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Deficits in Water Escape Performance and Alterations in Hippocampal Cholinergic Mechanisms Associated With Neonatal Monosodium Glutamate Treatment in Mice

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WONG, P. T.-H., L. H. NEO, W. L. TEO, H. FENG, Y. D. XUE AND W. H. LOKE. *Deficits in water escape performance and alterations in hippocampal cholinergic mechanisms associated with neonatal monosodium glutamate treatment in mice.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 383–388, 1997.—Mice treated neonatally with monosodium glutamate (MSG) were found to have learning and memory deficits in performing a non-spatial water escape task. Scopolamine impaired the water-escape performance of the control mice but not that of the MSG-treated mice. It was suggested that the water-escape performance deficit in the MSG-treated mice was a result of impaired central cholinergic mechanisms. As such, scopolamine was unable to further incapacitate an already impaired cholinergic system. This is strongly suppported by the decreased affinity of the sodium-dependent high-affinity choline uptake observed in the hippocampus. d-Cycloserine, a partial agonist at the glycine site of the NMDA receptor, did not affect the water-escape performance of the MSG-treated and control mice; nor did it alter the effects of scopolamine. This lack of effect of d-Cycloserine may imply that the NMDA receptors are not involved in non-spatial learning, in contrast to their reported involvement in spatial learning. © 1997 Elsevier Science Inc.

Monosodium glutamate Learning and memory Hippocampal cholinergic mechanisms NMDA receptors d-Cycloserine Scopolamine Water escape task

THE central effects of neonatal treatment with monosodium glutamate (MSG) in laboratory rodents have been quite extensively studied since the first report by Olney (27). This treatment causes lesions in many brain regions, such as the dentate gyrus of the hippocampus (28), the superior colliculi (3) and especially the arcuate nucleus of the hypothalamus (4). Profound developmental, behavioral and endocrinological changes (5,6,17,19) have been mainly attributed to the neuronal damages in the arcuate nucleus. Changes in various neurotransmitter or neuromodulator systems, including dopamine, GABA, glutamate/aspartate, nitric oxide, substance P, neuropeptide Y and beta-endorphin have been reported (7,9,15,33,38). In mice, such treatment with MSG leads to obesity, hypoactivity, reduced pituitary protein content, decreased ovarian weight, delayed puberty and elevated plasma corticosterone levels (19). In rats, such MSG treatment has also been shown to cause spatial learning deficits in a water maze task (12). These authors attributed this deficit to hippocampal damage since place learning appears to require normal hippocampal function (25). This implies that the MSG-associated learning deficits may involve an impairment of hippocampal cholinergic function in view of its well established role in learning and memory (11). For example, cholinergic blockade by scopolamine treatment has been shown to selectively impair spatial learning (34,35) as well as cue-learning in water escape tasks which do not require spatial navigation (30). Therefore, it is interesting to see whether or not the water escape performance is affected in mice treated with MSG

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and whether these mice show any alterations in cholinergic mechanisms in the hippocampus.

The glutamate neurotoxicity is predominantly mediated by the NMDA receptors (18). Moreover, the glutamatergic system, in particular the NMDA receptors, has also been implicated in modulating the phenomenon of long-term potentiation (LTP) and cognitive function (14,21). NMDA antagonists were found to impair both learning and memory and the induction of LTP (24,36). Glycine, acting on the strychnine-insensitive glycine modulatory site of the NMDA receptor, facilitated induction of LTP in the rat hippocampus (1,26). Interestingly, d-cylcoserine (DCS), which is a partial agonist at this glycine site, was reported to attenuate scopolamine-induced learning and memory deficits in rats (13). Therefore, one might expect that DCS would alleviate MSG-induced deficits especially if the deficits were due to cholinergic dysfunction. The present study was undertaken with three main objectives: (1) to study the effects of neonatal MSG treatment in mice on a water escape task, (2) to investigate whether DCS may alter the task performance of these mice, and (3) to investigate the effects of scopolamine on the task performance of these mice with or without coadministration of DCS. At the end of the experiments, neurochemical parameters of the glutamatergic and cholinergic systems were measured in various regions of the brain to see if neonatal MSG treatment had significantly altered these two systems.

