CHAPTER V

DISCUSSION

The objectives of this study were to examine the effects of chronic restraint stress on sweet food intake and dopaminergic neurotransmission, and the role of opioid receptor in stress-induced sweet food intake. The results demonstrated an increase sweet food intake in response to chronic restraint stress, whereas there was no alteration in the intake of standard rats chow in chronically-stressed rats (experiment 1). Interestingly, this sweet food intake can be suppressed by naloxone, the non selective opioid antagonist, whereas other selective opioid antagonists used in this study had no effect (experiment 2). Additionally, chronic stress rats had average daily weight gain and percent change of body weight lower than non stress rats. In rats submitted to sweet food intake, the average daily weight gain and percent change of body weight were higher than non sweet food intake rats. The adrenal hypertrophy and increase serum corticosterone observed in this study confirmed the effectiveness of this restraint stress model.

In animals study, adrenal gland weight was generally used as a parameter of chronic exposure to stress, as well as serum corticosterone levels. In this study, the adrenal gland weight and serum corticosterone in response to restraint stress were in agreement with previous studies in that prolonged stress lead to adrenal gland hypertrophy (Gamaro et al., 2003; Martin et al., 2007; Torres et al., 2002). This increased of adrenal gland and serum corticosterone in chronic restraint stress rats could be explained by that the prolong activation of the HPA axis by stressor can cause adrenal gland hypertrophy and elevation of corticosterone secretion (Tsigos et al., 2004). Interestingly, rats trained to eat sweet food, whether or not exposure to chronic restraint stress showed higher levels of serum corticosterone than control rats. The higher levels of corticosterone of these sweet food training rats could be elucidated by novel environment lead animals to stress, like acute stress response, since sweet food training paradigm was done in another room, new cage and new environment. There was a higher level of corticosterone but adrenal gland weight did not change. In

generals, when the same stressor is repeated the HPA axis response can adaptation such as desensitize or stable. Nevertheless, when the animals are challenged with a novel stressor following repeated exposures to stress, a sensitization of the HPA axis may occur (Bhatnagar et al., 2006). As in previous studies (Martin et al., 2007), they found that increased corticosterone levels in response to a novel environment in rats chronically exposed to restraint or variable stress. As well as Bhatnagar and Dallman (1998) have shown increased corticosterone levels in acute restraint stress in chronic exposure to cold stress rats. Thus, sweet food training paradigm may lead animals to stress like acute stress whereas acute stress did not affect to sweet food intake. Therefore, we conclude that 14 days of restraint stress are sufficiently severe or long to promote animals stress in these model at shown by change of serum corticosterone and adrenal gland weight in stress animals.

Previously, it has been shown that rats exposed to restraint stress 3 h/day, for 3 days or 11 days had a decrease in body weight gain and food intake (Harris et al., 1998; Marti et al., 1994; Rybkin et al., 1997). In this study, we also demonstrated that exposed to restraint stress for 14 days resulted in lower body weight gain and lower percent change of body weight when compared to non stress rats; however we did not find the different in daily food intake. In contrarily, some studies have reported that body weight gain and standard rats chow intake were unchanged in rat exposure to chronic restraint stress (Ely et al., 1997; Silveira et al., 2000; Torres et al., 2002). It is thus likely that stress could lead to either decrease or unchanged feeding, depending on duration or severity of stressor. In this study, it is quite interesting since the food intake between stress and non-stress rats was not different but body weight gain of stress rats was indeed lower than non-stress rats. This may occur through the difference in energy expenditure; in that the stress rats with heightened level of corticosterone may induce some calorigenic effects. As it is well known that corticosterone has a strong negative relationship between body weight and caloric efficiency (Dallman et al., 2003a; 2004). For example, corticosterone concentrations in the stress situation induced hepatic gluconeogenesis, lipolysis and proteolysis to provide energy for glucose synthesis, which can cause body weight loss (Dallman et al., 2003a; 2004; Tsigos et al., 2004). As well as, in studies using social stress, rats shown weight loss that has been attributed to

stress-induced enhancement of metabolic activity (Tamashiro et al., 2004). Therefore, from the present study, it may conclude that chronic restraint stress can cause body weight loss or decrease body weight gain through the effect of neurohormone (i.e. corticosterone) by regulating body metabolism. However, other factors such as animals illness or abnormal of gastrointestinal tract may also involved in these results (Boudry et al., 2007; Cameron and Perdue, 2005.).

