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Neonatal exposure to monosodium glutamate disrupts place learning ability in adult rats

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Abstract

The activation of glutamatergic NMDA receptors of the hippocampus is closely associated with expression of place learning. Neonatal exposure to monosodium glutamate leads to abnormal expression of NMDA receptor subunits in the hippocampus, but its effect on place learning is unknown. Place learning acquisition and retrieval were assessed in mature adult rats after subcutaneous injection of monosodium glutamate (4 mg/g body weight) in eight neonatal rat pups at postnatal days one, three, five, and seven. Eight untreated rats were used as controls. At four months of age, the rats were challenged over a period of nine days with a place learning task. The task used an acquisition–retrieval paradigm in a Morris maze. Place learning acquisition was impaired in the experimental rats, which were unable to reduce their escape latencies during the nine training days. Controls improved between the fifth and ninth days of training. Test trials showed that retrieval of spatial information was also impaired in the experimental animals. These results show that both place learning acquisition and retrieval abilities in mature rats are impaired by neonatal treatment with monosodium glutamate. These findings may be related to the abnormal expression of NMDA receptor subunits in the hippocampus.

Keywords: Hippocampus; Spatial orientation; Place learning; Monosodium glutamate; Neurotoxicity

1. Introduction

The hippocampus has an important role in spatial learning and memory (Compton, 2004; Eichenbaum and Otto, 1992; Poucet, 1993). Several changes in synaptic efficacy are closely related to memory consolidation (Harris, 1994), and these are mediated by glutamatergic neurotransmitter activity on dendritic spines (Segal, 1995; Kasai et al., 2003). Synaptic stimulation of the NMDA and AMPA ionotropic glutamate receptors on the dendritic spines of hippocampal CA1 pyramidal spiny neurons is necessary for the acquisition of several types of memory (Cammarota et al., 2004). NMDA receptor (NMDAR) activation is required for long-term potentiation (LTP) and long-term depression at hippocampal CA1 synapses (Liu et al., 2004; Muller et al., 2000; Shapiro, 2001). This observation suggests that some NMDAR subunits may be crucial for plasticity at the synaptic level (Liu et al., 2004).

Expression of the NR1 and NR2 (A–D) subunits of the NMDAR population increases in young adult rats after neonatal exposure to monosodium glutamate (MSG) (Beas-Zárate et al., 2002a,b, 2001). A moderate loss of hippocampal CA1 pyramidal cells and some cytoarchitectural changes in the surviving neurons were also observed following this treatment (Beas-Zárate et al., 2002a,b).

These changes at subcellular and cellular levels suggest that impairment in the integration of hippocampus-dependent place learning and memory could take place as a result of neonatal MSG exposure. This work examines the effect of neonatal MSG exposure on hippocampus-dependent place learning acquisition and retrieval in mature rats.

2. Materials and methods

Sixteen neonatal Sprague–Dawley male rats were used. These were obtained from the litters of eight normal female rats and were randomly divided into two groups. Eight experimental animals (MSG) were subcutaneously injected with a 50%

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aqueous solution of monosodium L-glutamic acid (SIGMA) (MSG) (4 mg/g of body weight) on postnatal days one, three, five and seven. The remaining eight rats were the untreated control group (Control). Animals from both groups remained with their original litter until weaning at 21 days and then were caged until they reached four months of age.

At four months of age, the rats were trained in a Morris maze for nine consecutive days with the following protocol. Each rat was given three trials daily, with a 2-min interval between each trial. Two of these three trials were conducted with a sunken glass platform whose surface was 1.5 cm below the water level. It was placed in one quadrant of the pool (the training trials). The platform was removed in the third trial (the test trial). The test trial was conducted either before or after the two training trials, at random, on each of the nine training days.

In each training trial, the rat was placed in the maze facing the wall of the pool, at different, randomly selected starting locations. The rat was then allowed to search for the platform for a period of 60 s. Once the rat found the platform, it was allowed to remain on it for 15 s. In the test trial, the rat was placed in the pool at different starting points and given 30 s to search for the (absent) platform.

