DISCUSSION

In this study, all rats showed exploratory behavior as soon as they were placed in a recording cage. After becoming adapted to the new environment, they started to groom and later fell asleep. In the control group of animals, the power in most of the EEG frequency bands began to increase at about 30 min after saline injection and the increase was significant after 75 min, except the higher frequency bands (beta-2 and gamma bands) in which no changes were observed. One might possibly think that these resulted from physiological saline injection. However, this is not possible since physiological saline was also used as solvent for methamphetamine in other groups of drug treatment and different results were obtained. In addition, this can be clearly explained by the observation that the animals, after saline injection, were habituated well to the environment and fell asleep thereafter.

From the results, it was demonstrated that methamphetamine inhibited the increase in most EEG frequency bands (delta, theta, alpha-1 and alpha-2 bands) therefore the power of these frequency bands was not different from their pre-injection or baseline levels. This implication is also noted from the results that, after comparing to the control group of animals, the increase in most EEG frequency bands was apparent at later periods after the injection (e.g., 165 min vs 75 min of control group). This also means that the low dose methamphetamine-treated animals could fell asleep at later time than the control group of animals. The inhibitory effects of methamphetamine is more pronounced and can be noted in the higher dose of methamphetamine-treated group (2.0 mg/kg BW) that alpha-1 and alpha-2 frequency bands were significantly decreased. However, it seems difficult to explain the effects of the highest dose of methamphetamine-treated group (4.0 mg/kg BW) on the power spectrum of EEG since the dose appeared to cause no changes in any power bands of the EEG spectrum. By such complexity of the observations in the present study, it might be possible at the present to simply suggest the effects to be due to the mechanism that moderate dose (2.0 mg/kg) and high dose (4.0 mg/kg) activate different

dopamine receptors. For locomotor activity, methamphetamine in low and moderate doses produced increases, but the high dose produced a seemingly multiple phase of locomotor activity. Regarding the effects on stereotyped behavior, all doses of methamphetamine increased the behavioral scores. Therefore, the effects of methamphetamine on EEG, locomotor activity, and stereotyped behavior were similar to those caused by amphetamine. Ferger et al. (1994) found that large dose of d-amphetamine produced a selective increase in power of the alpha-1 band with locomotor activation while the low dose produced a general lowering in power in all of the frequency bands. Furthermore, in the present study, the effects on locomotor activity were similar to the previous finding of Kuczenski and Segal (1999) that the acute administration of 0.5 mg/kg amphetamine enhanced locomotor response. On the other hand, regarding stereotyped behavior after low and moderate doses the results obtained in this study did not agree with the study of Kuczenski and Segal (1999) in which the absence of stereotyped behavior was found. This difference might probably come from the difference of definitions of stereotyped behavior between the two studies. This study regarded all stereotyped behavior including stereotyped sniffing and oral stereotyped behavior while those in Kuczenski and Segal (1999) only oral stereotyped behavior was scored. The data of stereotyped behavior after high dose of methamphetamine obtained in this study agree with the study of Ferger et al. (1994) in which both sniffing and oral stereotyped behavior were scored after 4.0 mg/kg BW amphetamine injection. Stereotyped behavior obtained in this study also agree with the studies of Groves and Tepper (1983) and Segal and Schuckit (1983). At a low dose of amphetamine, rats showed exploratory behavior and repetitive head movements. After a further high dose of amphetamine, rats showed oral stereotyped behavior such as licking or gnawing.

Though the involvements of other neurotransmitter systems, e.g. serotonin and noradrenergic mechanism (Itoh et al. 1990), cannot be excluded, the mechanism that methamphetamine affects EEG, locomotor activity, and stereotyped behavior is accepted to be mediated via dopaminergic system (Creese and Iversen 1974; Kelly et al. 1975; Kelly and Iversen 1976), particularly, dopamine receptors (Hisashi 1995). When methamphetamine is administered to animals, it causes dopamine release. The D_1 receptor

has higher affinity for dopamine than D_2 receptor (Leff and Creese 1985; Andersen et al. 1990) and the D_1 receptor is the most widespread dopamine receptor and is expressed at higher levels than any other dopamine receptor (Dearry et al. 1990; Fremeau et al. 1991; Weiner DM et al. 1991). When dopamine binds to D_1 receptor coupled with Gs protein, a kind of G protein coupled receptor, Gs protein activates adenylated cyclase (Kebabian et al. 1972; Kebabian and Calne 1979; Stoof and Kebabian 1984; Monsma et al. 1990; Dearry et al. 1990; Zhou et al. 1990). The produced cAMP activates protein kinase A enzyme that thereafter causes phosphorylation. Calcium channels are phosphorylated too. Then the calcium channels are caused to open and more calcium influxes. By the same time, potassium channels are closed and potassium efflux decreases (Kitai and Surmeier 1993; Missale et al. 1998). Then, intracellular positive ions increase and the cell is in state of hypopolarization. Finally, post-synaptic neuron increases excitability. In contrast, the membrane-bound D_2 receptor has Gi protein, a kind of G protein coupled receptor. If D_2 receptor is stimulated the activity of enzyme adenylate cyclase will be inhibited, and hence the amount of intracellular cAMP will decrease (Onali et al. 1984; Stoof and Kebabian 1981; Stoof and Kebabian 1984). Calcium channels are closed (Missale et al. 1998) and potassium channels are opened (Einhorn et al. 1990; Albert et al 1990; Missale et al. 1998). Then, intracellular positive ion decrease (Albert et al 1990). Finally, post-synaptic neuron excitability decreases (Valentijn et al 1993).

