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Spatiotemporal properties of locomotor activity after administration of central nervous stimulants and sedatives in mice

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ABSTRACT

In the present study, we investigated the spatiotemporal properties of locomotor activity after administration of CNS sedatives (pentobarbital and diazepam) and stimulants (theophylline and caffeine) in an open field test. The absolute and relative distances traveled in central or peripheral regions within 2 h were analyzed. We found that both pentobarbital and diazepam increased total travel distances, especially within the initial 30 min, when traveling was mainly in the peripheral region. Pentobarbital induced this hyperactivity at higher doses (maximum at 30 mg/kg); while diazepam at higher doses (4 and 8 mg/kg) mainly decreased the traveled distance during 0–1 h but increased that in the periphery during 1–2 h. On the other hand, both theophylline and caffeine generally increased the traveled distance in the central region; this effect lasted longer with increasing dose. Caffeine increased the traveled distance at lower doses (maximum at 10 mg/kg) but decreased it at higher doses (30 and 100 mg/kg) during the initial 1 h. Theophylline exhibited a similar but smaller decrease at higher doses. Thus, we revealed the spatiotemporal properties that sedatives decrease central locomotion but induce a dose-related peripheral hyperactivity while stimulants induce central hyperactivity with a bell-shaped dose–response relation.

1. Introduction

Evaluation of the sedative or stimulant action of drugs on the central nervous system (CNS) is frequently conducted by analyzing the locomotor activity of animals (usually rodents) in an open field (Dunne et al., 2007; Han et al., 2009; Himmel, 2008). Generally, CNS stimulation increases, and CNS depression decreases the amount of activity (Antoniou et al., 1998; Himmel, 2008; Uzbay et al., 2007). However, in some studies it has been reported that CNS depression might also increase the total amount of locomotion (Nakamura-Palacios et al., 1999; Turski et al., 1982).

The CNS depressant barbiturates (such as pentobarbital) induce general anesthesia at larger doses, hypnosis at middle doses, and sedation at lower doses. It was reported that sub-hypnotic doses (10–20 mg/kg) of pentobarbital significantly increase locomotor activity in the open field in mice (Vetulani et al., 1989; Sansone et al., 1992). The benzodiazepines (such as diazepam) are typical anxiolytics with sedative and hypnotic effects at higher doses. It was also reported that low doses of diazepam increase locomotor activity in mice (Turski et al., 1982; Johnston et al., 1989; Nakamura-Palacios et al., 1999; Huang et al., 2007). On the other hand, the methylxanthine derivatives (such as caffeine, theophylline and aminophylline) are typical central stimulants. Caffeine is used as a centrally active psychomotor stimulant (Fisone et al., 2004), while theophylline and its derivatives are usually used in the treatment of peripheral diseases, such as bronchial asthma, with adverse effects of CNS stimulation. The methylxanthines can increase locomotor activity in the open field (Kuribara et al., 1992; Nehlig et al., 1992; Haghgoo et al., 1995; Soares et al., 2009) with a bell-shaped dose–response relationship; i.e., they exert stimulating effects on locomotor activity at low-moderate doses, but less stimulating and even depressive effects at higher doses (El Yacoubi et al., 2000; Malec and Poleszak, 2006; Mumford and Holtzman, 1991; Uchiyama et al., 2010). Therefore, the actions of both CNS depressants and stimulants need further investigation, and the amount of locomotor activity in an open field cannot reflect their properties precisely.

It has been reported that the spatial and temporal organization of locomotion are most important parameters in addition to the amount of activity. In terms of spatial distribution, it is well known that rodents usually travel in the periphery, close to the walls in an open field, and refrain to travel in the central area (Eilam, 2003; Groenink et al., 2003; Haimovici et al., 2001; Paulus et al., 1999; Wesierska et al., 2003; Wang et al., 2003). Increased activity in the central region or a greater ratio of central/total locomotion is used as an indicator for evaluating anxiogenic or anxiolytic properties (Prut and Belzung, 2003). In terms of temporal structure, animals actively explore a new environment with a higher amount of activity immediately after being introduced into an open field (Drai and Golani, 2001; Eilam et al.,

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2003; Eilam and Golani, 1989, 1990; Golani et al., 1993; Haimovici et al., 2001). To date, the spatiotemporal properties of locomotor activity in the open field after administration of CNS sedatives and stimulants are poorly understood.

Using video tracking systems, we have found that focal cerebral ischemia does not alter the amount of locomotor activity in mice, but impairs the spatiotemporal activity – prolonging the initial hyperactivity and losing the regionally specific distribution of the activity (Zhang et al., 2006). In the present study, we used a video tracking system to assess the effects of sedatives (pentobarbital and diazepam) and stimulants (theophylline and caffeine) on the spatiotemporal organization of locomotor activity in mice. Our goals were (1) to reveal the spatiotemporal properties of locomotor activity after administration of CNS sedatives and stimulants in an open field test with a relatively long duration (2 h); and (2) to provide evaluation indicators for detecting the stimulant and sedative properties of these CNS drugs by analyzing the detailed spatiotemporal properties.

2. Materials and methods

2.1. Animals

Male Kunming mice weighing 25–30 g (Shanghai Experimental Animal Center, China, Certificate No. 22-001004) were housed socially in cages (290×178×160 mm; 5 mice per cage) under a controlled temperature (22±1 °C) and a 12-h light/dark cycle (lights off from 18:00 to 06:00), and allowed free access to food and water. The mice were left to acclimate to the vivarium room for 7 days and handled repeatedly before testing. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Animal Care Committee of Zhejiang University School of Medicine.

2.2. Drugs

Pentobarbital sodium (Guoyao Group of Chemical Reagents Ltd., China), diazepam (Jichuan Pharmaceutical Co., Ltd., Jiangsu, China), and caffeine (Wako Pure Chemical Industries, Japan) were dissolved in saline. Mice (15–18 per group) were injected intraperitoneally (i.p.) with saline (10 ml/kg), pentobarbital sodium (3.75, 7.5, 15, 30, or 45 mg/kg), diazepam (0.5, 1, 2, 4, or 8 mg/kg) or caffeine (1, 3, 10, 30, or 100 mg/kg). Theophylline (Wako Pure Chemical Industries, Japan) was dissolved in 20% dimethyl sulfoxide (DMSO). Mice (14–20 per group) were injected i.p. with 20% DMSO (as theophylline control) or theophylline (1, 3, 10, 30, or 100 mg/kg).