Behavioral evaluations were performed in a swimming pool, 140 cm in diameter, filled with water dyed blue by the addition of gentian violet, and maintained at 30 °C. All these experiments were conducted between 09:00–13:00 h, under natural day light and with free access to visuospatial cues, which were the current objects present in the lab.

All experimental procedures were conducted according to the Mexican Federal law for protection of animals (Official Newspaper, January 7, 1981), which is in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 8023, revised in 1985).

Video recordings were made of the behavioral tests, and from these the swimming routes were traced. The escape latencies were averaged for the two training trials for each day. Intragroup comparisons were made between the first day and the subsequent training days, using Friedman's ANOVA test and Wilcoxon's test post hoc. Intergroup comparisons were made using the Kruskal Wallis ANOVA test and the Mann–Whitney *U* test post hoc. The distance traveled in each trial was measured, and the swimming velocity was calculated by dividing the distance by the latency. Both distance and velocity were compared using the ANOVA test for repeated measurements and the Tukey test post hoc. For the trial test, the swimming paths were traced and the numbers of "crosses" through a circular area corresponding to the place where the platform had been located during the training trials were recorded. A comparison of the numbers of crosses was made with an ANOVA test for repeated measurements and the Tukey test post hoc.

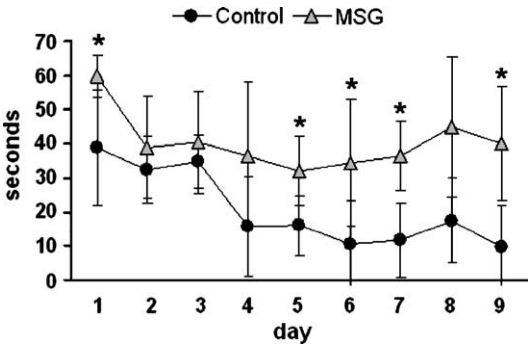

Fig. 2. Intergroup comparison of the escape latencies for each training day. Median ± Standard Error of the Median. ($P<0.05$).

3. Results

Control animals significantly reduced their escape latency [$X=23.25$, $P=0.003$] between the fifth and ninth days of training ($P=0.028$, $P=0.021$, $P=0.025$, $P=0.017$, and $P=0.012$; respectively), compared with the first day's latency. No reduction in escape latency was observed for the MSG group for any training day [$X=8.296$, $P=0.405$] (Fig. 1). The intergroup comparisons showed that the MSG-treated rats had longer escape latencies than the controls in the first [$Z=-2.237$, $P=0.036$], fifth [$Z=-2.359$, $P=0.015$], sixth [$Z=-2.265$, p=0.021], seventh [$Z=-2.598$, $P=0.008$], and ninth training days [$Z=-2.946$, $P=0.003$] (Fig. 2).

The number of crosses were averaged for the test trials over the nine days of training, and the control group had a higher average than the MSG-treated animals [F (1,15)=14.189, $P=0.002$] (Fig. 3). The control animals gradually increased their tendency to search the "correct" area during the training period, until this was significantly higher than for the MSG group by the ninth day of training [F (8,120)=2.873, $P=0.006$] ($P<0.001$). MSG-treated animals made fewer

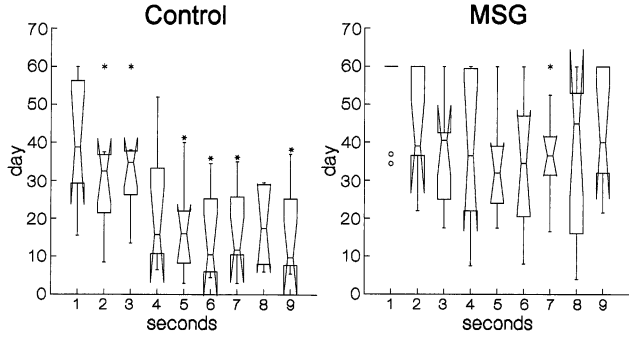

Fig. 1. Intragroup comparison of the escape latencies between the first and subsequent days of training. Median ± Standard Error of the Median. *: Significant difference vs. the first training day. ($P<0.05$).