METHODS

Swiss albino mice in advanced pregnancy were obtained from the Laboratory Animal Centre, National University of Singapore. Forty eight newborn mice (28 males, 20 females) were used in the study. Half of them (16 males, 8 females) were administered subcutaneously with MSG at doses of 2.2, 2.5, 2.8, 3.2, 3.4, 3.6, 3.8, 4.0, and 4.2 mg/g body weight on postnatal days 2–10, respectively (31). The other half of the littermates (12 males, 12 females) were injected subcutaneously with 0.9% saline and acted as controls. On weaning (day 21), the mice were housed in groups of 4-6 according to sex and treatment group. Their body weight was monitored at regular intervals. At ages of 4, 6 and 12 mo, their food and water consumptions were monitored over a 10-day period. Body conductivity, which is a measure of the fat-free lean mass of the body, was also determined at the beginning of this period by a non-invasive method using a Body Composition Analyzer (EM-SCAN Model SA-2).

Experiment 1

The mice, starting from about 4 mo of age, were required to perform a water escape task which was modified from the standard version of the Morris water maze (23). The task consisted of finding a circular transparent platform, measuring 8 cm in diameter. The platform, located at the centre of a heated (26±1°C) pool measuring 45×45×10 (depth) cm, was submerged 1 cm below the water surface. The water was colored brown with a nontoxic dye to make the platform invisible. The mice were always introduced at the same corner of the pool and the time taken to locate and mount the platform (the latency) was used as a dependent measure. Each mouse was tested individually over a period of 6 wk and two times a week 3–4 days apart (once per session), the mean latency was taken as its weekly performance score.

Experiment 2

After Experiment 1, the control and MSG-treated mice were each divided into 2 groups, 1 of the 2 groups received DCS (50 mg/kg in saline, IP) while the other received saline three times a week (Mon., Wed., and Fri.). The animals were tested twice a week (Tues. and Thurs.) over a period of 7 wk.

Experiment 3

The acute effects of scopolamine was studied with and without pretreatment with DCS. Mice that received DCS in Experiment 2 were injected with DCS (50 mg/kg, IP) while the others received saline. After 30 min, scopolamine (2 or 5 mg/kg, IP) was administered to all mice which were then subjected to the water escape task 30 min later. Pre-scopolamine latency was obtained by injecting saline in place of scopolamine one week before the scopolamine experiment.

Receptor Binding Assays

All mice were killed at about 1 year of age and their brain dissected. The hippocampus, cerebral cortex and striatum (pooled, when necessary, from several mice for 1 independent determination of K_d and B_{max} using 4 ligand concentration points, 5 or 6 independent determinations per group) were homogenized in 40 vol. of 50 mM Tris-acetate buffer, pH 7.4, centrifuged at 30,000 g for 20 min. The pellet was resuspended in equal volume of the same buffer and then incubated at 37°C for 20 min in a shaker bath followed by centrifugation as above. Triton-X100 (final concentration 0.05%) was added before the incubation only for MK-801 binding. The pellet was washed two more times and finally resuspended in 40 vol. of the Tris-acetate buffer.

For MK-801 binding, the final tissue preparation (0.2 ml) was incubated with [^{3}H]MK-801 (Du Pont, 0.1 μCi, final concentration 5–205 nM), glycine (10 μM) and glutamate (10 μM) (32) in a total volume of 1 ml at 30°C for 3 h. After incubation, bound radioligand was recovered by rapid filtration through glass fibre filters (Whatman GF/B), washed twice with ice-cold Tris-acetate buffer and then quantified by liquid scintillation spectrometry (Beckman LS 3801). Nonspecific binding was determined in the presence of 0.1 mM MK-801.

CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) binding assay was essentially the same as the MK-801 binding. The final tissue preparation was incubated with [^{3}H]CNQX (DuPont, 0.2 μCi, 7.5–207.5 nM). The incubation time was 1 h and nonspecific binding was determined in the presence of 0.1 mM DNQX (6,7-dinitroquinoxaline-2,3-dione) . For QNB (quinuclidinyl benzilate) binding, the final tissue preparation was resuspended in 40 vol. of 50 mM sodium potassium phosphate buffer, pH 7.4. Tissue (0.2 ml) was incubated with [^{3}H]QNB (Du Pont, 0.023–1.15 nM) in 1 ml of the same buffer at 25°C for 45 min. Nonspecific binding was determined in the presence of 0.1 mM atropine.

Uptake Assays.

