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Effects of the Systemic Administration of Kainic Acid and NMDA on Exploratory Activity in Rats

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GIMÉNEZ-LLORT, L., S. FERRÉ AND E. MARTÍNEZ. *Effects of the systemic administration of kainic acid and NMDA on exploratory activity in rats.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 205–210, 1995.—In spite of growing evidence for the involvement of the glutamatergic system in mammal's locomotion, studies on behavioural effects induced by the systemic administration of excitatory amino acids not associated to convulsions are lacking. In the present work, the effect of one single systemic administration of kainic acid (KA) (9 mg/kg, IP) or NMDA (100 mg/kg, IP) on exploratory activity in the rat during 6 consecutive days was studied. Separation of exploratory activity in fast (FM) and slow movements (SM) and rearings (R), together with the analysis of those variables during both the light and dark periods of the light–dark cycle, allowed finding specific drug-induced effects. KA produced an acute short-lasting increase in exploratory activity, only significant for FM. On the other hand, NMDA produced an acute short-lasting depressant effect on FM, SM, and R, followed during the next 2 days by a long-lasting increase in exploratory activity, only significant for FM during the dark period. These results underline the importance of using repeated testing during both light and dark periods of the light–dark cycle when analyzing drug-induced changes on exploratory activity.

Excitatory amino acids *N*-Methyl-D-aspartic acid Kainic acid Exploratory activity Rat

GLUTAMATE and, to a lesser extent, aspartate, are excitatory amino acids that are probably the neurotransmitters for most excitatory neurons in the brain. Glutamate's actions are mediated through both ionotropic and metabotropic receptors. Ionotropic excitatory amino acid receptors are now classified in NMDA, AMPA, and high-affinity kainate receptors, according to their different agonist selectivity. α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) is a selective agonist for AMPA receptors, which mediate most of the fast synaptic excitatory neurotransmission. Kainic acid (KA) is an agonist at both AMPA and high-affinity kainate receptors, which also mediate fast synaptic transmission. NMDA receptors are selectively stimulated by *N*-methyl-D-aspartic acid (NMDA) and mediate slow, Ca^{2+}-linked synaptic transmission [for reviews, see (15,18)].

Excitatory amino acids produce neuronal death when administered in sufficiently high doses, both locally or systemically, most probably as a consequence of excessive activation of excitatory amino acid receptors. Therefore, the term excitotoxin has been introduced (12,24). Many experimental data suggest that excitotoxicity contributes to the neuronal death associated to some acute pathologic situations, like cerebral ischemia (12). Furthermore, the involvement of excitotoxicity in chronic progressive degenerative diseases as amyotrophic lateral sclerosis and Huntington disease has also been suggested (12).

Most experimental studies about the behavioural effects induced by the systemic administration of excitatory amino acids agonists refer to those associated with convulsant activity, which seems to be invariably associated to excitotoxicity (21,25). Less is known, however, about those behavioural effects not associated with convulsions, which could reflect non-excitotoxic effects. The systemic administration of NMDA can increase motor activity in rodents without inducing convulsant activity (4,9,13,23). On the other hand, as far as we know, behavioural effects of systemically administered KA not associated to convulsant activity have not been reported.

In the present work we studied the effects of the systemic

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administration of NMDA and KA, excitatory amino acid ionotropic receptor agonists, on exploratory activity in rats, which did not develop seizures after their administration. A computerized method that allowed us to discriminate among different aspects of exploratory activity, under both light and dark conditions, was used.

METHOD

Animals

Male Wistar rats [WI(IOPS AF/Han), CRIFA, Lyon], initially weighing 250–300 g, were used. The animals were randomly assigned to the different groups and maintained, four per cage (Macrolon, 21.5 × 46.5 × 14.5 cm), under standard laboratory conditions (food and water ad lib, 22 ± 2°C and 12L : 12D cycles beginning at 0700 h). The animals were weighed once a day after each exploratory activity test during the light period.