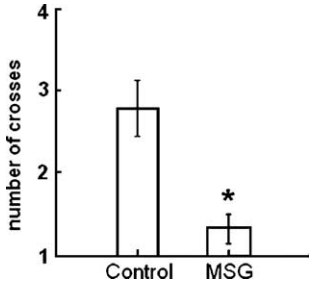

Fig. 3. Intergroup comparison of the number of crosses of the platform area showing averages of the nine test trials. Mean ± Standard Error of the Median. ($P=0.001$).

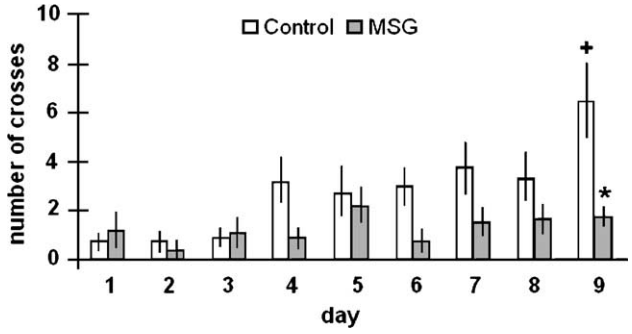

Fig. 4. Comparison of the number of crosses of the platform area for all nine training days. Mean±Standard Error of the Mean. *: Control vs. MSG; +: Significant difference vs. the first training day. ($P \leq 0.002$).

crosses in the ninth day than in the first day of training ($P < 0.001$) (Fig. 4).

Control rats were able to locate the platform throughout all nine trials (Fig. 5). Conversely, MSG-treated animals frequently swam in circles and did not find the platform until the last trials, suggesting that a spatially efficient strategy was not used to solve the task (Fig. 6).

No difference was observed in the swimming velocities of the two groups (data not shown).

4. Discussion

This study shows that both the acquisition and retrieval of spatial information were disrupted in adult rats that were exposed as neonates to the neurotoxic effects of MSG.

Cerebral cortex, striatum, and hippocampus are strongly involved in mnemonic information processing, and they are densely innervated by glutamatergic fibers. Glutamatergic activity is altered both in the cerebral cortex and hippocampus, and also in striatum but in a lesser extent; following neonatal MSG treatment (Ureña-Guerrero et al., 2003). In nature, different memory system-sustaining strategies are used to resolve a given spatial problem, depending on the available information. Taxon, praxis, and place strategies can be used in-parallel and independently, being the taxon (cue) and praxis (egocentric) striatum-dependent learning strategies (White and McDonald, 2002), while place learning has been proposed to depend on the dorsal (Moser et al., 1995) hippocampal activity (White and McDonald, 2002).

Prefrontal cortex activity has been related to spatial or non-spatial working memory process, as well as to egocentric learning. Deficits in delayed-response spatial alternation in a T-maze, and spatial reversal; have been reported (Larsen and Divac, 1978; Silva et al., 1986). However, these findings have been proposed to be related rather to a short-term memory processing deficit, than to a spatial information processing deficit (De Bruin et al., 1994). The Morris maze paradigm used in the present study evaluates reference, but not working memory. Lesions of the medial prefrontal cortex induced a slow learning pattern in the Morris maze, but not inability (Kolb et al., 1982, 1983), as seen in the present study. In addition, no significant deficiencies in reference memory have been reported after lesion of several working memory-organizing prefrontal regions (De Bruin et al., 1994). Thus,