Synaptosomal sodium-dependent glutamate and choline uptake was performed exactly as described previously (37) using the appropriate radioligands. Tissues were pooled, when necessary, from several mice for 1 independent determination of kinetic parameters using 5 substrate concentration points, 5 or 6 independent determinations per group. Crude synaptosomal preparations were obtained by homogenizing tissues in 10 vol. of 0.32 M sucrose solution and centrifuged at 1000 g for 10 min, the pellets were resuspended in 0.32 M sucrose

TABLE 1

BODY WEIGHT AND CONDUCTIVITY, AND FOOD AND WATER INTAKE OF CONTROL AND MSG-TREATED MICE AT THREE DIFFERENT AGES

	4-mo-old		6-mo-old		12-mo-old	
	Control	MSG	Control	MSG	Control	MSG
Body weight	35 ± 1	41 ± 1	37 ± 1	45 ± 1	39 ± 1	47 ± 1
Body conductivity	39 ± 2	24 ± 2	41 ± 2	28 ± 3	50 ± 2	35 ± 2
Food intake	4.2 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.6 ± 0.2	4.0 ± 0.1	3.8 ± 0.2
Water intake	6.1 ± 0.2	4.0 ± 0.3	4.5 ± 0.2	3.7 ± 0.1	5.7 ± 0.1	4.6 ± 0.1

Data are expressed as mean ± SEM of 24 mice. Body weight is in g, conductivity is in arbitrary unit. Food and water intake is in g/mouse/day. ANOVA (repeated measures): Body weight—$F(1, 41) = 33.52$, $P < 0.001$, for treatment; $F(2, 82) = 11.44$, $P < 0.001$, for age; $F(2, 82) = 0.23$, $P > 0.7$, for treatment by age. Body conductivity—$F(1, 41) = 51.43$, $P < 0.001$, for treatment; $F(2, 82) = 20.34$, $P < 0.001$, for age; $F(2, 82) = 0.11$, $P > 0.8$, for treatment by age. Food intake—$F(1, 85) = 1.37$, $P > 0.2$, for treatment; $F(2, 172) = 11.36$, $P < 0.001$, for age; $F(2, 172) = 5.03$, $P < 0.01$, for treatment by age. Water intake—$F(1, 85) = 22.11$, $P < 0.001$, for treatment; $F(2, 172) = 39.49$, $P < 0.001$, for age; $F(2, 172) = 1.87$, $P > 0.1$, for treatment by age. Daily variations of food and water intake during the observation periods were insignificant.

(30–40 ml/g original tissue). This fraction (0.1 ml containing approx. 0.12 mg protein) was preincubated (5 min, 37°C) with 1 ml of a Krebs-Ringer phosphate buffer (sodium phosphate, 16.2 mM; NaCl, 118; KCl, 4.7; $CaCl_2$, 1.8; $MgSO_4$, 1.2; d-glucose, 5.8; pH 7.4; saturated with pure oxygen) or of a sodium-free buffer (as above with sodium phosphate and NaCl replaced by potassium phosphate and choline chloride, respectively). Uptake was started by the addition of 0.1 ml [^{3}H]choline (0.5 μCi, 0.1-5 μM) or [^{3}H]glutamate (0.1 μCi, 0.5-20 μM). After 10 min, incubation was terminated by filtration (0.45 fm pore size cellulose nitrate filter) and the filters were washed with ice-cold physiological saline. Radioactivity retained on filters was determined by liquid scintillation spectrometry.

Protein Assays.

Protein was assayed according to Lowry et al. (20) using the Folin-Ciocalteus phenol reagent with bovine serum albumin as standard

Statistics

All statistical analyses were performed by either SPSSPC (water escape latencies) or SPSS for Windows (neurochemical parameters).

RESULTS

The body weight of the MSG-treated mice was significantly greater than that of the control mice by 17, 22 and 21%, respectively, at 4, 6 and 12 mo of age, $F(1, 41) = 33.52$, $P < 0.001$ using ANOVA with repeated measures (Table 1). Body weight also varied significantly with age [$F(2, 82) = 11.44$, $P < 0.001$]. In contrast, their body conductivity was markedly lower (30–38%) at the three ages, $F(1, 41) = 51.43$, $P < 0.001$ for treatment and $F(2, 82) = 20.34$, $P < 0.001$ for age. Food intake varied significantly only by age [$F(2, 172) = 11.36$, $P < 0.001$] but not by treatment [$F(1, 85) = 1.37$, $P > 0.2$]. However, treatment by age interaction was significant [$F(2, 172) = 5.03$, $P < 0.01$] indicating that the treatment effect differed at different ages. Analysis of the data separately showed that food intake was significantly increased only at 6 mo of age [$F(1, 107) = 12.29$, $P < 0.002$]. On the other hand, water intake was consistently reduced (18–34%) at all three ages, $F(1, 85) = 22.11$, $P < 0.001$ for treatment and $F(2, 172) = 39.49$, $P < 0.001$ for age with insignificant interaction. In both food and water intake measurements, the daily variations during the observation periods were insignificant.