2.3. Apparatus and procedure

Locomotor activity of mice was recorded and analyzed using a Mouse and Rat Spontaneous Activity Video Analysis System (JLBehv-LAG-4, Shanghai Jiliang Software Technology Co., Ltd.). Four adjacent enclosures (40×40×60 cm each) with 4 video cameras on the tops were connected to a computer. The activity of 4 mice (each in a separate enclosure) was recorded simultaneously. Each enclosure was arbitrarily divided into a central region (20×20 cm) and peripheral regions (Fig. 1).

To assess the spatial organization of locomotor activity, we calculated the following parameters: (a) total travel distance (m); (b) traveled distance in central or peripheral regions (m); (c) central or peripheral ratio (distance traveled in central or peripheral regions/total distance). To assess the temporal aspect, the parameters (the total travel distance and traveled distance in each region) were calculated at 0.5-h intervals.

All experiments were conducted between 10:00 and 17:00. Each mouse was placed gently into the center of the enclosure 10 min after administration of drugs. The locomotor tracks in the open field were continuously recorded by video camera for 2 h and analyzed. After each testing session, the enclosures were thoroughly cleaned with 70% ethanol and water.

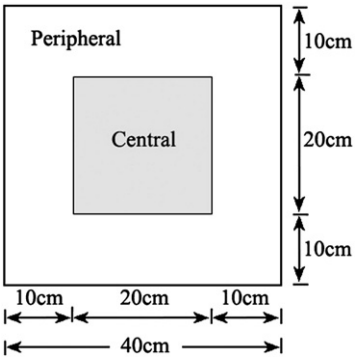

Fig. 1. Schematic diagram of the open field (40×40 cm) divided into central (20×20 cm) and peripheral regions.

2.4. Statistical analysis

Data are reported as mean±SEM, and were analyzed using SPSS version 16. The significance of differences between control and individual drug groups was determined by independent sample *t*-test; $P<0.05$ was considered to be statistically significant. One-way ANOVA was performed for each drug with the dose as the factor. Differences between doses, time (different intervals), and the time×dose interaction were analyzed by two-way ANOVA or MANOVA; $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of sedatives, pentobarbital and diazepam

To determine the optimal doses of the sedatives, we determined the hypnotic doses of pentobarbital and diazepam required to induce immobility. The results showed that the maximal non-hypnotic dose was 30 mg/kg for pentobarbital and 16 mg/kg for diazepam (Table 1). Therefore, we used pentobarbital at 3.75, 7.5, 15, and 30 mg/kg and diazepam at 0.5, 1, 2, 4, and 8 mg/kg in the following experiments.

3.1.1. Effect of pentobarbital

Saline-treated control mice were most active during the first 0.5 h after being introduced to the novel environment. Then, the activity gradually decreased during the second 0.5 h (0.5–1 h), and reached a steady state of less activity during 1–2 h. Traveled distance was mainly distributed in the periphery (Fig. 2A).

To determine the dose–response relationship, we analyzed traveled distance at 0.5-h intervals and the total travel distance

Table 1
Hypnotic and sub-hypnotic doses of pentobarbital and diazepam.

Groups	Dose (mg/kg)	*n*	Number of immobile rats	Total distance during 0–0.5 h (m)
Control 1	0	16	0	83.2±5.9
Pentobarbital	30	17	0	148.1±24.9**
Pentobarbital	40	17	2 (11.8%)	64.7±18.9
Pentobarbital	45	16	8 (50%)	35.3±14.4*
Pentobarbital	60	13	11 (84.6%)	0.34±0.20**
Control 2	0	14	0	78.4±7.4
Diazepam	12	14	0	32.7±4.9**
Diazepam	16	14	0	15.9±7.3**
Diazepam	20	11	4 (36.4%)	9.9±8.4**
Diazepam	25	12	8 (66.7%)	0.47±0.23**

Data are expressed as mean±SEM; *$P<0.05$, **$P<0.01$ *vs.* control, analyzed by one-way ANOVA. Immobile: traveled distance <0.5 m during the first 0.5 h.