Exploratory Activity Recording

Exploratory activity [initial displacement movements shown by the animal when placed in a new environment (6,22)] was recorded with a video-computerized system (Videotrack 512, View Point, Lyon) by using a tracking image analysis. Three different kinds of exploratory movements were defined. Fast (FM) and slow (SM) ambulatory movements consisted of horizontal displacements with a speed greater than 25 cm/s and between 12 cm/s and 25 cm/s, respectively. The percentage of the total session time engaged by the rat in each of these movements was determined. Vertical displacements were measured as total number rearings (R) per session. Four cages (polyglass, 35.5 × 35.5 × 35.5 cm) were analyzed simultaneously in a soundproof, temperature-controlled (22 ± 2°C) experimental room. Exploratory activity was recorded immediately after the animals were placed in the cages without any acclimatization period. To record under light or dark conditions, the experimental room was uniformly illuminated with two incandescent lamps (100 W; located 1 m above the floor) or with red lamps, respectively.

Exploratory Activity in Nontreated Rats

To determine the optimum period of time engaged in exploratory activity (i.e., with more horizontal and vertical displacements and with less time engaged in grooming), the following analysis was made: FM, SM, R, and presence or absence of grooming were recorded in 12 animals during 10 min and analyzed in two 5-min periods, under light conditions, during the light period of the light-dark cycle. The optimum period of time was then used to study exploratory activity in another group of 35 rats. They were tested for exploratory activity twice a day for 7 days, during both the light and dark periods of the dark-light cycle (from 0800 to 1000 h and from 2000 to 2200 h), under light and dark conditions, respectively.

Exploratory Activity After Treatment With Excitatory Amino Acid Receptor Agonists

The previously analyzed nontreated rats were administered on the eighth day and only once with either NMDA (N = 8; 100 mg/kg, IP), KA (N = 15; 9 mg/kg, IP), or saline (N = 6). Six animals were excluded due to missing values. NMDA and KA doses were chosen from a pilot study, which showed that almost all animals developed convulsions or died with higher doses of both compounds. KA and NMDA (both purchased from Sigma, St. Louis, MO) were dissolved in saline. NMDA solution was adjusted to pH 7.4 with NaOH. Immediately after the injection, exploratory activity (FM, SM, and R) with a 5-min session was determined. The test was done twice a day for 6 days, during both the light and dark periods of the dark-light cycle (the same schedule as that used during the nontreated period). In the first session the animals were left in the cage for 2 h, to observe the appearance of convulsions. Convulsant rats were excluded from the study.

Statistical Analysis

Student's paired t-test and analysis of variance (ANOVA) with post hoc Newman-Keuls comparisons were used to ana-