Results of Experiment 1 were presented in Fig. 1. The MSG-treated and control mice started at the same level of perfor-

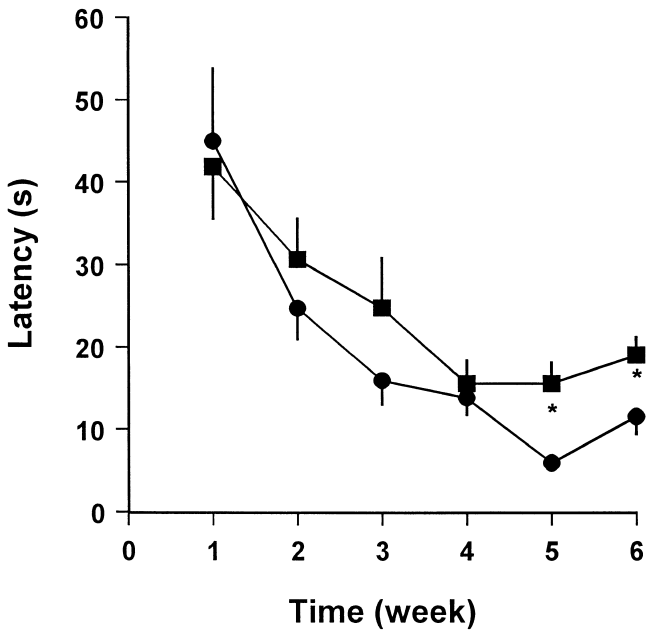

FIG. 1 .Water escape latencies for control (●) and MSG-treated (■) mice for a 6-week period. Analysis by ANOVA with repeated measures: $F(1, 46) = 4.72$, $P < 0.05$, for main treatment effect; $F(5, 230) = 14.21$, $P < 0.001$, for main time effect; $F(5, 230) = 0.59$, $P > 0.05$, for treatment by time interaction. Follow-up analysis showed significant time effect for both control [$F(5, 115) = 10.26$, $P < 0.001$] and MSG-treated [$F(5, 115) = 4.91$, $P < 0.001$] mice. *Significant difference from control mice by two-tailed t-test in week 5 ($t = 3.52$, $P < 0.005$, df = 26.63 adjusted due to unequal variance) and week 6 ($t = 2.27$, $P < 0.05$, df = 46). These remained significant after applying the Bonferroni-Holm correction for multiple comparison.

TABLE 2

THE MEAN WATER ESCAPE LATENCIES (s) OF CONTROL AND MSG-TREATED MICE OVER A 7-WEEK PERIOD WITH d-CYCLOSERINE (DCS) ADMINISTRATION

	Without DCS	With DCS
Control mice	10 ± 1	13 ± 1
MSG-treated mice	18 ± 1	19 ± 3

ANOVA: $F(1, 44) = 13.61$, $P < 0.001$ for main (MSG) treatment effect, but insignificant DCS effect [$F(1, 44) = 1.13$] and interaction [$F(1, 44) = 0.22$].

mance in the water escape task and improved in subsequent weeks. Analysis by ANOVA showed significant treatment effect [$F(1, 46) = 4.72$, $P < 0.05$] and time effect [$F(5, 230) = 14.21$, $P < 0.001$], but insignificant interaction. Follow-up analysis showed significant time effect for both control [$F(5, 115) = 10.26$, $P < 0.001$] and MSG-treated [$F(5, 115) = 4.91$, $P < 0.001$] mice. Significant difference in the latencies between the control and treatment groups were shown only in weeks 5 and 6 where $t = 3.52$, $P < 0.005$ and $t = 2.27$, $P < 0.05$, respectively. The MSG-treated mice had a mean latency time of 17 ± 2 s over weeks 5 and 6 as compared to 9 ± 3 s for the control mice.

Results of Experiment 2 were presented in Table 2. DCS treatment did not affect the latency times of both the control and MSG-treated mice. The latency times obtained during this period also did not differ from the pre-DCS treatment latency. Therefore, chronic DCS treatment had no apparent effects on the water escape performance of both control and MSG-treated mice. Consistent with results in Experiment 1, the effect of MSG-treatment was significant, $F(1, 44) = 13.61$, $P < 0.001$.