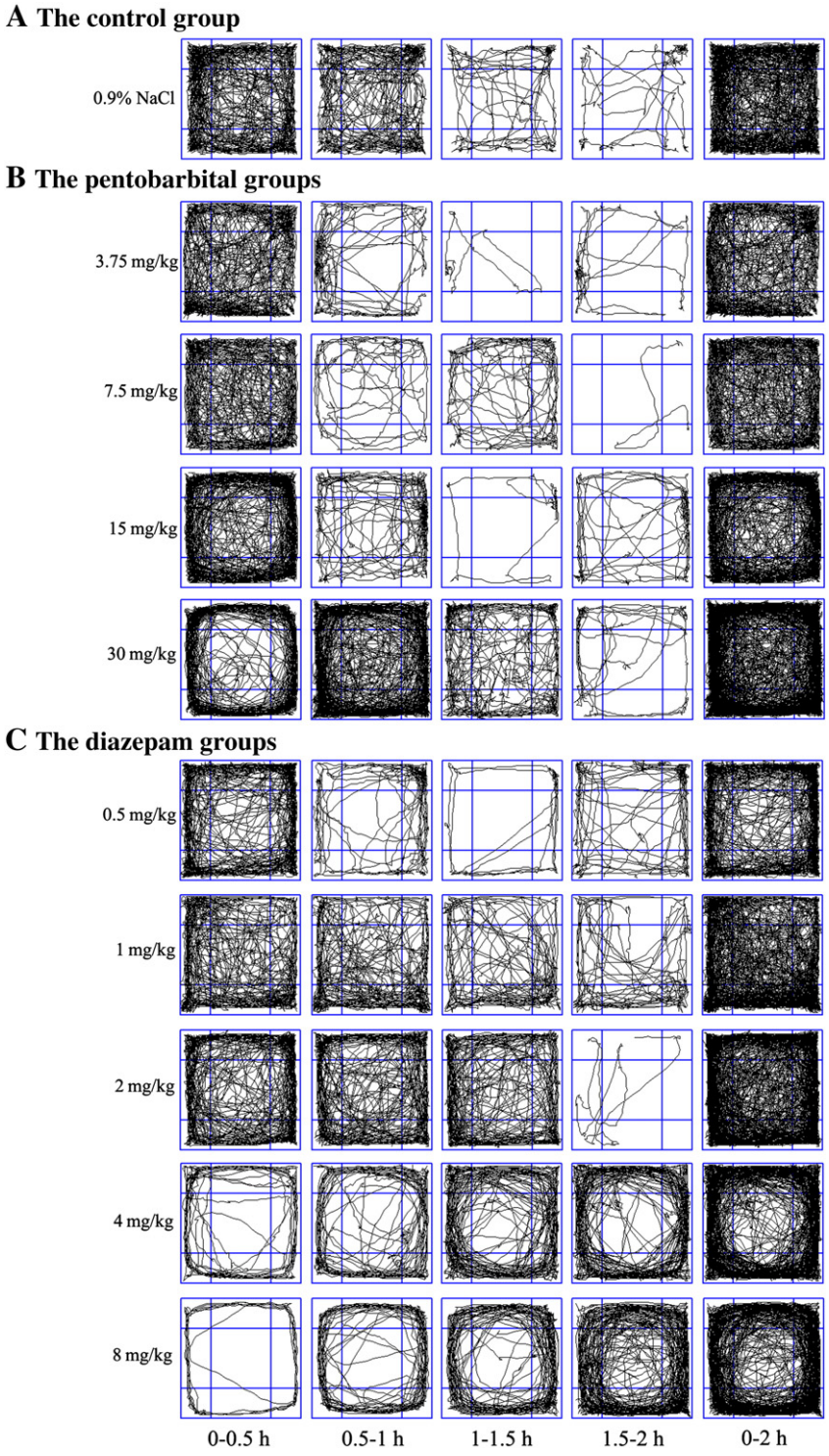

Fig. 2. Typical locomotor tracks at 0.5-h intervals after administration of 0.9% NaCl (A, control), pentobarbital (B) and diazepam (C).

during 2 h (Fig. 3A). There were significant changes in the total travel distance after treatment with pentobarbital ($F(4, 78) = 24.251$, $P<0.001$). Pentobarbital at the smallest dose (3.75 mg/kg) caused a small but significant reduction in the total travel distance ($P<0.05$, t-test); however, the higher doses (15 and 30 mg/kg) increased the distance in a dose-dependent manner (Fig. 3A). A significant difference was found in the total travel distance induced by various doses ($F(4, 312) = 23.199$, $P<0.001$).

Time course: the locomotor pattern after pentobarbital treatment was similar to control mice, i.e. an initial increase followed by a gradual decrease (Fig. 3A). Significant differences were found in time ($F(3, 312) = 186.054$, $P<0.001$) and in time × dose interaction ($F(12, 312) = 5.292$, $P<0.001$).

Spatial distribution: traveled distance mainly increased in the periphery after treatment with higher doses of pentobarbital (Fig. 3A). Pentobarbital decreased the central ratio during the initial hour or overall 2 h, in spite of decreased or increased amounts of traveled distance (Figs. 2B, 3A and 4A). In terms of regional distribution during 2 h (Fig. 4A), pentobarbital significantly decreased the central ratio ($P<0.05$ or 0.01, t-test); only a relatively low dose (7.5 mg/kg) did not