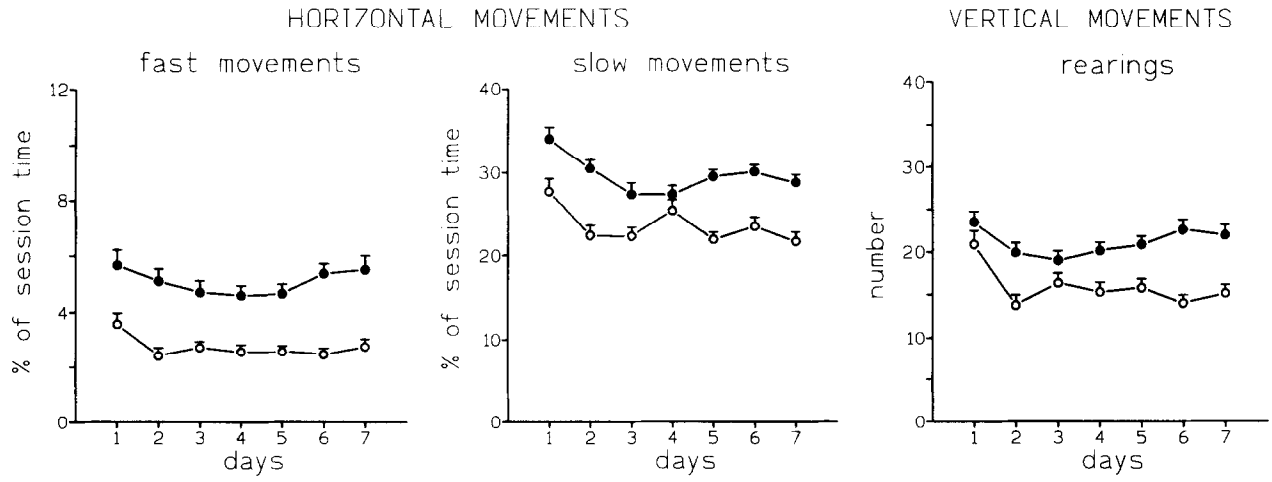

FIG. 1. Repeated testing (5 min/session) of exploratory activity in nontreated rats (n = 35) during the light period (open circles) and the dark period (closed circles). For fast (left graph) and slow (central graph) movements results are expressed as means ± SEM of the percentage of the session time the animal is engaged in each of these movements. For rearings (right graph) results are expressed as means ± SEM of number per session. Repeated-measures ANOVA with two within factors (day and light–dark) shows a significant effect of both factors for each variable ($p < 0.01$ in all cases). The day factor significance is due to the first day values (post hoc Newman-Keuls comparisons, $p < 0.01$).

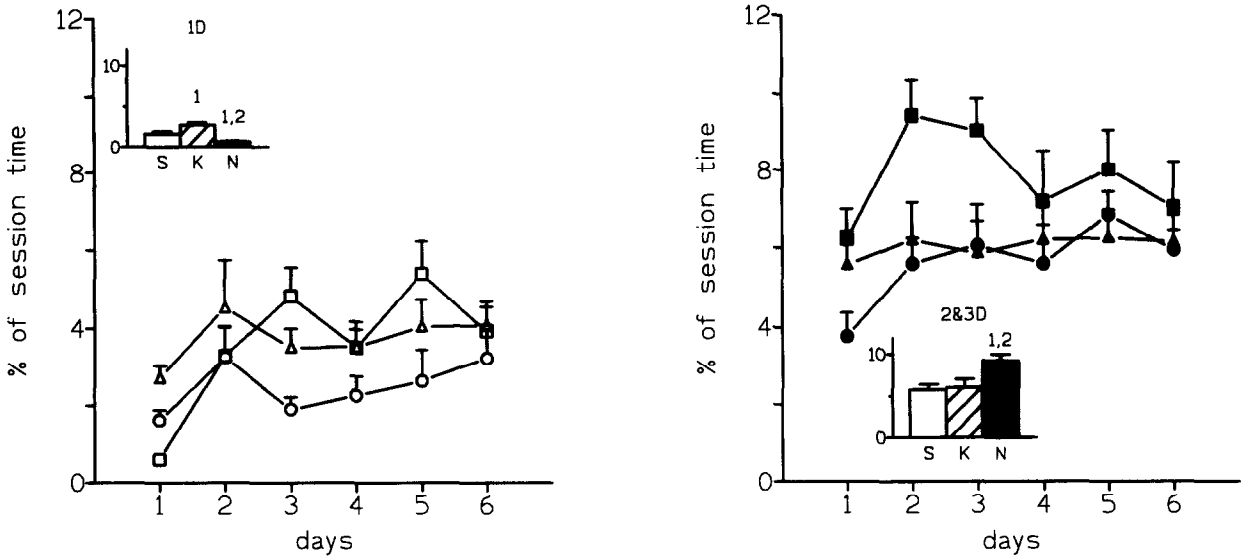

FIG. 2. Repeated testing (5 min/session) of fast movements in rats administered saline (circles; $n = 6$), KA 9 mg/kg IP (triangles; $n = 8$), or NMDA 100 mg/kg IP (squares; $n = 7$). Results are expressed as means ± SEM of the percentage of the session time the animal is engaged in this movement during the light period (left graph) and the dark period (right graph). Insets: means ± SEM of fast movements in rats administered saline (white bars), KA (striped bars), or NMDA (black bars), during the first day (1D) and during the second-third day period (2&3D). [1]Significantly different compared to the saline group, [2]significantly different compared to the KA group (ANOVA with post hoc Newman-Keuls comparisons: $p < 0.01$ in all cases).