Table 3 showed the results of Experiment 3. As in experiment 2, DCS showed no significant effects on the water escape latencies of both control and MSG-treated mice after scopolamine treatment. Therefore, the data obtained in the presence and absence of DCS were pooled together. Data analysis by ANOVA (repeated measures) showed a significant scopolamine (2 mg/kg, 30 min before the water escape task) effect [$F(1, 41) = 5.76$, $P < 0.05$] due to the increase in latency from 11 ± 1 to 19 ± 3 s in the control mice. In contrast, the MSG-treated mice were not affected by scopolamine. The effect of MSG-treatment was insignificant while interaction of the two treatments was significant [$F(1, 41) = 4.74$, $P < 0.05$], possibly reflecting the difference between the pre-scopolamine latencies for control and MSG-treated mice.

Data presented in Table 4 showed that both the K_t and V_{max} of choline uptake in the hippocampus of MSG-treated mice (2.6 ± 0.5 μM and 1.1 ± 0.3 nmol/mg protein/h, respectively) were increased significantly as compared to control mice (5.1 ± 0.8 μM and 2.6 ± 0.4 nmol/mg protein/h, respectively), $t = 2.92$ and 3.04 respectively, $P < 0.02$. The choline uptake in the cerebral coxtex and striatum (data not presented) were not changed. [^{3}H]QNB binding to muscarinic receptors, [^{3}H]MK-801 binding to NMDA receptors, [^{3}H]CNQX binding to non-NMDA glutamate receptors and the sodium-dependent glutamate uptake were all unchanged between control and MSG-treated mice in the three brain regions.

TABLE 3

THE EFFECTS OF SCOPOLAMINE (2 mg/kg) ON THE WATER ESCAPE LATENCY (s)

	Pre-scopolamine	Post-scopolamine
Control mice	11 ± 1	19 ± 3
MSG-treated mice	18 ± 2	18 ± 3

Pre-scopolamine latency was obtained the week before the scopolamine experiment. ANOVA with repeated measures: $F(1, 41) = 5.76$, $P < 0.05$, for scopolamine treatment effect; $F(1, 41) = 4.74$, $P < 0.05$, for interaction, but insignificant MSG effect [$F(1, 41) = 1.92$].

TABLE 4

GLUTAMATE AND MUSCARINIC RECEPTOR BINDING, AND GLUTAMATE AND CHOLINE UPTAKE IN THE HIPPOCAMPUS OF CONTROL AND MSG-TREATED MICE

		Control	MSG-treated
[^{3}H]MK-801 binding	K_d	78 ± 28	68 ± 20
(NMDA)	B_{max}	10 ± 4	9 ± 3
[^{3}H]CNQX binding	K_d	92 ± 5	136 ± 38
(non-NMDA)	B_{max}	7 ± 1	6 ± 2
[^{3}H]QNB binding	K_d	0.15 ± 0.04	0.11 ± 0.03
(muscarinic)	B_{max}	0.7 ± 0.1	0.8 ± 0.1
[^{3}H]Glutamate	K_t	5 ± 1	9 ± 2
uptake	V_{max}	29 ± 4	33 ± 4
[^{3}H]Choline	K_t	2.6 ± 0.5	5.1 ± 0.8*
uptake	V_{max}	1.1 ± 0.3	2.6 ± 0.4**

K_d and B_{max} of the receptor bindings are expressed in nM and pmol/mg protein, respectively. K_t and V_{max} of the transport processes are expressed in μM and nmol/mg protein/h, respectively. Data are mean ± SEM of 5 or 6 independent determinations. Significantly different from control by two-tailed *t*-test, *$t = 2.92$, df = 9, $P < 0.02$; **$t = 3.04$, df = 9, $P < 0.02$.

DISCUSSION

The MSG-treated mice were significantly heavier than controls by 4 mo of age, and this persisted until 1 year of age. However, their body conductivity, which is a measure of the lean mass, was significantly reduced despite the greater total body mass. This indicated that the increase in body weight was due entirely to an increase in fat content. Their food intake was observed to be the same as control mice except at 6 mo of age when it was apparently higher. Since the heavier body weight was well established by the age of 4 mo, the increase in food intake at 6 mo was not a cause of the increased body weight. In contrast, water intake of the MSG-treated mice was significantly lower than that of the control mice consistently. It has been reported that a single neonatal injection of MSG caused hypophagia and hypodipsia in the subsequently obese mice and these effects have been attributed to damages in the arcuate nucleus (7). The present results failed to demonstrate any hypophagia but confirmed the hypodipsic effect of neonatal MSG-treatment.