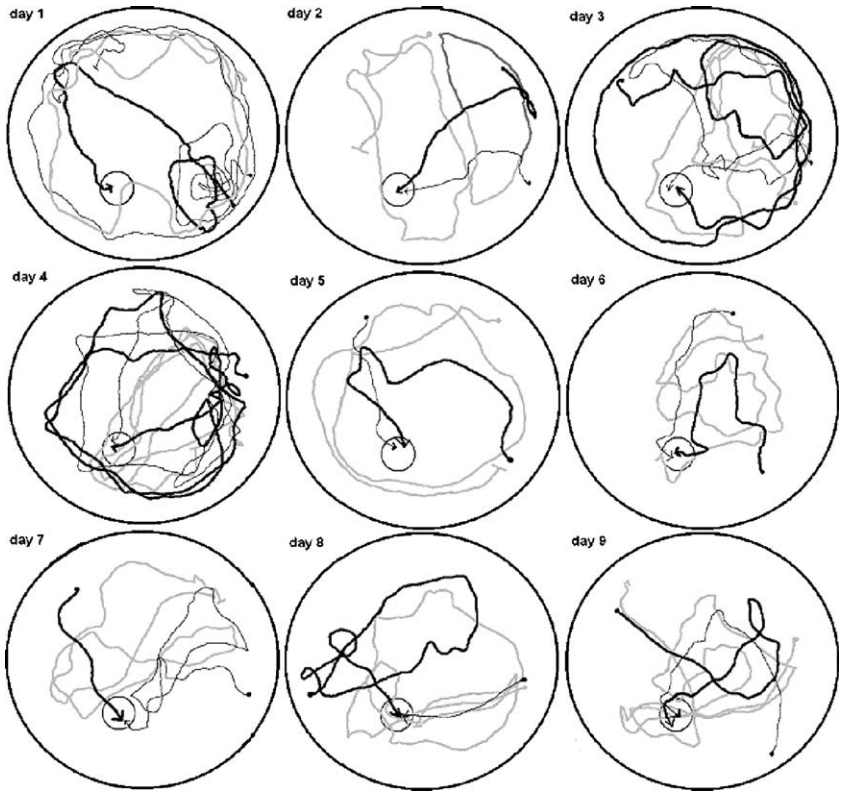

Fig. 5. Swimming routes of a representative control animal for each of the nine training days. Thin black line: first training trial; thick black line: second training trial; gray line: test trial; circle: location of the platform during the training trials.

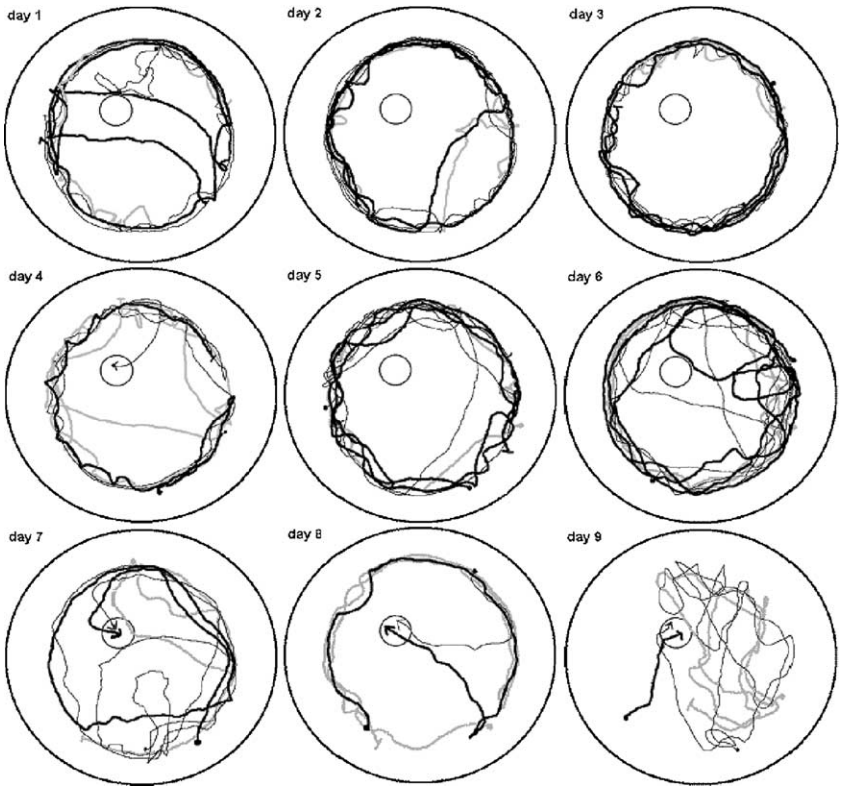

Fig. 6. Swimming routes of a representative MSG-treated rat for each of the nine training days. Thin black line: first training trial; thick black line: second training trial; gray line: test trial; circle: location of the platform during the training trials.

place learning deficiencies in the reference memory paradigm reported in the present work could most likely be due to alterations in the integrative activity of the dorsal hippocampus induced by neonatal MSG treatment. Thus, MSG effects on other brain regions involved in mnemonic information processing could contribute secondarily, and further behavioral studies must be conducted to test this hypothesis.