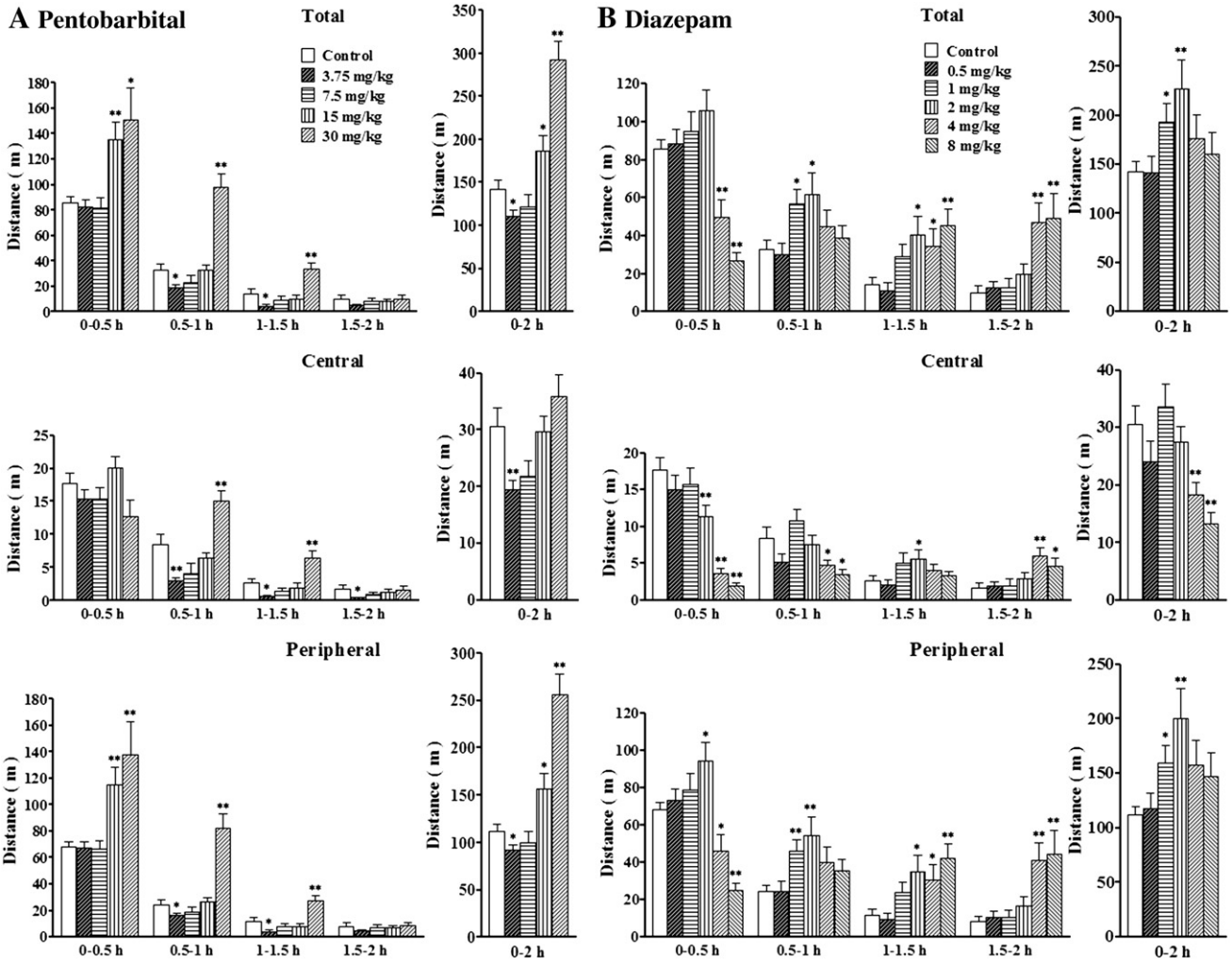

Fig. 3. Traveled distances in central and peripheral regions at 0.5-h intervals and within 2 h after administration of pentobarbital (A) and diazepam (B). Data are expressed as mean ± SEM; $n = 15–18$ mice; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* control, analyzed by *t*-test.

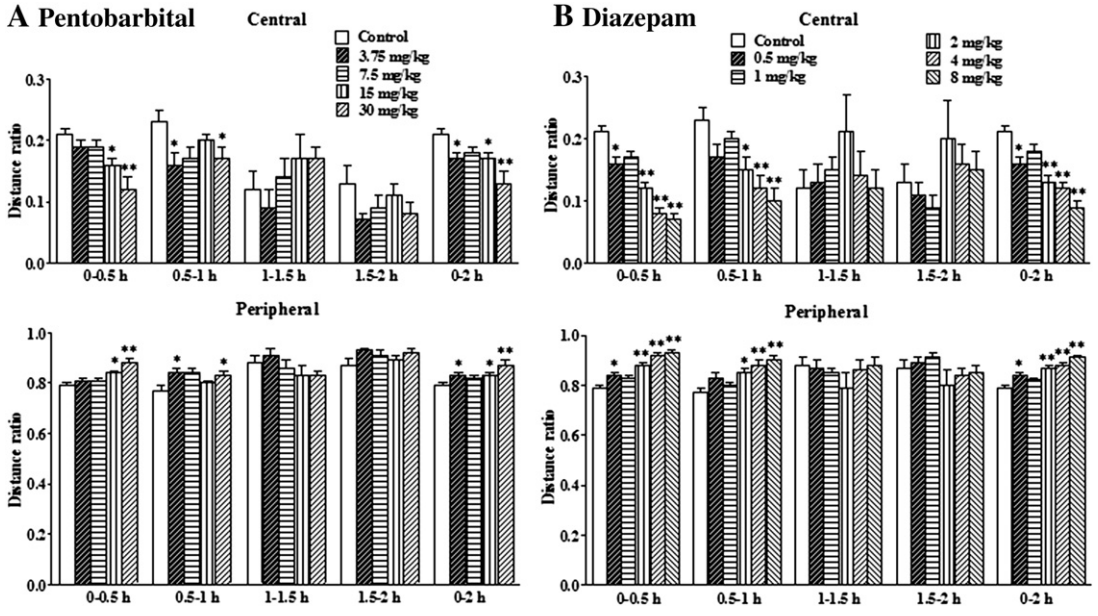

Fig. 4. Distance ratios in central and peripheral regions at 0.5-h intervals and within 2 h after administration of pentobarbital (A) and diazepam (B). Data are expressed as mean ± SEM; $n = 15–18$ mice; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* control, analyzed by *t*-test.

show a significant decrease. Overall, a significant decrease was found in the central ratio during 2 h with doses of pentobarbital ($F(4, 78) = 4.462$, $P<0.01$). In parallel, the peripheral ratio was increased after treatment with pentobarbital (Fig. 4A).

Individual intervals and doses: pentobarbital dose-dependently decreased the central ratio during 0–0.5 h with significant decreases at 15 and 30 mg/kg ($P<0.05$ or 0.01, t-test). It significantly decreased the ratio during 0.5–1 h at 3.75 and 30 mg/kg ($P<0.05$, t-test), and did not show significant changes thereafter. Significant decreases were found in the central ratio at doses ($F(4, 312) = 2.914$, $P<0.05$) and in time ($F(3, 312) = 15.695$, $P<0.001$). However, the varied insignificant changes in the second hour masked the significance of changes in time × dose interaction ($F(12, 312) = 1.727$, $P>0.05$). In addition, the peripheral ratio was reversed (Fig. 4A).

3.1.2. Effect of diazepam

In traveled distance at 0.5-h intervals and the total travel distance during 2 h (Fig. 3B), diazepam-treated rats exhibited a biphasic dose–response relationship. In general, during 2 h diazepam increased the total travel distance at 1 and 2 mg/kg ($P<0.05$ or 0.01, t-test), but did not significantly affect the distance at 4–8 mg/kg (Fig. 3B). There were significant changes in the total travel distance during 2 h ($F(5, 93) = 2.519$, $P<0.05$).