In this study, the increase in sweet food intake following chronic restraint stress was evident and similar to previous investigations (Ely et al., 1997; Silveira et al., Torres et al., 2002). Moreover, Oliver's group (2000) and Zellner's group (2006) have reported that stress not only increases sweet food consumption but also shifts their food choice from lower sweet to higher sweet foods. The increase sweet food intake after exposure to chronic stress was possibly caused by glucocorticoids, the stress hormone, which could promote the motivation to eat high-sweet food (Dallman et al., 2003a, b). Bhatnagar and co-workers (2000) reported that corticosterone induced saccharine intake in adrenalectomized rats. The effect of corticosterone and sweet food consumption may mediate via other systems such as mesolimbic dopamine system.

Previously, Barr and coworkers (2000) suggested that glucocorticoids have a considerable impact on the mesolimbic dopamine system; that the glucocorticoids may have a direct or an indirect effect on the dopamine system. This based on their finding that dopamine levels in nucleus accumbens of adrenalectomized rats were decreased in response to hypothalamic self stimulation, and could be restored by corticosterone treatment. The dopamine levels in nucleus accumbens have been recognized as a stimulant for sweet food intake, the increase in dopamine levels by blocking the dopamine re-uptake transporter through nomifensine or cocaine can enhance sucrose intake (Hajnal and Norgren, 2001; Masi et al., 2000). This could be one mechanism responsible for the increase in sweet food intake in chronic restraint stress rats in the current study.

To further confirm the above mechanism, the measurement of dopamine levels in nucleus accumbens using HPLC-EC technique demonstrated that stress rats had significant higher level of dopamine in nucleus accumbens compared to non-stress rats. Additionally, the sweet food consumption was tended to increase dopamine levels in

nucleus accumbens in both stress and non-stress rats. This finding is not surprisingly since it had been shown previously that sucrose consumption can increase dopamine release as measure with microdialysis method (Hajnal and Norgren, 2001). Previously, a number of studies have shown that the extracellular of dopamine in nucleus accumbens was decreased in chronic stress rats (Gambarana et al., 1999, 2003; Masi et al., 2000; Scheggi et al., 2002). These data were somewhat differed from the present study, our results suggested that dopamine output was reduced in chronically stress rat as evident by the lower dopaminergic activity, unchanged DOPAC or HVA levels with higher level of dopamine. This difference could be at least explained in part by that the different in the method, we measured dopamine and its metabolites from the homogenate brain tissue not an *in vivo* microdialysis technique. The used method was actually a summation of changes, both intra- and extra-cellular, since they failed to capture the *in vivo* dynamics of the dopaminergic transmissions. Therefore, final results of dopamine levels in this study means intra- and extracellular dopamine levels. Moreover, the higher levels of dopamine in chronically stress rat could be owing to the effect of corticosterone on dopamine synthesis. It has been shown that corticosterone can increase tyrosine hydroxylase enzyme protein, the rate-limiting enzyme for the dopamine synthesis specifically in nucleus accumbens (reviewed by Czyrak et al., 2003). Thus, the conversion of tyrosine to L-DOPA by tyrosine hydroxylase was increased, and led to enhance dopamine levels.