Hippocampus is one of the most vulnerable brain regions to glutamate-mediated excitotoxic effects (Schmidt-Kastner and Freund, 1991). However, normal performance in a spatial memory task is sustainable by only about 20% of intact dorsal hippocampus, after ibotenic acid-induced lesion (Moser et al., 1995). Neonatal MSG treatment leaded to 12% reduction of pyramidal cells from the dorsal hippocampal CA1 field (Beas-Zárate et al., 2002a,b), and also to the severe impairments in place learning reported in the present study. Altogether, these findings could be related differentially to the pathophysiological and thereafter plastic events in mature or newborn rats; which, evidently sustained enough the spatial orientation ability, or leaded to place learning deficits; respectively. In this sense, further studies are needed to characterize the biological parameters of the surviving neurons underlying the MSG-inducing spatial memory deficits.

Glutamatergic NMDA receptors appear to be very important in spatial learning (Wong et al., 1997), as suggested by the observation that NMDA-injected rat pups take longer to learn the platform location in a water maze paradigm when tested nearly four months later (Stafstrom and Sasaki-Adams, 2003).

Behavioral training leads to a rapid, transient increase in the expression of NMDA NR1 subunits (Cammarota et al., 2000). Despite the increase of NMDA NR1 subunit expression observed in adult rats following neonatal MSG administration (Beas-Zárate et al., 2001), a deficit in place learning has been shown in this study after MSG treatment. This result may be related to the concomitant increase in the expression of NMDA NR2 subunits, which is also observed following MSG treatment (Beas-Zárate et al., 2002a,b). The behavioral consequences of the differential expression of these two subunits are unknown, but GluR2 knock-out mice do not appear to form place fields (a term for the cognitive representations of the locations of objects in the environment) (Yan et al., 2002). In long-term paradigms such as the one used in this study, stabilization of newly established firing fields by hippocampal place cells requires normal NMDAR functioning; this stabilization may be related to LTP and therefore to spatial learning (Kentros et al., 1998; Shapiro, 2001). LTP induction depends on normal NMDAR functioning (Shapiro, 2001), and neonatal MSG treatment leads to a failure to maintain and consolidate LTP (Sanabria et al., 2002). This evidence suggests that the molecular changes previously observed in the NMDAR subunits after neonatal MSG treatment (Beas-Zárate et al., 2002a,b, 2001) may be responsible for the cognitive impairments observed in this study, by hindering the formation of stable hippocampal place fields.

In conclusion, we propose that the impaired behavioral performance in the MSG-treated rats is closely related to an abnormal, NMDA-mediated pathway for spatial information in

the hippocampus. A possible role for the differential expression of NMDAR subunits in hippocampal-dependent place learning requires further investigation.

References

Beas-Zárate C, Rivera-Huizar SV, Sanchez-Ruiz MY, Martínez-Contreras A, Feria-Velasco A, Armendariz-Borunda J. Changes in NMDA-receptor gene expression are associated with neurotoxicity induced neonatally by glutamate in rat brain. Neurochem Int 2001;39:1–10.

Beas-Zárate C, Flores-Soto ME, Armendariz-Borunda J. NMDAR-2C and 2D subunits gene expression is induced in brain by neonatal exposure of monosodium L-glutamate to adult rats. Neurosci Lett 2002;321:9–12.

Beas-Zárate C, Perez-Vega MI, Gonzalez-Burgos I. Neonatal exposure to monosodium L-glutamate induces cell death and cytoarchitectural impairments in hippocampal CA1 pyramidal neurons of adult rats. Brain Res 2002;952:275–81.

