CHAPTER VI
CONCLUSION

The overall objective of the present study was to investigate the effects of estrogen deprivation on anxiety-like behaviors, $GABA_A$ receptor subunit gene expression and function, and serotonergic activity in brain area associated with anxiety utilizing ovariectomized rat as a model. Firstly, the time required to induce anxiety was studied along with the changes in $GABA_A$ receptor subunit gene expression and serotonergic activity in brain area associated with anxiety. The rats were ovariectomized and later tested for anxiety with elevated T-maze; the brains were then collected for the measurement of $GABA_A$ receptor subunit gene expression and serotonin levels with real time PCR and HPLC techniques, respectively. Secondly, the $GABA_A$ receptor sensitivity was tested by injecting different dosages of benzodiazepine agonist, diazepam; concurrently, the $GABA_A$ alterations affect serotonergic activity was also investigated. The first and the second would provide the information on the roles of estrogen in causing and preventing anxiety. Finally, the role of estrogen in treating anxiety was studied. In this part, anxiety was first induced by ovariectomized and later supplemented with estrogen, the behavioral data along with the changes in $GABA_A$ receptor sensitivity and serotonin were investigated.

The conclusions that can be drawn from this dissertation are as follows.

1. Anxiety could be uniformly induced in female rats after ovariectomized for at least 3 weeks. The longer the lacking of estrogen, the higher the anxiety was demonstrated.
2. The alterations of $GABA_A$ receptor subunit gene expressions i.e. $\alpha 2$, $\alpha 3$ and $\alpha 4$ were found esp. in the midbrain of the estrogen-deprived rat. These alterations were likely to affect $GABA_A$ receptor sensitivity as these rats showed higher responsiveness to benzodiazepine agonist compared to estrogen supplemented ovarictomized rats. The changes in $GABA_A$ receptor subunit gene expressions and sensitivity were found in relation to the changes in behavior.

3. The serotonergic activity in the brain areas associated with anxiety as determined from serotonin, its metabolite and the ratio of its metabolite and serotonin indicated that there were age dependent as the levels of serotonin and its metabolite were increased as the rat aged. Despite the age effect, the serotonin turnover rates (the ratio of its metabolite and serotonin) in the estrogen supplemented rats were relatively stable; while it was more fluctuated in the estrogen deprived rats.

   From all above, the preventive effect of estrogen was therefore supported through the behavioral data, the alterations of $GABA_A$ receptor subunit and function, and the serotonergic activity.
4. Following the induction of anxiety by ovariectomy, subsequent estrogen supplementation could reduce anxiety as seen in behavioral data from the last part of the experiment. The reduction in anxiety was probably due to the regulative effect of estrogen on GABAergic and/or serotonergic system as demonstrated by the different in benzodiazepine agonist responsive between ovariectomized rats with or without estrogen supplementation. However, due to the limitation of this study, it could not rule out whether the alteration of $GABA_A$ receptor function after ovariectomy affected the serotonergic activity in brain areas associated with anxiety.

Conclusively, this dissertation provided the first evidence of the time of estrogen deprivation on anxiety levels; the more understanding mechanism in relevant to the timing of estrogen decline and the molecular mechanism of estrogen on the regulation of GABAergic system, which has important role in modulating emotion the brain was established.