Other than the modulation of dopaminergic system in the nucleus accumbens of stress rat, we also found that HVA level in the hippocampus of stress rat was lower than non-stress rat. The dopaminergic activity, shown by ratio of DOPAC/DA and HVA/DA was significantly lowered in the amygdala of stress rats compared to non-stress rat. The alteration of DOPAC/DA and HVA/DA ratio in amygdala, suggesting a decreased of dopamine release, which may decrease catabolism of DA to DOPAC and HVA, consequently. These results are in agreement with previous study of Konstandi and co-workers (2000), they found that the catabolism of DA to DOPAC or HVA was altered as demonstrated by a decreased DOPAC/DA ratio in amygdala after stress. These findings suggested that the chronic restraint stress may alter brain structures related to reward pathway and such a sweet food preference.

The sweet food (i.e. froot loops®) also has an impact on mesolimbic dopamine system. There is evidence that this system, which contains key components of the reward pathway, plays a critical role in the rewarding effect of drugs of abuse including sweet food preference (MacDonald et al., 2003). Several studies reported that a highly sweet food elicits an increase DA release in nucleus accumbens (Hajnal and Norgren, 2001; Masi et al., 2000; Pothos and Sulzer, 1998); similarly, we observed the increase in dopamine levels in nucleus accumbens of stress rat received sweet food about 4 times higher than non-stress rats not receiving sweet food (control). In addition, HVA, the dopamine metabolite in nucleus accumbens was significantly increased in stress rats receiving sweet food. Moreover, sweet food can increase the dopamine activity (DOPAC/DA) in hippocampus and tended to increase in frontal cortex in nucleus accumbens

The involvement of opioid system in regulating dopamine systems is well established (MacDonald et al., 2003) through the studies of pain modulation (Suarez-Roca et al., 2006; Torres et al., 2003; Towett et al., 2006) and drug addiction (Del Rosario Capriles and Cancela, 2002; Grakalic et al., 2006) during stress conditions, it is thus interesting to see whether opioid plays a role in sweet food intake following chronically restraint stress. In our study, different opioid antagonists was given before sweet food intake test, and we found that only naloxone, non-selective opioid antagonist (0.5 mg/kg, s.c.) can inhibit sweet food intake in stress rat with no effect on non-stress rat, suggesting that opioid receptor is modulated by stress and opioid itself is indeed partially regulating sweet food intake. This effect of naloxone was similarly to study by O'Hare et al. (2004). They found that naloxone at a dosage 1 and 3 mg/kg suppressed sucrose intake. Further supported by Boggiano and co-workers (2005), they found that naloxone (1 mg/kg) suppressed high palatable food intake in rat exposed to stress. In order to dissect the types of opioid receptor responsible for this finding, naltridole (δ-opioid receptor antagonist), nor-BNI (κ- opioid receptor antagonist) or CTOP (μ-selective opioid receptor antagonist) was injected to rats before submitting to sweet food intake test. Unfortunately, none of these antagonists was able to alter sweet food intake following restraint stress, it may be then suggested that δ- and κ- opioid receptor antagonists were not involved in regulating sweet food intake in stress condition.

However, it is still inconclusive for μ- opioid receptor for the following reasons; firstly, CTOP, μ-selective opioid receptor antagonist, used in this study may be unable to cross blood brain barrier and thus fail to exert its effect. This is owing to that the structure of CTOP, which composed of 8 amino acids when injected subcutaneously, was then unable to cross blood brain barrier and reach its target. Secondly, naloxone, the non-selective opioid antagonist, at low dose it was shown to bind with higher affinity to μ-opioid receptor than other types (Leslie, 1987). Therefore, stress-induced sweet food intake in this study may be a consequence of up- or down regulation of μ- opioid receptor in mesolimbic system, which leads to behavioral change. Previous studies demonstrated that chronic stress can up-regulate μ- opioid receptor mRNA of in VTA and midbrain (Nikulina et al., 2005; Yamamoto et al., 2003), whereas density of opioid receptor in cortex and hippocampus was decreased in chronic stress rats (Dantas et al., 2005). All of this results indicated that chronic restraint stress could modify opioid system that led to behavioral change. Therefore, in order to elucidate the role of μ-opioid receptor in regulating sweet food intake in chronic stress condition, the CTOP, the only known available μ- opioid receptor antagonist, may need to be injected directly into specific brain area.