Cammarota M, de Stein ML, Paratcha G, Bevilaqua LR, Izquierdo I, Medina JH. Rapid and transient learning-associated increase in NMDA NR1 subunit in the rat hippocampus. Neurochem Res 2000;25:567–72.

Cammarota M, Bevilaqua LR, Bonini JS, Rossatto JI, Medina JH, Izquierdo N. Hippocampal glutamate receptors in fear memory consolidation. Neurotoxicol Res 2004;6:205–12.

Compton DM. Behavior strategy learning in rat: effects of lesions of the dorsal striatum or dorsal hippocampus. Behav Processes 2004;67:335–42.

De Bruin JPC, Sánchez-Santed F, Heinsbroek RPW, Donker A, Postmes P. A behavioural analysis of rats with damage to the medial prefrontal cortex using the morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. Brain Res 1994;652:323–33.

Eichenbaum H, Otto T. The hippocampus—what does it do? Behav Neural Biol 1992;57:2–36.

Harris KM. Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu Rev Neurosci 1994;17:341–71.

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H. Structure–stability–function relationships of dendritic spines. TINS 2003;26:360–8.

Kentros C, Hargreaves E, Hawkins RD, Kandel ER, Shapiro M, Muller RV. Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. Science 1998;280:2121–6.

Kolb B, Pittman K, Sutherland RJ, Wishaw IQ. Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic nucleus to spatially guided behaviour in the rat. Behav Brain Res 1982;6:365–78.

Kolb B, Sutherland RJ, Whishaw IQ. A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. Behav Neurosci 1983;97:13–27.

Larsen JK, Divac I. Selective ablations within the prefrontal cortex of the rat and performance of delayed alternation. Physiol Psychol 1978;6:15–7.

Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, et al. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 2004;304:973–4.

Moser MB, Moser EI, Forrest E, Andersen P, Morris RGM. Spatial learning with a minislab in the dorsal hippocampus. Proc Natl Acad Sci U S A 1995;92:9697–701.

Muller D, Toni N, Buchs PA. Spine changes associated with long-term potentiation. Hippocampus 2000;10:596–604.

Poucet B. Spatial cognitive maps in animals: new hypotheses on their structure and neural mechanisms. Psychol Rev 1993;100:163–82.

Sanabria ER, Pereira MF, Dolnikoff MS, Andrade IS, Ferreira AT, Cavalheiro EA, et al. Deficit in hippocampal long-term potentiation in monosodium glutamate-treated rats. Brain Res Bull 2002;59:47–51.

Segal M. Morphological alterations in dendritic spines of rat hippocampal neurons exposed to N-methyl–D-aspartate. Neurosci Lett 1995;193:73–6.

Schmidt-Kastner R, Freund TF. Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 1991;40:599–636.

Shapiro M. Plasticity, hippocampal place cells, and cognitive maps. Arch Neurol 2001;58:874–81.

Silva MG, Boyle MA, Finger S, Numan B, Bouzrara AA, Almli CR. Behavioral effects of large and small lesions of the rat medial prefrontal cortex. Exp Brain Res 1986;65:176–81.

Stafstrom CE, Sasaki-Adams DM. NMDA-induced seizures in developing rats cause long-term learning impairment and increased seizure susceptibility. Epilepsy Res 2003;53:129–37.

Ureña-Guerrero ME, López-Pérez SJ, Beas-Zárate C. Neonatal monosodium glutamate treatment modifies glutamic acid decarboxilase activity during rat brain postnatal development. Neurochem Int 2003;42:269–76.

White NM, McDonald RJ. Multiple parallel memory systems in the brain of the rat. Neurobiol Learn Mem 2002;77:125–84.

Wong PT, Neo LH, Teo WL, Feng H, Xue YD, Loke WH. Deficits in water escape performance and alterations in hippocampal cholinergic mechanisms associated with neonatal monosodium glutamate treatment in mice. Pharmacol Biochem Behav 1997;57:383–8.

Yan J, Zhang Y, Jia Z, Taverna FA, McDonald RJ, Muller RU, et al. Place-cell impairment in glutamate receptor 2 mutant mice. J Neurosci 2002;22(RC204):1–5.