The MSG-treated mice failed to attain the same level of competence as the control mice in the water escape task (Fig. 1). This lower level of competence was unlikely to be due to physical defects because there was no evidence of any slowness in movement in the water or any difficulty in mounting the target platform despite their obesity. Preliminary study showed that the swim speed of control and MSG-treated mice did not differ significantly at 0.38 ± 0.03 and 0.33 ± 0.02 m/s, respectively, when swimming in a straight path. In support, Fisher et al. (12) have previously reported water maze perfor-

mance deficit in MSG-treated rats which did not show any obesity. This deficit was ameliorated by enriched housing condition presumably due to increased sensory experience associated with enriched housing.

Fisher et al. (12) further attributed this deficit in water maze performance to MSG toxicity on the developing hippocampus since place navigation as required in the standard spatial version of the Morris water maze (23) appears to be highly sensitive to hippocampal damage (25). The task performed in the present study involved finding a centrally located hidden platform with a constant starting point. Thus this task, unlike the Morris maze, can be solved without using spatial information but simply by learning to use the same response on every trial. It has been reported (10) that rats lesioned in the fornix to impair hippocampal function were capable of learning to find a hidden platform under the constant-start condition. The mean escape latency for these fornix-lesioned rats was nonetheless significantly longer than that of the control. Under the novel-start condition, however, fornix-lesioned rats were markedly impaired whereas control rats were not. Similar findings have been reported (29) for DBA/2 mice which are an inbred strain impaired on hippocampal-dependent tasks. Thus, the MSG-treated mice appeared to show learning and memory deficits possibly due to impairment of hippocampal function.

There may be other contributing factors that need to be considered. Firstly, neonatal MSG-treatment affects the development of the retina leading to abnormal electroretinogram (31) and deficits in similtaneous brightness discrimination (2). If the distal vision of these mice were affected, it is likely to impair their performance in the water escape task. However, it has been shown that such MSG-treatment did not impair simple visual discrimination (2). Secondly, if MSG-treated mice had increased levels of anxiety, they would be more inhibited to swim to the center of the pool as it is anxiety provoking. Thirdly, these mice might be, for some reason, not as motivated to escape from the water. Our observations suggest that increased anxiety and lack of motivation are unlikely to be important as MSG-treated mice were not seen to stay close to the walls and they swam continuously once they were in the water. In support, the mean escape latencies obtained in the first week did not differ significantly between the control and MSG-treated mice (Fig. 1).

Cholinergic receptor blockade by muscarinic antagonists impaired place learning in the Morris water maze task (34,35). It has also been reported that these drugs impaired cued learning in water escape task (30), suggesting that the central cholinergic mechanism is involved in simple, non-spatial learning processes as well as spatial learning. Consistently, the present results showed that scopolamine significantly increased the escape latency of the control mice. It was surprising that scopolamine had no effect on the water escape performance of the MSG-treated mice. No effect was observed even when the scopolamine dose was increased from 2 to 5 mg/kg. In addition, scopolamine increased the latency of the control mice to the same level as that of the MSG-treated mice. One interpretation of these results is that the water escape performance deficit in the MSG-treated mice was a result of impaired central cholinergic mechanisms. As such, scopolamine was unable to further incapacitate an already impaired cholinergic system. This is strongly suppported by the observed changes in the cholinergic parameters in the hippocampus. The choline transport process was found to have shifted to a lower affinity state with a corresponding increase in the uptake capacity. As the sodium-dependent high-affinity choline uptake (HACU) into nerve endings is rate-limiting in the synthesis of acetylcholine (ACh) (16), a decrease in the high-affinity uptake would lead to a decrease in ACh synthesis. These may represent a significant reduction in cholinergic actions in the hippocampus. Interestingly, hippocampal HACU has been reported (8) to decrease after 4 days of place-training but not cue-training in the water maze. This decrease occurred during the acquisition phase of learning and was related to the rate of acquisition for individual animals, strongly suggesting the existence of a functional link between HACU and the water maze task performance.