It is obvious that sweet food intake of non-stress (control) rats in experiment 2 was somewhat higher than those in experiment 1. This may be explained by that the vehicle injected to these rats was ethanol, which was known that it can activate mesolimbic dopamine system by increased dopamine levels in this area (Yim and Gonzales, 2000). Consequently, this increase dopamine was then stimulated the sweet feeding in these rats and resulting in higher number of sweet food pellets consumed. Similarly, Hajnal and Norgren (2001) have previously demonstrated that increased dopamine levels in nucleus accumbens led to an increase of sucrose intake. Additionally, the measurement of dopamine levels reveals that dopamine levels in the brain especially nucleus accumbens of vehicle injected rats (in experiment 2) were higher than its counterpart in experiment 1. Based on this evidences, we were then able to conclude that increased sweet food intake in control animal in experiment 2 was at least a result of ethanol induced dopamine release.

In the second experiment, we found that when compared to vehicle injected rats, we found different effects of opioid antagonists on the function of dopaminergic neuron in control and stress animals. In control animals, nor-BNI, κ- opioid receptor antagonist, tends to decreased dopamine levels, whereas other opioid antagonists did not affect. On the other hand, naltridole, δ- opioid receptor antagonist, could suppress dopamine levels in chronic stress animals but nor-BNI has no effect. From these differences, it is suggested that chronic exposure to stress causes alteration of opioid system by either decreased in number or function of κ- opioid receptor and/or decreased in number or function of δ- opioid receptor which can modify dopaminergic activity in the nucleus accumbens. However, it should keep in mind that the levels of dopamine and its metabolites in this study cannot easily explain since the neurotransmitter levels were not only a summation of intra- and extra-cellular as stated previously, but also an integrated effect of sweet food which can in turn stimulate dopamine release in the same way as ethanol.

Interestingly, by comparing the neurotransmitter levels in either stress or non-stress rat receiving sweet food between the first and second experiment (given ethanol as a vehicle before sweet food intake test), we observed an increased in dopamine levels in the nucleus accumbens and amygdala of non-stress animals after treated with ethanol (vehicle). In the stress rat, we observed the increased in dopamine level in nucleus accumbens and frontal cortex in the one received ethanol as a vehicle (experiment 2). It should be noted that DOPACDA and HVA/DA ratio was not affected. It is likely that frontal cortex was more sensitive to ethanol when exposure to chronic stress. These results indicated that chronic restraint stress can modify differed parts of brain which may affect feeding behavior.

In conclusion, this study confirmed that chronic restraint stress cause an increase sweet food intake and stress is differentially affected the activity of central dopaminergic neurotransmission of reward system and the stress regulatory system which are nucleus accumbens, hippocampus, and amygdala. This sweet food intake is partially regulated by opioid system since it can be suppressed by naloxone, the non-selective opioid receptor. Although the types of specific opioid receptors responsible for sweet food intake could not be ruled out, it is suggested that μ- opioid receptor

antagonist may be a candidate for this effect. Moreover, the adaptations of δ- or κ-opioid receptors should be taken into account for these changes since the dopaminergic neurotransmission is altered following selective opioid receptor antagonists. Nevertheless, this study is able to establish the interrelation between stresses and opioid system in modulating mesolimbic dopamine system, the reward system. In the future, the study of this relation in depth, by injecting a selective opioid antagonist into a specific brain area or *in vivo* microdialysis during behavioral test would be more useful and may reveal some unraveled mechanisms. Further, the molecular techniques to measure the alteration of opioid receptors by mean of mRNA or protein expression levels, or the function of these receptors (i.e. ligand binding) is also promising. Finally, the alteration of opioid receptors in the brain structure related to reward system may suggest a new drug target for treatment of stress-related diseases in the future.