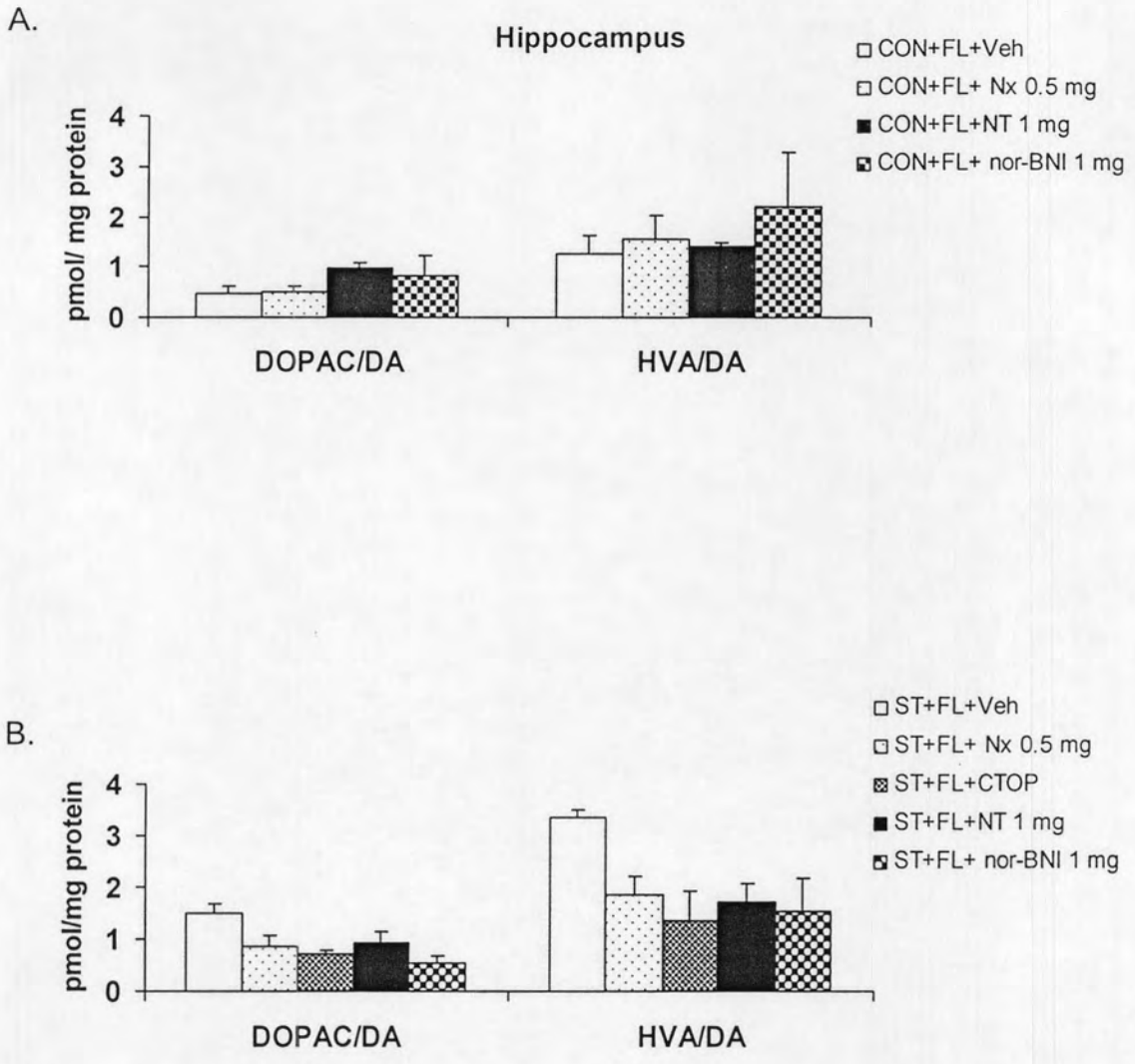
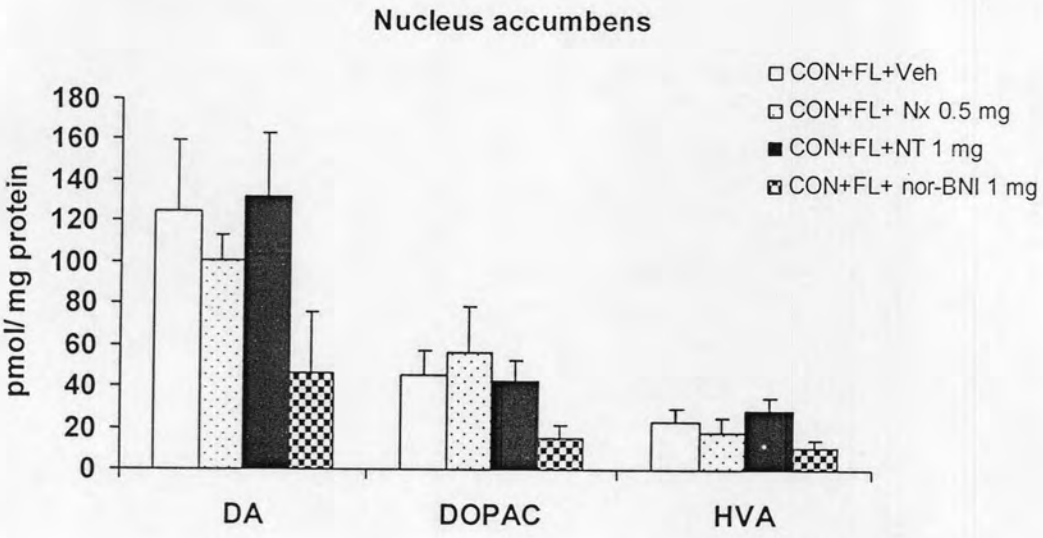