In addition to the cholinergic receptors, the NMDA receptors were implicated in place learning as the antagonist AP5 was found to cause selective impairment of place learning in the Morris water maze (24). Conversely, DCS, which is a partial agonist at the glycine site of the NMDA receptor, was found to enhance performance (22) as well as to attenuate scopolamine-induced deficits (13) in rats. However, the present results showed that DCS neither enhanced the water escape performance in control mice nor ameliorated the deficit in MSG-treated mice (Table 2). This is supported by the lack of change in the glutamatergic parameters in the hippocampus (Table 4) as well as the cerebral cortex. Also, DCS did not alter the deleterious effect of scopolamine in control mice. This observed lack of effect of DCS, contrary to its reported effects on place learning in rats (22), is not readily explanable but is unlikely to be due to insufficient dosage. In fact, the DCS dose of 50 mg/kg used was higher than those employed previously (13,22). One possible reason for the discrepancy is that there is a species difference between rat and mouse. On the other hand, this lack of effect of DCS may imply that the NMDA receptors are not involved in non-spatial learning, in contrast to their involvement in spatial learning (22).

In conclusion, mice neonatally treated with MSG showed considerable learning and memory deficit in a water escape task. This deficit may be due to altered cholinerigic mechanisms in the hippocampus.

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REFERENCES

1. Abe, K.; Xie, K.; Watanabe, Y.; Saito, H. Glycine facilitates induction of long term potentiation in rat hippocampus. Neurosci. Lett. 117:87–92; 1990.
2. Berry, H. K.; Butcher, R. E.; Elliot, L. A.; Brunnerm R. L. The effect of monosodium glutamate on the early biochemicaland behavioral development of the rat. Dev. Psychobiol. 7:165–173; 1974.
3. Brankack, J.; Klingberg, F. Permanent changes of the visually evoked responses in the superior colliculus after early postnatal treatment with monosodium-L-glutamate. Biomed. Biochim. Acta 49:481–487; 1990.
4. Burde, R. M.; Scheinke, B.; Kayes, J. Acute effects of oral and subcutaneous administration of monosodium glutamate on the arcuate nucleus of the hypothalamus in mice and rat. Nature (Lond.) 233:58–60; 1971.
5. Dawson, R. Jr. Developmental and sex-specific effects of low dose neonatal monosodium glutamate administration on mediobasal hypothalamic chemistry. Neuroendocrinology 42:158–166; 1986.