lyze exploratory activity in nontreated rats. Pearson's correlation coefficients were used to study the existence of linear relationships between motor variables. To study the differences among the saline group and the KA-and NMDA-treated groups, the "summary measures" method (11) was used. In this case, the first day's values, the mean of the second and third days' values, and the mean of the fourth to the sixth days' values were used as the summary statistics, and they were subsequently analyzed by ANOVA with post hoc Newman-Keuls comparisons.

RESULTS

Exploratory Activity in Nontreated Rats

FM and SM values (analyzed as percent of the total session time the animal was engaged in each of these movements) and R values (analyzed as number per session) were significantly higher during the first 5-min period (means ± SEM: 3.2 ± 0.3, 31.3 ± 2.7, and 23.0 ± 1.7 for FM, SM, and R, respectively) than during the second 5-min period (1.9 ± 0.2, 22.3 ± 1.8, and 14.2 ± 2.1) (Student's paired t-test: $p < 0.01$ in all cases). Furthermore, grooming was recorded in 50% and 92% of the animals during the first and second 5-min periods of observation, respectively. Therefore, the first 5-min period of observation was used in subsequent experiments, as it was considered optimum to evaluate exploratory activity.

Stable values of FM, SM, and R were found with repeated testing during 1 week, during both the light and the dark periods of the light-dark cycle. A repeated-measures ANOVA with two within factors (a day factor, with seven levels and a light-dark factor, with two levels) showed a significant effect of both factors for each variable ($p < 0.01$ in all cases). The day factor significance was due to the values obtained during the first day, which were found to be significantly different to most of those values obtained during the next 6 days (with post hoc Newman-Keuls comparisons). The light-dark factor significance was due to higher FM, SM, and R values during the dark than during the light period of the light-dark cycle (Fig. 1). Horizontal and vertical components of motor activity (FM vs. SM, FM vs. R, and SM vs. R) showed a significant positive correlation during both the light and the dark periods of the light-dark cycle; FM values during the light period also showed a significant positive correlation with FM values during the dark period (Pearson's coefficient > 0.5 and $p < 0.05$ in all cases).

Exploratory Activity After Treatment With Excitatory Amino Acid Receptor Agonists

Convulsions appeared in seven rats treated with KA and one rat treated with NMDA, and they were excluded from the study. Data corresponding to FM, SM, and R for 6 days are represented in Figs. 2-4. A two-factor ANOVA with a within factor (a light-dark factor, with two levels) and a between factor (a treatment factor, with three levels) applied, separately, for the first day values, the mean of the second and third days' values, and the mean of the fourth to the sixth days' values was used, to determine differences in the three dependent variables FM, SM, and R. In all cases a light-dark factor significance was found ($p < 0.01$ in all cases). A significant treatment effect was obtained for the first day FM values, with a significant increase induced by KA and a significant decrease induced by NMDA compared to the saline-treated group (one-way ANOVA with post hoc Newman-Keuls comparisons: $p < 0.01$ in both cases) (Fig. 2). A significant treatment effect was also found for the first day SM and R values, with a significant decrease in the NMDA-

