CHAPTER V

Discussion

Glutamate NMDAR1 receptor

The present study demonstrated that the expression of NMDAR1 levels was significantly increased in the rat striatum and frontal cortex after methamphetamine administration. The results of the present study are in agreement with the previous reports on a raise in NMDAR1 subunit in cerebral cortex following administration of psychostimulant such as cocaine (Ghasemzadeh et al., 1999; Toda et al., 2002; Hemby et al., 2005). Although the mechanisms responsible for this methamphetamine–induced increases in NMDAR1 receptors are unknown, it is known that methamphetamine increases extracellular glutamate in the striatum (Mark KA et al., 2007), cerebral cortex (Shoblock et al., 2003), frontal cortex (Kim et al., 1981), prefrontal cortex (Stephans and Yamamoto, 1995) and hippocampus (Raudensky and Yamamoto, 2007). Additionally, increases in vesicular glutamate transporter VGLUT1 occur in response to facilitate methamphetamine-induced elevations in glutamate releasing (Mark et al., 2007). As methamphetamine is an abused psychostimulant, these results provide a support of psychostimulant effects on glutamatergic system. Dysfunctions of glutamatergic system may be derived from changes in the functions of dopaminergic system which are induced by psychostimulant effects (Baker et al., 2003; Karler et al, 2003). Moreover, an impairment of monoamine neurotransmission such as dopamine after repeated METH produces changes in amino acid homeostasis, probably leading to an overstimulation of NMDA receptor (Bustamante et al., 2002).

The findings of this study showed an increase of NMDAR1 in striatum in both acute and chronic methamphetamine administrations. It is well established that amphetamine is a dopamine agonist which can direct affect the striatum. Therefore, an alteration of NMDAR1 expression had occurred in striatum after acute methamphetamine administration, and its effect on a change of NMDAR1 expression remains occurrence following repeated administration. It has been reported that both acute and chronic methamphetamine administration induces dysfunction of neurotransmitter system such as monoaminergic system in the striatum which is

associated with behavioral changes including hyperactivity (Reynolds et al., 1998) and stereotypy (Reynolds et al., 1998; Mori et al., 2002). Thus, the results of this study provide an evidence to support that methamphetamine-induced glutamatergic dysfunction may be involved in abnormalities of behaviors expressed in methamphetamine dependence. This may be affected from monoaminergic system dysfunction in striatum in methamphetamine dependence.

In frontal cortex, a significant alteration in NMDAR1 expression was observed only in rats treated with chronic methamphetamine administration. The finding of the present study can be speculated that neurons in frontal cortex received the synaptic input indirectly from striatum, a specific area of drug abuse and amphetamine response, via several parts of brain such region as pallidum and thalamus. A malfunction in frontal cortex produces cognitive impairments (Paulas et al., 2002) and compulsive behaviors (Volkow et al., 2001c) after long term exposure to methamphetamine. Thus, this finding supports the effects of methamphetamine which contribute malfunction of neurotransmitter that is associated with behavioral changes, and this phenomenon correspond to neuronal circuitry and duration of methamphetamine administration.

Although no alteration in NMDAR1-IR in the hippocampal formation, which was not identified a sub-region of the hippocampus, was observed in the present study after methamphetamine treatment, the finding is consistent with a previous study that has revealed no change and a slightly decrease of NMDAR1 mRNA expression in dentate gyrus after exposure to morphine and cocaine, respectively (Turchan et al., 2003). Moreover, no differences in the NMDAR1, 2A and 2B immunoreactivities were observed in the hippocampus of methamphetamine -sensitized rats (Yamamoto et al., 1999). Taken together, these observations provide further support for a regional specific of glutamatergic dysfunction after methamphetamine administration. However, this result needs to be repeated in subregions of hippocampal formation as it is known that the hippocampal formation composed of three compartments including dentate gyrus, hippocampus proper (CA1-CA3), and subiculum, which can communicate and form interconnections within hippocampal formation on with other brain regions (Witter, 2003).

Neuronal glutamate EAAT3 transporter

The present study demonstrated an increase in EAAT3 expression following repeated administration of methamphetamine in hippocampal formation which may be a compensatory response to an elevation in glutamate in hippocampus (Kim et al., 1981; Rocher and Gardier, 2001; Raudensky and Yamamoto, 2007). This upregulation of EAAT3 may suggest a protective mechanism of neurons in this brain region to regulate the rise in extracellular glutamate and consequently prevent excitotoxicity.

Alternatively, an increase in EAAT3 expression following chronic methamphetamine treatment in hippocampal formation observed in this study may be due to the internalization of this transporter which may contribute to the excessive extracellular glutamate via the action in the reverse direction (Boeck et al., 2005). It is well known that the hippocampal formation consists of three compartments including dentate gurus, hippocampus proper (CA1-CA3), and subiculum. These compartments form connections within hippocampal formation which receive major cortical inputs from the entorhinal cortex and send dense direct projection or indirect reciprocity to the prefrontal cortex (Witter, 2003; Kelly, 2004). Therefore, the upregulation of EAAT3 in hippocampal formation may reflect adaptive changes in neuronal glutamate transporter activity within each of these compartments of this brain region after exposure to methamphetamine.