Individual intervals and doses: for diazepam, the effect was significant in time ($F(3, 372) = 52.186$, $P<0.001$), at doses ($F(5, 372) = 4.573$, $P<0.001$) and in time × dose interaction ($F(15, 372) = 7.280$, $P<0.001$). During 0–0.5 h, diazepam at lower doses (0.5–2 mg/kg) increased traveled distance in the periphery but tended to decrease the distance in the central region (significant only at 2 mg/kg; $P<0.01$, t-test); while at higher doses (4 and 8 mg/kg) it significantly decreased the distance in both central and peripheral regions ($P<0.05$ or 0.01, t-test). During 0.5–1 h, diazepam at lower doses (1 and 2 mg/kg, Fig. 3B) significantly increased the total travel distance ($P<0.05$, t-test), which resulted from an increase in the periphery, while it significantly decreased the distance in the central region at higher doses (4 and 8 mg/kg; $P<0.05$, t-test). However, during the second hour, diazepam at higher doses (4 and 8 mg/kg) significantly increased the total travel distance ($P<0.05$ or 0.01, t-test) that resulted especially from the distance in the central region (Fig. 3B). The moderate dose of 2 mg/kg increased the total travel distance and traveled distance in the periphery during 1–1.5 h (Fig. 3B; $P<0.05$, t-test).

Spatial distribution: the central ratio during 2 h was significantly decreased after treatment with diazepam (Fig. 4B, $F(5, 93) = 13.094$, $P<0.001$). During the first hour, diazepam induced a relatively clear dose-dependent decrease in the central ratio, with the exception of a small but not significant decrease at 1 mg/kg. During the second hour, no significant changes were found in the regional differences because of relatively large variability (Fig. 4B). Significant decreases were found in the central ratio at doses ($F(3, 312) = 3.125$, $P<0.01$) and in interval × dose interaction ($F(15, 372) = 2.583$, $P<0.01$), but not in time ($F(3, 372) = 1.174$, $P>0.05$). In parallel, the peripheral ratio increased after treatment with diazepam (Fig. 4B).

3.1.3. Similarities and differences in the effects of the sedatives, diazepam and pentobarbital

Increasing doses of pentobarbital and diazepam gradually increased traveled distance mainly in the periphery, but diazepam decreased the distance (mainly in the central region) during the first hour at higher doses (4 and 8 mg/kg).

3.2. Effects of stimulants, theophylline and caffeine

3.2.1. Effect of theophylline

The solvent for theophylline (20% DMSO) did not affect locomotor activity (Fig. 5A). The time course after theophylline treatment at most doses was similar to control mice, i.e. the maximum was in the initial 30 min, and then gradually decreased. The total travel distance during 2 h was significantly increased after treatment with 3–100 mg/kg of theophylline with the maximum at 10 and 30 mg/kg (Fig. 6A, $F(5, 105) = 13.372$, $P<0.001$).

Individual intervals and doses: higher doses of theophylline (30 and 100 mg/kg) continually increased the total travel distance over 2 h; but the increase during the first hour was a little less than the moderate dose (10 mg/kg). The lower doses of 1 and 3 mg/kg increased the traveled distance in the periphery only during the first 0.5 h but not during 0.5–2 h (1 mg/kg) and 1–2 h (3 mg/kg). The moderate dose (10 mg/kg) increased the traveled distance during 0–1.5 h both in the central and peripheral regions (Fig. 6A). There was a significant increase in the traveled distance in time ($F(3, 420) = 190.978$, $P<0.001$), at doses ($F(5, 420) = 36.159$, $P<0.001$), and in time × drug interaction ($F(15, 420) = 3.562$, $P<0.001$).

Spatial distribution: theophylline increased the traveled distance in the central region ($F(5, 105) = 6.835$, $P<0.001$). The central ratio during 2 h (Fig. 7A) was significantly increased at higher doses (30 and 100 mg/kg; $P<0.01$, t-test). The central ratio during 0.5–1 h increased dose-dependently. During 0–0.5 h and 1–2 h, the central ratio showed a trend to increase, and was significantly increased only at 30 mg/kg (0–0.5 h; $P<0.05$, t-test) or 100 mg/kg (1–1.5 h; $P<0.01$, t-test; Fig. 7A). There were significant changes in time ($F(3, 420) = 16.826$, $P<0.001$) and at doses ($F(5, 420) = 13.291$, $P<0.001$), but no significant changes in time × drug interaction ($F(15, 420) = 0.629$, $P>0.05$). In parallel, the peripheral ratio was decreased (Fig. 7 A).

3.2.2. Effect of caffeine

Generally, caffeine significantly increased the total travel distance during 2 h (Fig. 6B, $F(5, 97) = 11.425$, $P<0.001$). During 2 h, it increased the total travel distance at 1–30 mg/kg ($P<0.05$ or 0.01, t-test) with a maximum at 10 mg/kg; but there was no significant effect at the largest dose (100 mg/kg), which resulted from the temporally different changes (Fig. 6B).

Individual intervals and doses: caffeine-treated rats exhibited a drastically different spatiotemporal pattern at lower and higher doses. During the first hour, the lower doses of caffeine (1 and 3 mg/kg) increased the total travel distance ($P<0.05$ or 0.01, t-test); while the higher doses increased less (30 mg/kg), had no effect or even decreased the distance (100 mg/kg during 0–0.5 h). However, during the second hour, the lower doses (1 and 3 mg/kg) did not affect or even decreased the total travel distance; but the higher doses (30 and 100 mg/kg) significantly increased it ($P<0.05$ or 0.01, t-test). Different from the lower and higher doses, the moderate dose (10 mg/kg) caused the maximal increase, which was continuously maintained over the 2 h of observation ($P<0.01$, t-test; Fig. 6B). Overall, significant changes were found in the total travel distance in time ($F(3, 388) = 228.315$, $P<0.001$), at doses ($F(5, 388) = 25.691$, $P<0.001$), and in time × drug interaction ($F(15, 388) = 6.632$, $P<0.001$).