Figure 4-17 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DOPAC/DA and HVA/DA ratios in hippocampus (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M.

A.



B.

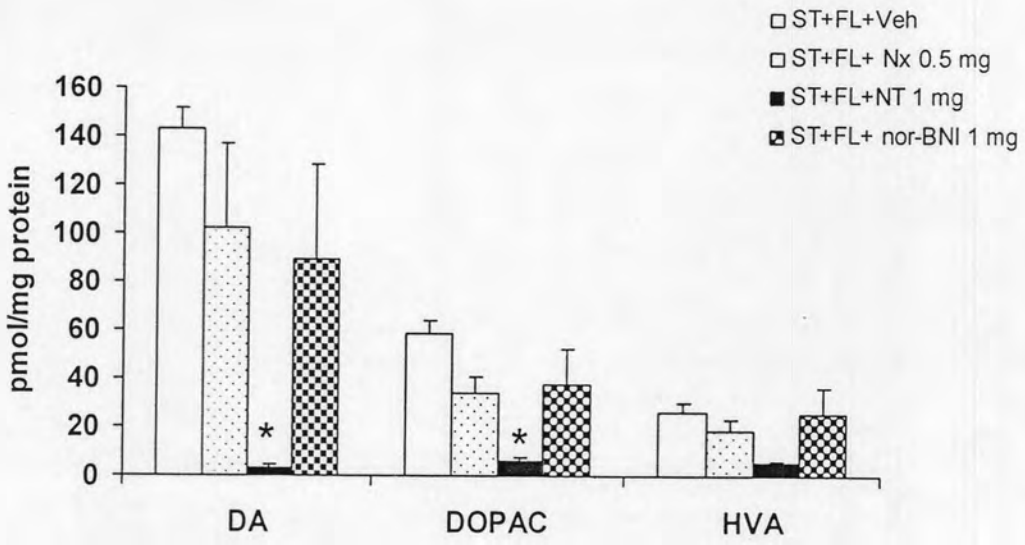
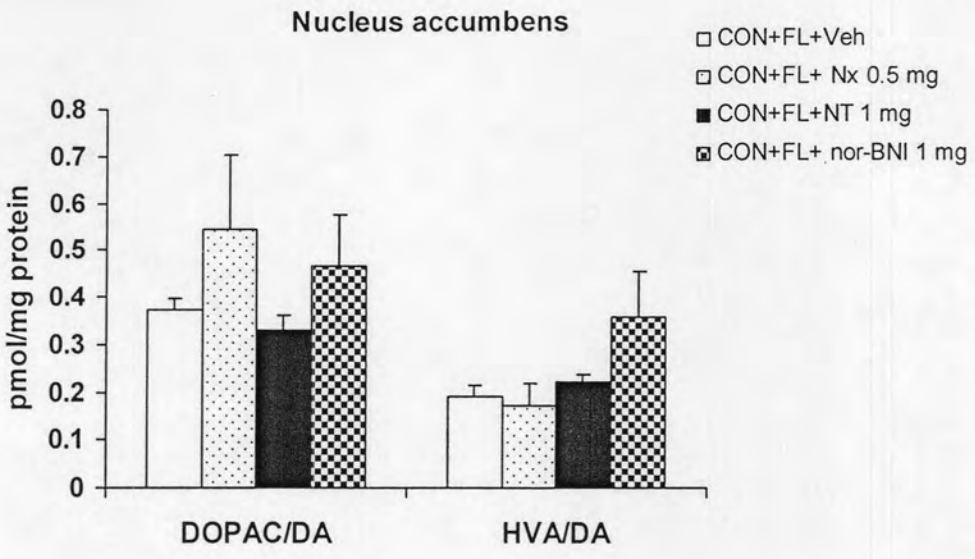