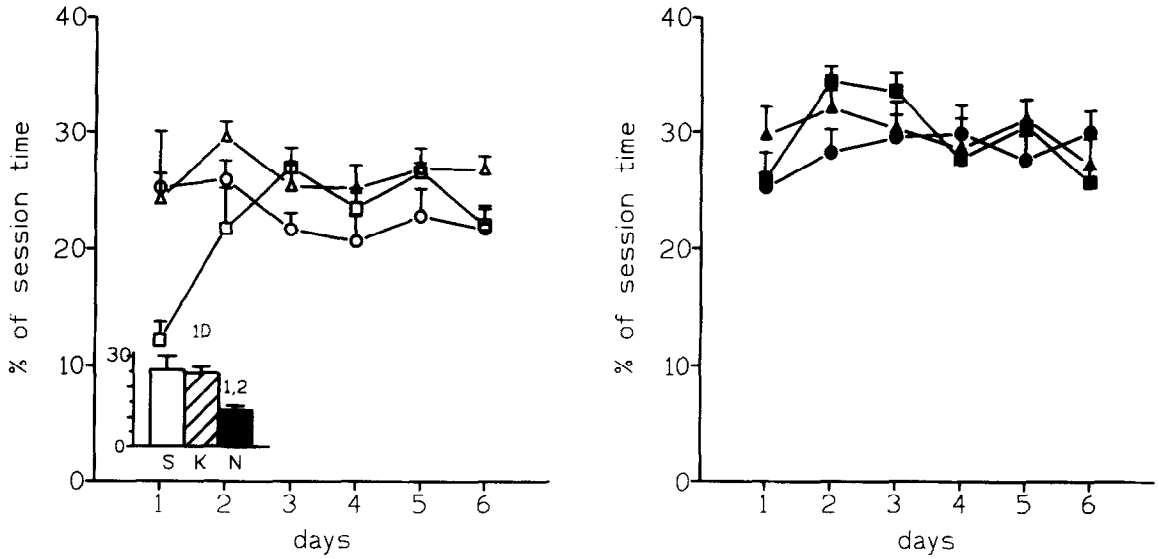

FIG. 3. Repeated testing (5 min/ session) of slow movements in rats administered saline (circles; $n = 6$), KA 9 mg/kg IP (triangles; $n = 8$), or NMDA 100 mg/kg IP (squares; $n = 7$). Results are expressed as means ± SEM of the percentage of session time the animal is engaged in this movement during the light period (left graph) and the dark period (right graph). Insets: means ± SEM of slow movements in rats administered saline (white bars), KA (striped bars), or NMDA (black bars), during the first day (1D). [1]Significantly different compared to the saline group, [2]significantly different compared to the KA group (ANOVA with post hoc Newman-Keuls comparisons: $p < 0.01$ in all cases).

treated group (one-way ANOVA with post hoc Newman-Keuls comparisons: $p < 0.01$ in both cases) (Figs. 3 and 4). For the second and third days' FM values, a significant treatment effect with a significant increase in the NMDA-treated group was obtained (one-way ANOVA with post hoc Newman-Keuls comparisons: $p < 0.01$ in both cases) (Fig. 2). The treatment effect was not found significant for the fourth to sixth days' FM, SM, or R values (Figs. 2-4). All correlations between FM, SM, and R were maintained in the NMDA-treated group. On the other hand, the correlation between FM and R was lost in the KA-treated group, during both the light and the dark periods (Pearson's coefficient around 0.1 in both cases). Body weight gain was not significantly different among the three differently treated groups (data not shown).