On the other hand, a significant decrease and a trend towards decrease of EAAT3 expression in striatum and frontal cortex, respectively, after methamphetamine administration found in the present study were supported by previous reports that chronic methamphetamine administration enhances glutamate efflux in striatum (Kim et al., 1981; Del Arco et al., 1999; Mark et al., 2004 and Mark et al., 2007), prefrontal cortex (Staphan and Yamamoto, 1995; Shoblock, 2003), and frontal cortex (Kim et al., 1981). Several studies demonstrated that methamphetamine-induced neuronal cell death in both striatum (Deng and Cadet, 2000; Deng et al., 2001; Xu et al., 2005; Zhu, 2006) and frontal cortex (Deng et al., 2001). Since neuronal EAAT3 transporter is expressed on both pre- and postsynaptic neurons (Rothstein et al., 1994), therefore, a reduction in EAAT3 expression in both striatum and frontal cortex may be an effect of methamphetamine-induced neuronal cell death. Another possibility is that methamphetamine may induce a decrease of EAAT3 expression

consequence lead to neuronal cell death. Moreover, this finding may be supported by a recent report that methamphetamine can produce an increase in glutamate release in the corticostriatal pathway which can lead to exitotoxicity (Mark et al., 2004). Therefore, these findings provide further support for glutamatergic dysfunction in methamphetamine dependence, with abnormalities involving a transporter which contributes to maintaining appropriate synaptic glutamate levels. However an investigation into possible underlying mechanism between neuronal glutamate transporter dysfunction and neurotoxicity is warranted.

The present study revealed different alterations of EAAT3 expression in hippocampal formation, striatum and frontal cortex after acute and chronic methamphetamine administration. It was shown that acute and chronic methamphetamine administration decreased EAAT3 expression in the striatum, although a reduction seen in frontal cortex did not reach statistical significance compared with control. In contrast, an increase in EAAT3 expression following repeated administration of methamphetamine was observed in hippocampal formation. Similar with the present study, the differential changes in glutamatergic neurotransmission has been reported in the hippocampus and striatum in the methamphetamine-sensitized rats (Yamamoto et al., 1999).

The correlation between NMDAR1 and EAAT3 expressions

In the present study, a significant increase of NMDAR1 expression was observed in striatum of acute methamphetamine group, in both frontal cortex and striatum of chronic methamphetamine group, and a trend toward increase in hippocampal formation of both acute and chronic methamphetamine groups. On the other hand, a significant decrease was observed in striatum of both acute and chronic methamphetamine groups, and a trend toward decrease of EAAT3 expression was observed in frontal cortex of both acute and chronic methamphetamine groups. However, a significant increase of EAAT3 expression was observed in hippocampal formation of chronic methamphetamine group, although there was no an alteration of EAAT3 expression in acute methamphetamine group. There was no significant correlation between NMDAR1 and EAAT3 expression in either acute or chronic methamphetamine groups in hippocampal formation, frontal cortex and striatum,

although a trend toward significant correlation between NMDAR1 and EAAT3 expressions was seen in striatum of both acute and chronic methamphetamine groups.

It could be speculated that methamphetamine produces excitotoxic neuronal cell damage as well as neuronal death leading to a depletion of glutamate content which is contribute to increase in extracellular glutamate (Kim et al., 1981; Staphan and Yamamoto, 1995; Shoblock, 2003; Mark et al., 2007), and a consequent upregulation of NMDAR1 expression during methamphetamine dependence. Another possibility is that a malfunction of glutamate uptake to normalize glutamatergic transmission from synaptic cleft by glutamate transporters may increase in extracellular concentrations of glutamate as evidenced by the present observation that methamphetamine can reduce EAAT3 expression in striatum and frontal cortex. Thus it is possible that the combination of overexpression of NMDAR1 receptor and a reduction of glutamate uptake site into the neuronal compartment may induce abnormalities of glutamate transmission and consequently results in excitotoxic neuronal damage following methamphetamine administration. Although there was no correlation between NMDAR1 and EAAT3 expressions in hippocampal formation, taken with the results that an increase of EAAT3 expression but not NMDAR1 expression in both acute and chronic methamphetamine exposure. These finding indicate a protective mechanism of the hippocampal formation to the dosage and duration of methamphetamine treatment in this study. It is interesting to study the effects of methamphetamine on expression of NMDAR1 and EAAT3 as well as the relationship between NMDAR1 and EAAT3 in higher dose and longer duration than that of the present study. The findings of this study suggest that methamphetamine may induce the differential changes in glutamategic transmission, and the brain regions are differentially vulnerable to the neurotoxic effects of methamphetamine (Eisch et al., 1992).