Spatial distribution: caffeine generally increased the central ratio over the two-hour observation ($F(5, 97) = 5.053$, $P<0.001$), and the increase was significant at the higher dose (30 mg/kg) (Fig. 7B). During the first hour, caffeine significantly increased the central ratio at 10 and 30 mg/kg ($P<0.05$ or 0.01, t-test); during 1–1.5 h, it did not significantly affect the ratio; during 1.5–2 h, it significantly decreased the ratio at 3 mg/kg ($P<0.01$, t-test) but increased it at 100 mg/kg ($P<0.05$, t-test; Fig. 7B). There were significant changes in the central ratio in time ($F(3, 388) = 22.099$, $P<0.001$), at doses ($F(5, 388) = 15.502$, $P<0.001$) and in time × drug interaction ($F(15, 388) = 1.926$, $P<0.05$). In parallel, the peripheral ratio showed reverse changes after treatment with caffeine (Fig. 7B).

3.2.3. Similarities and differences in the effects of the stimulants, theophylline and caffeine

Theophylline and caffeine generally increased the total travel distance and traveled distance mainly in the central region. There was

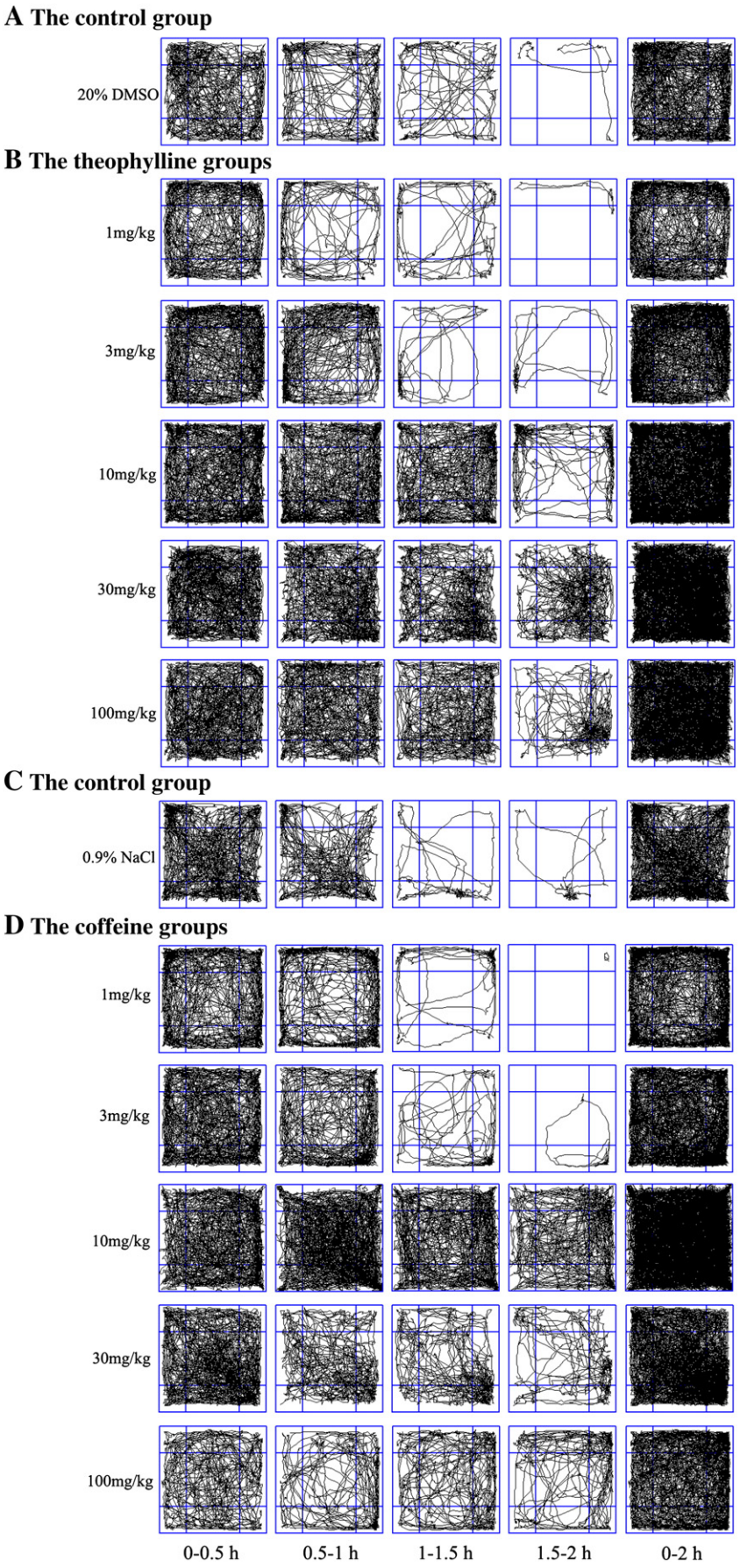

Fig. 5. Typical locomotor tracks at 0.5-h intervals after administration of 20% DMSO (A), theophylline (B), 0.9% NaCl (C) and caffeine (D).

