MATERIALS AND METHODS

Animals

Male Wistar rats (National Laboratory Animal Center, Salaya Campus, Mahidol University) weighing about 300 g were used in the experiments. The animals were housed under controlled environmental conditions of constant temperature (25 $^{\circ}$C) with a 12-h light/12 h dark cycle (lights on 06.00 - 18.00 h). Laboratory food and water were given ad libitum.

EEG and locomotor activity were not recorded in the same rat because the EEG tool and locomotor activity tool were interfered each other. So, the EEG experiment and the locomotor activity experiment were separated. But the stereotyped behavior was observed in the EEG experiment and the locomotor activity experiment.

Electrode Implantation

At 5-7 days before the day of the experiment, the animals were implanted with stainless steel screw electrode over the cerebral cortex under pentobarbital sodium anesthesia (40 mg/kg IP). After fixed in a stereotaxic apparatus, the exposed skull was cleared of the periosteum then four small cavities were drilled into the cranial bone, without perforating the dura. Two screw electrodes for recordings of cortical field potential differences were chronically implanted into the prepared cavities of the cranial bone. These two electrodes were positioned symmetrically on both side at 3 mm laterally from the sagittal fissure and at midline between bregma and lambda. The reference electrode was located on 2 mm laterally from midline and 3 mm anterior to the bregma. One fixing screw was implanted at 2 mm rostral and 2 mm lateral to the lambda, on left side. All electrodes were fixed in place by cementing their upper portions to the skull

with dental cement. After implantation, the rats were returned to the laboratory house, but in separate cages to prevent from gnawing of the implanted electrodes by cage mates.

EEG Recording Procedure

On the day of the experiments, EEGs were recorded by means of suspended recording cables coupled to the implanted screw electrodes via stainless steel clips (PowerLab 4/20, ADInstruments Corporation). The sampling rates of the amplified and digitized EEG signal was 400/s. The digitized EEG signal were connected to an EEG microcomputer system coupled with a Fast Fourier transformation: basis sweeps of 4 second were averaged as spectra of power density ($\mu V^2/Hz$) on the monitor and stored in a file. Data obtained in successive time-blocks of 3 min each were summed up again and averaged to obtain periods of 15 min each. The mean values of the four 15 min pre-drug periods (from -60 to -15 min) were regarded as the baseline activities, which were assumed as a value of 100%. The EEG spectra of 15 min-period each was calculated into % of baseline EEG power and were used for analysis of this study. In order to perform a power spectral analysis of each frequency band, the spectra obtained were divided into seven frequency bands: 1.25-4.50 Hz (delta band), 4.75-6.75 Hz (theta band), 7.00-9.50 Hz (alpha-1 band), 9.75-12.50 Hz (alpha-2 band), 12.75-18.50 Hz (beta-1 band), 18.75-35.00 Hz (beta-2 band) (Ferger et al. 1994; Stahl et al. 1997), and 35.25-60 Hz (gamma band).

Before starting the EEG recording, animals were adapted to the experimental conditions for about 15 min in order to get stable EEG signal. Animals with abnormal EEG activities were discarded. During the following 60 min, a drug-free baseline activity was recorded. Immediately afterwards, the drug was injected. Since then, data acquisition had continued for 3 hours. All EEG patterns were returned to the normal condition in 3 hours.

Locomotor Activity

The locomotor activity of each rat was measured with Automex-II-2SD animal activity monitor (Columbus Instruments International Corporation). The apparatus detected movement by capacitance of animal. It can set separated sensitivity between horizontal and vertical movements. In this experiment, vertical movements such as rearing and head movement were discarded, the apparatus selectively recorded horizontal movement (locomotor activity).

Locomotor activity was counted every minute from the beginning of baseline activity to the end of the experiments for 240 minutes. The locomotor activity counts were summed for a period of 15 minutes each. The mean values of the four 15 minutes pre-drug periods from -60 to -15 min) were regarded as the baseline activities, which were assumed as a value of 100%. The locomotor activity counted for a period of 15 minutes each was calculated into % of baseline locomotor activity and were used for analysis.

Quantitative Stereotyped Behavior Analysis

In an experiment complementary to the EEG recording, the behavioral effects of drug treatment were quantified. On the experiment, each rat was scored for the first 10 seconds of every 1-minute period from the beginning of baseline activity to the end of the experiments for 240 minute. A slightly modified scoring system of Costal and Naylor (1973) for classification and evaluation of stereotyped behavior was utilized. The following time values in bracketed represented the minimal time, during which the stereotyped behavior occured during the period of 10 second: stereotyped score 1 corresponded to discontinuous stereotyped sniffing (5-7 second), and the score 2 corresponded to continuous stereotyped sniffing (8-10 second). Stereotyped sniffing was defined by rhythmic movement of the snout and head along a cage wall or floor, accompanied by rapid movement of the vibrissae. The score of 3 was given for discontinuous licking (2-7 second), and the score of 4 was given for continuous licking (8-10 second). Stereotyped licking was defined as protrusion of the tongue against the cage wall or floor. The score of 5 was given for discontinuous gnawing (2-7 second), and

the score of 6 was given for continuous gnawing (8-10 second). Stereotyped gnawing was defined by gripping the wire of the cage wall or floor between the teeth. In the cases that different stereotypy patterns coexisted in the same observation period, only the highest score was taken into calculation. Stereotyped behavior score minute by minute was summed up for a period of 15 minutes each. This summed score was used as score of stereotyped behavior for analysis of this study.

Drug

The drug used was methamphetamine HCl provided by courtesy of the Drug Control National Police Bureau, Thailand. Methamphetamine was dissolved, with physiological saline. These drug solutions were administered intraperitoneally (IP) at a volume of 1 ml/kg body weight of the rats. The four conditions of the methamphetamine dosage were made by an amount of injection: 0.5 mg/kg (low dose), 1 mg/kg (low dose), 2 mg/kg (moderate dose) and 4 mg/kg (high dose). Rats with physiological saline only were assumed as control groups.

Statistical Analysis

The data from the experiments were expressed as means $\pm$ standard error. All results of the EEG recordings and locomotor activity from the time of drug injection were analyzed using a two-way ANOVA for repeated measurements with following Duncan test. The results of the stereotyped behavior experiments were analyzed by Kruskal-Wallis H test with following Mann-Whitney U test. The correlation between EEG, locomotor activity, and stereotyped behavior data at 30 minutes after drug administration were estimated by Spearman's rank order correlation coefficient.