Figure 4-18 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DA and its metabolites in hippocampus (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M., significant difference was assessed with ANOVA followed by multiple-comparison test of Dunnett. * $P<0.05$ compared to ST+FL+Veh group.

A.



B.

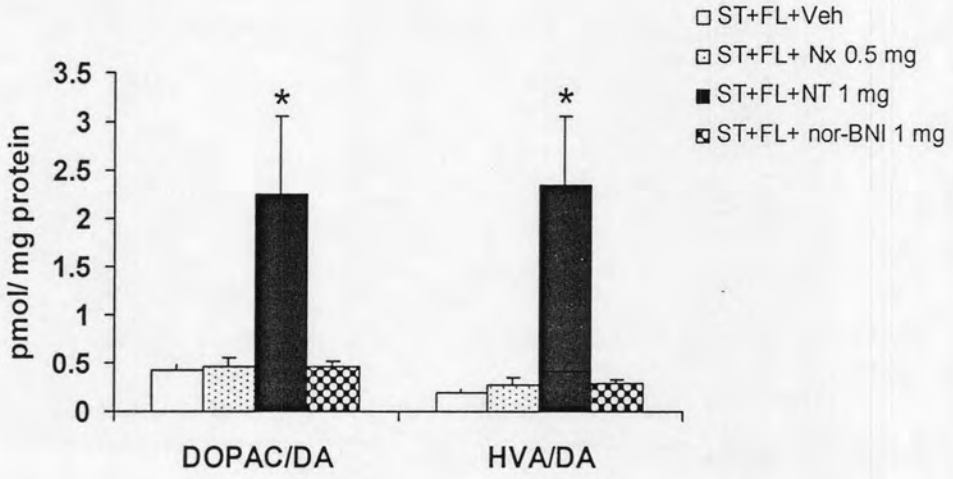


Figure 4-19 Histograms illustrate effect of naloxone (NX), naltridole (NT) and nor-binaltorphinmine (nor-BNI) on DOPAC/DA and its metabolites in nucleus accumbens (A) control with froot loops (CON+FL) and (B) stress with froot loops (ST+FL) rats. Data express as mean ± S.E.M., significant difference was assessed with ANOVA followed by multiple-comparison test of Dunnett. * $P<0.05$ compared to ST+FL+Veh group.