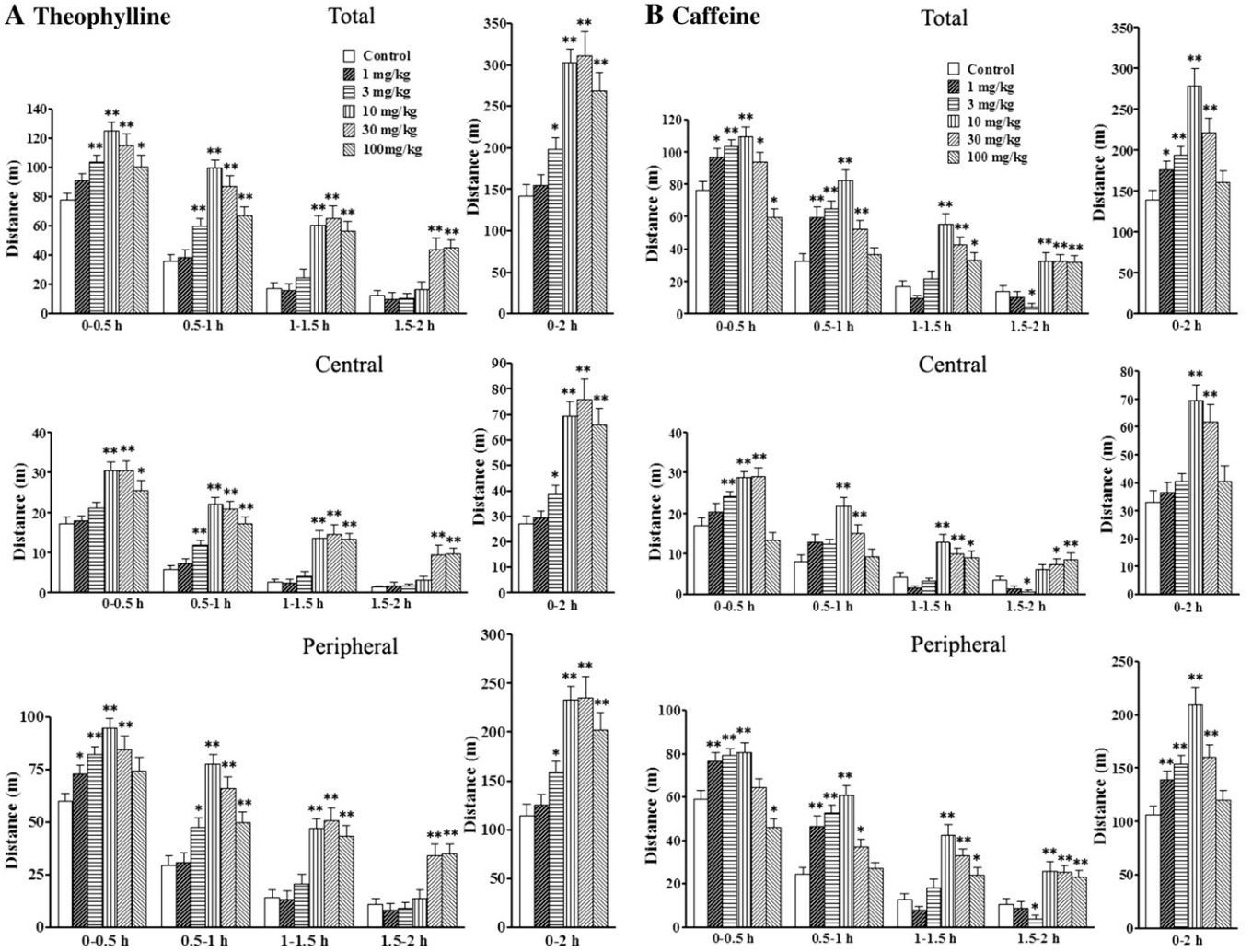

Fig. 6. Traveled distances in central and peripheral regions at 0.5-h intervals and within 2 h after administration of theophylline (A) and caffeine (B). Data are expressed as mean ± SEM; $n = 15–20$ mice; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* control, analyzed by *t*-test.

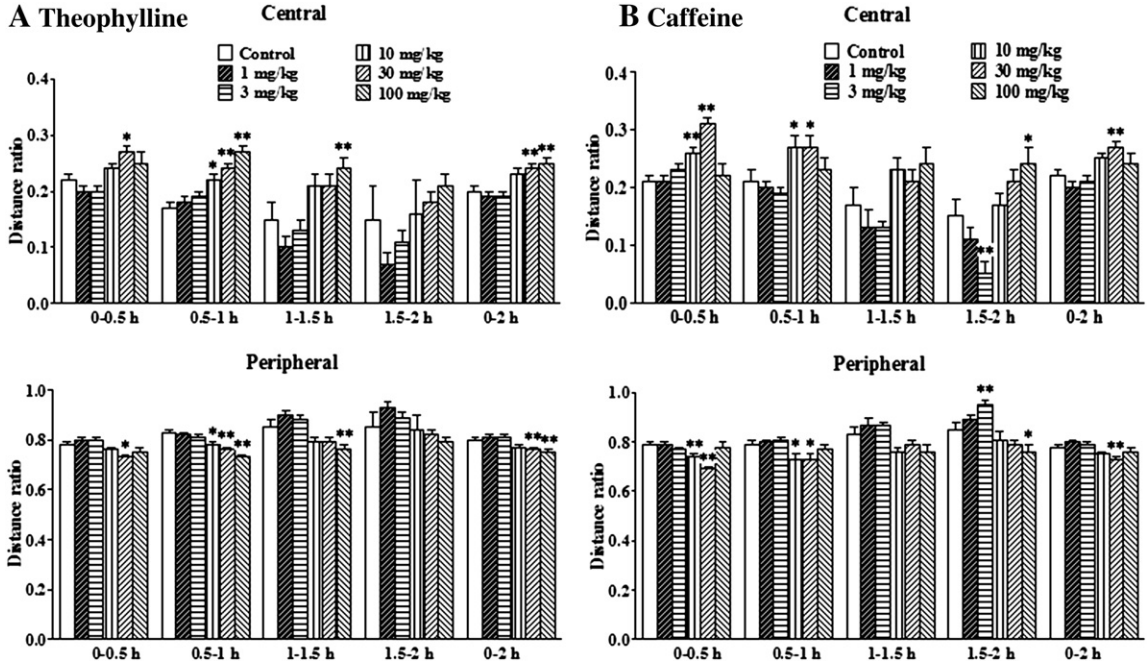

Fig. 7. Distance ratios in central and peripheral regions at 0.5-h intervals and within 2 h after administration of theophylline (A) and caffeine (B). Data are expressed as mean ± SEM; $n = 15–18$ mice; $^{*}P<0.05$, $^{**}P<0.01$ *vs.* control, analyzed by *t*-test.

a bell-shaped dose–response relation, i.e. lower doses within 10–30 mg/kg gradually increased the distances (maximum at 30 mg/kg of theophylline or 10 mg/kg of caffeine), while higher doses (100 mg/kg of both, or 30 mg/kg of caffeine) increased the distances less.