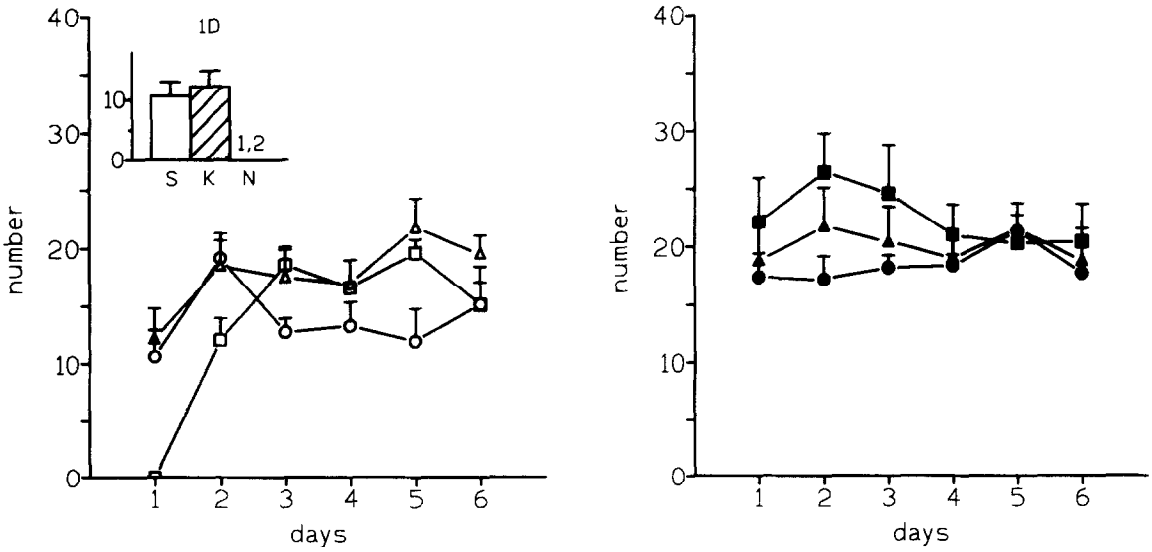

FIG. 4. Repeated testing (5 min/session) of rearings in rats administered saline (circles; $n = 6$), KA 9 mg/kg IP (triangles; $n = 8$), or NMDA 100 mg/kg IP (squares; $n = 7$). Results are expressed as means + SEM of number per session during the light period (left graph) and the dark period (right graph). Insets: means ± SEM of rearings in rats administered saline (white bars), KA (striped bars), or NMDA (black bars), during the first day (1D). [1]Significantly different compared to the saline group, [2]significantly different compared to the KA group (ANOVA with post hoc Newman-Keuls comparisons: $p < 0.01$ in all cases).

DISCUSSION

Most exploratory activity is produced during the first minutes after the animal is placed in a new environment (6,22). In the present study, a short session time (5 min) was found to be optimum for analyzing exploratory activity with three components: fast movements, slow movements, and rearings. Fast and slow movements and rearings were found to be very stable with repeated testing, during both the light and dark periods of the light-dark cycle. Only the first day values were significantly different compared with those of the other days. These results suggest that when repeated testing is needed, exploratory activity during the first tests should not be used for comparisons. In fact, it has been reported that measurements of locomotor activity during the first day are poor predictors of the activity of subsequent daily tests (6). In agreement with the literature, exploratory activity was significantly higher during the dark than during the light cycle (16,17).

Separation of exploratory activity in fast and slow movements and rearings, together with the analysis of those variables during both the light and dark periods of the light-dark cycle, allowed finding specific drug-induced effects. KA produced an increase in exploratory activity after its administration in the first test during the light period, and this effect was only significant for fast movements. On the other hand, NMDA produced in the same test a decrease in exploratory activity, which was significant for fast and slow movements and for rearings. However, this initial motor depression induced by NMDA was followed during the next 2 days by an increased exploratory activity. Another differential effect between both glutamatergic agonists was a disruption of the correlation between fast movements and rearings after KA treatment.

The systemic administration of high doses of KA has been shown to induce seizures followed by motor hyperactivity and impairment of learning tasks (8,14). KA-induced convulsant activity is invariably associated to both excitotoxic brain lesions (2,14,21) and weight loss (10,21). In a previous work (21) we showed, using conventional staining procedures, that only some rats that did not present convulsions after KA administration (9 mg/kg, IP) displayed some minor histological alterations. The animals used in the present study to analyze exploratory activity did not present convulsions or weight loss after KA administration, which suggests that direct stimulation of ionotropic excitatory amino acid receptors that mediate fast neurotransmission (KA and or AMPA receptors) induces an increase in exploratory activity. The same markers for excitotoxicity (seizures and weight loss) were used for NMDA, and consequently, we are not sure about the absence of an excitotoxic component of the effects of NMDA on exploratory activity. Nevertheless, the convulsant response developed by rats after NMDA (one animal out of eight) was lower than that developed by KA-treated rats (7 animals out of 15).