Different affinities for dopamine of dopamine receptor subtypes allow different receptors to be activated at low or high levels of dopamine release or injection (Gingrich and Caron 1993). When the low to moderate amount of methamphetamine were administered, a limited amount of dopamine was released and bound to D_1 receptor only. On the other hand, high dose of methamphetamine caused more release of dopamine. Some of this released dopamine binds to D_2 receptor in addition to D_1 receptor. Supporting this explanation is the suggestion that concurrent activation of both D_1 and D_2 receptors is required for full expression of the effects of drugs such as methamphetamine (Delfs and Kelly 1990; Xu et al. 1994; Kuczenski and Segal 1999). For example, pretreatment with a D_1 agonist followed by administration of a D_2 agonist promoted the expression of locomotor activity and stereotyped behavior to a higher degree than either

drug administered alone (Mashurano and Waddington 1986).

Regarding locomotion, low and moderate doses of methamphetamine stimulate D_1 receptor and hence more action potential or signals to locomotion center in mesolimbic dopamine pathway, which would make an increase of locomotor activity. On the other hand, the high dose stimulates D_2 receptor in addition to D_1 receptor. As a result, an increase of locomotor activity was observed first, followed by its decrease. This indicates that after the level of methamphetamine declined to some extent, it could not stimulate D_2 receptor but D_1 receptor. This can be observed that locomotor activity increased, and after that decreased and finally increased again before gradually decreased to the baseline. Such type of multiphasic dose-dependent behavioral effects is similar to those caused by amphetamine which differential stimulation of mesolimbic or striatal brain regions was suggested. Specifically, the mesolimbic brain region was expected to mediate the increased locomotor activity and stereotyped behaviors observed in response to low dose of amphetamine. For the high dose, increased stereotyped behaviors are believed to be mediated largely through the nigrostriatal pathway (Creese and Iversen 1974; Kelly and Iversen 1976; Kelly et al. 1975). Electrophysical studies have also demonstrated such complexity of the dose-dependent effects of amphetamine that seemed to agree with these behavioral responses (Groves and Robec 1976; Robec and Zimmerman 1980). In addition, amphetamine was previously shown to induce the biphasic dose-dependent effects on in vivo striatal dopamine synthesis (Kuczenski 1977).

The results on gamma power band were not the same pattern as for other power bands. After low and moderate dose of methamphetamine, the power of gamma band did not increase significantly. This might be because these doses stimulated a limit amount of D_1 receptor. High dose of methamphetamine increased significantly power of gamma band because the dose stimulated both D_1 and D_2 receptors. This dose stimulated D_1 receptor up to its saturation and higher amount than low and moderate doses of methamphetamine.

Regarding the time-course of response, in the control group, after 75 min following saline injection, all of the EEG power bands except beta-2 and gamma bands increased, locomotor activity decreased, and stereotyped behavior was not detectable. The effect probably can be explained by the observation that the rats, after treatment with

physiological saline, were gradually adapted to the environment and fell asleep. When sleep was shown, EEG pattern was in spindle shape. This is the reason why most of the lower frequency EEG power bands except beta-2 and gamma bands increased during most of the post-injection period.

Regarding the correlation between various EEG frequency bands, locomotor activity and stereotyped behaviors, various levels of correlation were obtained. Though the relatively high level of correlation coefficients of 0.8 and -0.8 were obtained between two frequency bands of EEG (alpha-1 and gamma bands respectively) and locomotor activity and stereotyped behavior, the correlations were not significant. In contrast, the correlation of 1.0 level between locomotor activity and stereotyped behaviors was significantly observed. This suggests that whenever the animals show high locomotor activity the high score of stereotyped behavior will definitely be obtained. However, the strong correlation might be due to the relatively high sensitivity of the locomotor activity meter used in the present study since it was observed oftenly that when the animals showed stereotyped sniffing and/or orally stereotyped behaviors such as licking and gnawing the locomotor activity meter counted and detected the activity mistakenly as locomotor activity. This can be verified later.

From the present study, although methamphetamine is suggested to modulate the EEG, locomotor activity and stereotyped behaviors via the dopaminergic mechanisms, there appears to be no simple quantitative relationship between all of them. In addition, there is evidence that the quantitative and qualitative features of behavioral response to ampheatmine-like substance in rats can be dissociated from the dopamine response (Kuczenski and Segal 1999). In those study, the dissociation was evident in the temporal profiles of the extracellular dopamine and stereotypy response to high dose of amphetamine (4.0 mg/kg BW). It was observed the persistence of intense stereotypies after acute amphetamine administration during the rapid decline in synaptic dopamine and amphetamine and the intense stereotyped behavior in response to the relatively low dose of amphetamine injection. It was suggested that the process of sensitization of stereotyped behavior contributed to the dissociation observed.

In conclusion, the present study shows that methamphetamine modulated the EEG activity, locomotor activity and stereotyped behaviors. The low and moderate doses of methamphetamine caused an EEG activity with decreases in all EEG frequency bands and this is likely to be due to D_1–like dopamine receptor activation. With relatively high dose of methamphetamine, the EEG pattern of characteristic with an increase in alpha-1 frequency band was produced and this suggests activation of D_2-like dopamine receptors. This response with a particular characteristic to increase the alpha-1 frequency band of EEG in response to high dose of methamphetamine suggests that the EEG might be used as a sensitive tool to indicate the D_2-like dopamine receptor activation.