4. Discussion

In the present study, the most important finding was that the centrally active drugs showed distinct spatiotemporal patterns of locomotor activity in the open field test. The sedative drugs pentobarbital and diazepam increased traveled distance in the periphery of the enclosure at moderate or higher doses, and the effects of higher doses lasted longer. The CNS stimulants theophylline and caffeine increased the total travel distance and traveled distance in the central region, and the dose–response relationship was bell-shaped. These findings showed the spatiotemporal properties that sedatives and stimulants induce dose- and time-related peripheral hyperactivity and central hyperactivity, respectively, in mice.

We first evaluated the sub-hypnotic doses of the sedative drugs pentobarbital and diazepam because they have hypnotic effects at large doses. Therefore, the sedative doses used in the present study excluded the apparent hypnotic effects. In this dose range, we confirmed the reported phenomenon of locomotor hyperactivity induced by pentobarbital (Vetulani et al., 1989) and diazepam (Huang et al., 2007; Turski et al., 1982). We also revealed that this hyperactivity was mainly distributed in the periphery of the open field. Diazepam at higher doses (4 and 8 mg/kg) induced an initially decreased activity mainly in the central region, and a delayed hyperactivity in the periphery although total activity was unchanged. The delayed hyperactivity might be due to its active metabolite, desmethyldiazepam (Friedman et al., 1986) that may prolong its action, especially after administration of higher doses.

Diazepam, an agonist of the benzodiazepine receptor, can enhance $GABA_A$ receptor-mediated inhibitory neurotransmission in the CNS, but the mechanism underlying hyperactivity induced by diazepam is unknown. It has been reported that diazepam (2.5 mg/kg) increases the release of the neurotransmitter dopamine (DA) in the medial prefrontal cortex (Finlay et al., 1995), and increased DA may produce hyperactivity. In DA transporter knockdown mice, enhanced dopaminergic function results in locomotor hyperactivity that occurs in the periphery of an open field (Ralph-Williams et al., 2003). Thus, diazepam-increased DA levels in the brain may explain the hyperactivity. However, pentobarbital might induce hyperactivity through different mechanisms because it inhibits DA release in the brain induced by ketamine (Masuzawa et al., 2003), L-dopa (Adachi et al., 2006), and K^+ (Hirota et al., 2000).

The stimulants, theophylline and caffeine, had a bell-shaped dose–response relationship of their effects on locomotor activity, i.e. stimulating effects at low-moderate doses, but less stimulating and even depressive effects at higher doses, which has also been reported elsewhere (El Yacoubi et al., 2000; Malec and Poleszak, 2006; Mumford and Holtzman, 1991; Uchiyama et al., 2010). Both agents have been reported to be non-specific antagonists of adenosine receptors ($A_{2A} > A_1 = A_{2B} > A_3$) (Moreau and Huber, 1999). The stimulant effect of lower doses of caffeine may be mediated by blocking the adenosine A_{2A} receptor, while the depressant effect at higher doses may be mediated by blocking the A_1 receptor (El Yacoubi et al., 2000). In the present study, we also found the property of regional distribution; namely, activity in the central region increased after administration of theophylline and caffeine. Consistently, cocaine, an inhibitor of monoamine neurotransmitter transporters with central stimulant activity, also increases central locomotion (Muller et al., 2008).

However, an increase of locomotion in the central region of an open field without modification of total locomotion can be interpreted as an anxiolytic-like effect, while a decrease is associated with anxiogenic effects (Prut and Belzung, 2003; Ramos, 2008). Diazepam and pentobarbital are anxiolytic agents, and caffeine and theophylline have anxiogenic activity (Kulkarni et al., 2007; Vitale et al., 2008). In our open field experiments, both types of drugs exhibited contradictory profiles against the general notions for evaluating anxiety mentioned above. The inconsistency of the effects of anxiolytic agents in the open field was also reported in other studies (Prut and Belzung, 2003). Generally, anxiety and anxiolytic agents are assayed by a series of behavioral tests that detect various conditioned and unconditioned responses in rodents (Bourin et al., 2007). In animal models of anxiety, central locomotion in the open field test is largely influenced by various factors including the animals' emotional states (Ramos, 2008) and test conditions (Bourin et al., 2007). Actually, in rats perinatally exposed to caffeine, the central occupation in an open field increases, and this is decreased by diazepam (Fisher and Hughes, 1996). This result and our findings in the present study suggest that our experimental conditions were not suitable for evaluation of anxiety-related changes.

Another factor impacting the results of locomotor activity in the open field is the novelty of the environment. Exposure to a new and unfamiliar environment can induce novelty exploration and stress-induced anxiety-like behaviors in animals (Dunne et al., 2007; Viggiano, 2008; Allan et al., 2007). According to the changes in locomotor activity in the open field, our results suggested that the activity at the first 0.5 h might involve a greater component of novelty exploration, and then gradually move to basal locomotion as mice habituated to the environment. Thus our present findings reflected the complicated behaviors of basal locomotion, novelty exploration and stress-induced anxiety. This may be a reason for the contradictory anxiety-related effects of the drugs mentioned above. As an alternative method of the open fiend test, observation of locomotion in the home cage has been used to avoid the influence of exploration and stress (Dunne et al., 2007; Viggiano, 2008).

In the present study, we found that central locomotion might be more important than total travel distance; however, the time spent in central or peripheral regions was not a sensitive and reliable parameter (especially for pentobarbital and diazepam) although the changes showed profiles similar to the parameter of distance (data not shown). This might result from larger variations in speeds in different regions at different times after drug administration. Our findings also showed that observation duration is also important, especially for long-acting agents and higher doses, since higher doses of diazepam decreased total distance in the first 0.5 h but increased the distance during the second hour.

In summary, we described the spatiotemporal properties of CNS drug effects on locomotor activity in an open field test in mice. Sedatives decreased central locomotion but induced a dose-related hyperactivity mainly in the periphery, while stimulants induced hyperactivity mainly in the central region with a bell-shaped dose–response relation. These findings will be useful in evaluation of the CNS depressant or stimulant profiles of drugs using a more detailed analysis in addition to development of substitute methods other than the open field test (Dunne et al., 2007). The parameter, total amount of locomotor activity, in the open field test is limited to more completely clarify the behavioral effects of drugs, while analysis of the spatiotemporal profiles of drug effects is more important.

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