In agreement with the present results, we have recently shown that NMDA induces in mice an initial motor depression of about 1 h of duration followed, in the next hour, by an increased motor activity (5). Von Lubitz et al. (23) found that systemically administered low doses of NMDA in mouse induces a decrease in motor activity. Those authors suggested that the depressant effect of NMDA could be mediated by an NMDA-induced release of adenosine (7), which inhibits motor activity by counteracting dopaminergic neurotransmission [for a review, see (4)]. In reserpinized mice an NMDA-induced dopamine-independent motor activation was also found (5). We have previously suggested that the striatum, a basal ganglia structure involved in the production of motor activity, might be the main locus of action in the brain responsible for the excitatory effects of NMDA on motor activity (5). We suggest that this brain structure could also be involved in KA-induced motor activation. In fact, the striatum (caudate-putamen, nucleus accumbens, and olfactory tubercle) contains, by far, the highest densities of all types of excitatory amino acid receptors in the basal ganglia (1). Furthermore, it has been shown that the local injection of both KA or NMDA in the nucleus accumbens or in the ventral pallidum (a target nucleus for the nucleus accumbens) induces a pronounced motor activation (19,20). However, we cannot exclude a peripheral component in the effect of systemically administered excitatory amino acids.

In summary, systemic administration of excitatory amino acid ionotropic receptor agonists produce an increase in exploratory activity in rats that is probably not linked to their excitotoxic action. Although a significant increase of exploratory activity induced by NMDA was only obtained for fast movements during the dark period of the light-dark cycle, a nonsignificant increase was observed for fast movements during the light period and for slow movements and rearings during both the light and dark periods. These results suggest that NMDA induces a general long-lasting increase in exploratory activity during the whole light-dark cycle and not a specific effect on fast movements during the dark period. The stronger exploratory activity during the dark compared to the light period, which in fact is specially different for fast movements (Fig. 1), could facilitate the demonstration of significant differences. The long-lasting effect of NMDA could be related to its well-known long-lasting synaptic effects (3).

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REFERENCES

1. Albin, R. L.; Makowiec, R. L.; Hollingsworth, Z. R.; Dure IV, L. S.; Penney, J. V.; Young, A. B. Excitatory amino acid binding sites in the basal ganglia of the rat: A quantitative autoradiographic study. Neuroscience 46:35–48; 1992.
2. Ben-Ari, Y.; Tremblay, E.; Riche, D.; Ghilini, G.; Naquet, R. Electrographic, clinical and pathologic alterations following systemic administration of kainic acid, bicuculine or pentetrazole: metabolic mapping using the deoxiglucose method with special reference to the pathology of epilepsy. Neuroscience 6:1361–1391; 1981.
3. Collingridge, G. L.; Singer, W. Excitatory amino acid receptors and synaptic plasticity. Trends Pharmacol. Sci. 11:290–296; 1990.
4. Ferré, S.; Fuxe, K.; Von Euler, G.; Johansson, B.; Fredholm, B. B. Adenosine-dopamine interactions in the brain. Neuroscience 51(3):501–512; 1992.
5. Ferré, S.; Giménez-Llort, L.; Artigas, F.; Martínez, E. Motor activation in short- and long-term reserpinized mice: Role of *N*-methyl-D-aspartate, dopamine D_1 and dopamine D_2 receptors. Eur. J. Pharmacol. 255:203–213; 1994.
6. Geyer, M. A. Approaches to the characterization of drug effects on locomotor activity in rodents. In: Modern methods in pharmacology, vol. 6, testing and evaluation of drugs of abuse. New York: Wiley-Liss, Inc.; 1990:81–99.
7. Hoehn, K.; White, T. D. *N*-methyl-D-aspartate, kainate and quis-