6. Dawson, R. Jr.; Annau, Z. A behavioral assessment of arcuate nucleus damage after a single injection of monosodium glutamate. Neuorbehav. Toxicol. Teratol. 5:399–406; 1985.
7. Dawson, R. Jr.; Wallace, D. R.; Gabriel, S. M. A pharmacological analysis of food intake regulation in rats treated neonatally with monosodium glutamate (MSG). Pharmacol. Biochem. Behav. 32:391–398; 1989.
8. Decker, M. W.; Pelleymounter, M. A.; Gallagher, M. Effects of training on a spatial memory task on high affinity choline uptake in hippocampus and cortex in young adult and aged rats. J. Neurosci. 8:90–99; 1988.
9. Desjardins, G. C.; Brawer, J. R.; Beaudet, A. Monosodium glutamate-induced reductions in hypothalamic beta-endorphin content result in mu-opioid receptor up-regulation in the medial preoptic area. Neuroendocrinology 56:378–384; 1992.
10. Eichenbaum, H.; Stewart, C.; Morris, R. G. M. Hippocampal representation in place learning. J. Neurosci. 10:3531–3542; 1990.
11. Fibiger, H. C. Cholinergic mechanisms in learning, memory and dementia: A review of recent evidence. Trends Neurosci. 14:220–223; 1991.
12. Fisher, K. N.; Turner, R. A.; Pineault, G.; Kleim, J.; Saari, M. J. The postweaning housing environment determines expression of learning deficit associated with neonatal monosodium glutamate (M.S.G.). Neurotoxicol. Teratol. 13:507–513; 1991.
13. Fishkin, R. J.; Ince, E. S.; Carlezon, W. A. Jr.; Dunn, R. W. d-Cycloserine attenuates scopolamine-induced learning and memory deficits in rats. Behav. Neural Biol. 59:150–157; 1993.
14. Izquierdo, I. Role of NMDA receptors in memory. Trends Pharmacol. Sci. 12:128–129; 1991.
15. Jessop, D. S.; Chowdrey, H. S.; Biswas, S.; Lightman, S. L. Substance P and substance K in the rat hypothalamus following monosodium glutamate lesions of the arcuate nucleus. Neuropeptides 18:165–170; 1990.
16. Jope, R. S. High affinity choline transport and acetylcoA production in brain and their roles in the regulation of acetylcholine synthesis. Brain Res. Rev. 1:313–344; 1979.
17. Klingberg, H.; Brankack, J.; Klingberg, F. Long-term effects on behaviour after postnatal treatment with monosodium-L-glutamate. Biomed. Biochim. Acta 46:705–711; 1987.
18. Lehmann, A.; Jonsson, T. MK-801 selectively protects mouse arcuate neurons in vivo against glutamate toxicity. Neuroreport 3:421–424; 1992.
19. Lorden, J. F.; Caudle, A. Behavioral and endocrinological effects of single injections of monosodium glutamate in the mouse. Neuorbehav. Toxicol. Teratol. 8:509–519; 1986.
20. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
21. Madison, D. V.; Malenka, R. C.; Nicoll, R. A. Mechanisms underlying long-term potentiation of synaptic transmissions. Ann. Rev. Neurosci. 14:379–397; 1991.
22. Monahan, J. B.; Handelmann, G. E.; Wood, W. F.; Cordi, A. A. d-Cycloserine, a positive modulator of the N-methyl-D-aspartate receptor, enhances performance of learning tasks in rats. Pharmacol. Biochem. Behav. 34:649–653; 1989.
23. Morris, R. G. M. Spatial localization does not require the presence of local cues. Learn & Motiv. 12:239–260; 1981.
24. Morris, R. G. M.; Anderson, E.; Lynch, G. S.; Baudry, M. Selective impairment of learning and blockade of long-term potentiation by and N-methyl-D-aspartate receptor antagonist, AP5. Nature (Lond.) 319:774–776; 1986.
25. Morris, R. G. M.; Garrud, P.; Rawlins, J. N. P.; O Keefe, J. Place navigation impaired in rats with hippocampal lesions. Nature (Lond.) 297:681–683; 1982.
26. Oliver, M. W.; Kessler, M.; Larson, J.; Schottler, F.; Lynch, G. Glycine site associated with the NMDA receptor modulates long-term potentiation. Synapse 5:265–270, 1990.
27. Olney, J. W. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science 164:719–721; 1969.
28. Olney, J. W. Brain damage and oral intake of certain amino acids. Adv. Exp. Med. Biol. 69:497–506; 1976.
29. Paylor, R.; Baskall, L.; Wehner, J. M. Behavioral dissociations between C57BL/6 and DBA/2 mice on learning and memory tasks: A hippocampal-dysfunction hypothesis. Psychobiology 21: 11–26; 1993.
30. Paylor, R.; Rudy, J. W. Cholinergic receptor blockade can impair the rat s performance on both the place learning and cued versions of the Morris water task: The role of age and pool wall brightness. Behav. Brain Res. 36:79–90; 1990.
31. Potts, A .M.; Modrell, R. W.; Kingbury, C. Permanent fractionation of the electroretinogram by sodium glutamate. Am. J. Ophth. 50:900–907; 1960.
32. Ransom, R. W.; Eng, W.; Burns, H. D.; Gibson, R. E.; Solomon, H. F. (+)-3-[123I]Iodo-MK-801: Synthesis and characterization of binding to the N-methyl-D-aspartate receptor complex. Life Sci. 46:1103–1110; 1990.
33. Stoeckel, M. E.; Tappaz, M.; Hindelang, C.; Seweryn, C.; Porte, A. Opposite effects of monosodium glutamate on the dopaminergic and GABAergic innervations of the median eminence and the intermediate lobe in the mouse. Neurosci. Lett. 56:249–255; 1985.
34. Sutherland, R. J.; Whishaw, I. Q.; Regehr, J. C. Cholinergic receptor blockade impairs spatial localization by use of distal cues in the rat. J. Comp. Physiol. Psychol. 96:563–573; 1982.
35. Whishaw, I. Q. Cholinergic receptor blockade in the rat impairs locale but not taxon strategies for place navigation in a swimming pool. Behav. Neurosci. 99:979–1005; 1985.
36. Whishaw, I. Q.; Auer, R. N. Immediate and long-lasting effects of MK-801 on motor activity, spatial navigation in a swimming pool and EEG in the rat. Psychopharmacology 98:500–507; 1989.
37. Wong, P. T. -H.; Teo, W. L. The effect of phenytoin on glutamate and GABA transport. Neurochem. Res. 11:1379–1382, 1986.
38. Xue, Y.-D.; Wong, P. T.-H.; Leong, S.-K. Nitric oxide synthase, NMDA receptor, glutamate and aspartate immunoreactive neurons in the mouse arcuate nucleus: Effects of neonatal treatment with monosodium glutamate. (submitted).