qualate release endogenous adenosine from rat cortical slices. Neuroscience 39:441–450; 1990.
8. Kish, J. S.; Sperk, G.; Hornykiewicz, O. Alteration in benzodiazepine and GABA receptor binding in rat brain following systemic injection of kainic acid. Neuropharmacology 22(11):1303–1309; 1983.
9. Mares, P.; Velísek, L. *N*-methyl-D-aspartate (NMDA)-induced seizures in developing rats. Dev. Brain Res. 65:185–189; 1992.
10. Martínez, E.; de Vera, N.; Artigas, F. Differential response of rat brain polyamines to convulsant agents. Life Sci. 48:77–84; 1991.
11. Matthews, J. N. S.; Altman, D. G.; Campbell, M. J.; Royston, P. Analysis of serial measurements in medical research. Br. Med. J. 230:730; 1990.
12. Meldrum, B.; Garthwaite, J. Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol. Sci. 11:379–387; 1990.
13. Metha, A. K.; Ticku, M. K. Role of *N*-methyl-D-aspartate (NMDA) receptors in experimental catalepsy in rats. Life Sci. 46: 37–42; 1990.
14. Milgram, N. W. Deficits in spontaneous behaviour and cognitive function following systemic administration of kainic acid. Neurotoxicology 9(4):611–624; 1988.
15. Nakanishi, S. Molecular diversity of glutamate receptors and implications for brain function. Science. 258:597–603; 1992.
16. Norton, S.; Culver, B.; Mullenix, P. Development of nocturnal behavior in albino rats. Behav. Biol. 15:317–331; 1975.
17. Reiter, L. W.; MacPhail, R. C. Factors influencing motor activity measurements in neurotoxicology. In: Micthell, C. L., ed. Nervous system toxicology. New York: Raven Press; 1982:45–65.
18. Seeburg, P. H. The molecular biology of mammalian glutamate receptor channels. Trends Pharmacol. Sci. 14:297–303; 1993.
19. Shreve, P. E.; Uretsky, N. J. Effect of GABAergic transmission in the subpallidal region on the hypermotility response to the administration of excitatory amino acids and picrotoxin into the nucleus accumbens. Neuropharmacology 27(12):1271–1277; 1988.
20. Shreve, P. E.; Uretsky, N. J. AMPA, kainic acid and *N*-methyl-D-aspartic acid stimulate locomotor activity after injection into the substantia innominata/lateral preoptic area. Pharmacol. Biochem. Behav. 34:101–106; 1989.
21. Vera, de, N.; Artigas, F.; Serratosa, J.; Martínez, E. Changes in polyamine levels in rat brain after systemic kainic acid administration: Relationship to convulsant activity and brain damage. J. Neurochem. 57(1):1–8; 1991.
22. Vetulani, J.; Marona-Lewicka, D.; Michaluk, J.; Antkiewicz-Michaluk, L.; Popik, P. Stability and variability of locomotor responses of laboratory rodents. II. Native exploratory and basal locomotor activity of wistar rats. Pol. J. Pharmacol. Pharm. 39: 283–293; 1987.
23. Von Lubitz, D. K. J. E.; Paul, I. A.; Carter, M.; Jacobson, K. A. Effects of N^6-cyclopenthyl adenosine and 8-cyclopenthyl-1,3-dipropylxanthine on *N*-methyl-D-aspartate induced seizures in mice. Eur. J. Pharmacol. 249:265–270; 1993.
24. Westbrook, G. L. Glutamate receptors and excitotoxicity. In: Wasman, S. G., Molecular and cellular, vol. 3, approaches to the treatment of neurological disease. New York: Raven Press; 1993: 35–49.
25. Winn, P.; Stone, T. W.; Latimer, M.; Hastings, M. H.; Clark, A. J. M. A comparison of excitotoxic lesions of the basal forebrain by kainate, quinolate, ibotenate, *N*-methyl-D-aspartate or quisqualate, and the effects on toxicity of 2-amino-5-phosphonovaleric acid and kynurenic acid in the rat. Br. J. Pharmacol. 102: 904–908; 1991.