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The effect of glial glutamine synthetase inhibition on recognition and temporal memories in the rat

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HIGHLIGHTS

- MSO, a glutamine synthetase inhibitor, induced temporal memory deficit.
- However, it did not alter spatial recognition memory.
- This suggests that glia also modulates some aspects of mnemonic processes.

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ABSTRACT

The glutamate neurotransmitter is intrinsically involved in learning and memory. Glial glutamine synthetase enzyme synthesizes glutamine, which helps maintain the optimal neuronal glutamate level. However, the role of glutamine synthetase in learning and memory remains unclear. Using associative trace learning task, we investigated the effects of methionine sulfoximine (MSO) (glutamine synthetase inhibitor) on recognition and temporal memories. MSO and vehicle were injected (i.p.) three hours before training in separate groups of male Wistar rats ($n = 11$). Animals were trained to obtain fruit juice after following a set of sequential events. Initially, house-light was presented for 15 s followed by 5 s trace interval. Thereafter, juice was given for 20 s followed by 20 s inter-presentation interval. A total of 75 presentations were made over five sessions during the training and testing periods. The average number of head entries to obtain juice per session and during individual phases at different time intervals was accounted as an outcome measure of recognition and temporal memories. The total head entries in MSO and vehicle treated animals were comparable on training and testing days. However, it was 174.90% ($p = 0.08$), 270.61% ($p < 0.05$), 143.20% ($p < 0.05$) more on training day and 270.33% ($p < 0.05$), 157.94% ($p < 0.05$), 170.42% ($p < 0.05$) more on testing day, during the house-light, trace-interval and inter-presentation interval phases in MSO animals. Glutamine synthetase inhibition did not induce recognition memory deficit, while temporal memory was altered, suggesting that glutamine synthetase modulates some aspects of mnemonic processes.

1. Introduction

The glutamate neurotransmitter is involved in various neural processes including learning and memory [14,19]. Neuronal plasticity and augmented synaptic strength provide the cellular/molecular substrates for learning and memory [9]. It has been found that glutamatergic receptors are selectively upregulated in those synapses which obtain synaptic strength in response to correlated pre- and postsynaptic activity, a phenomena strongly associated with learning and memory [13]. Further, inhibition of glutamatergic receptors induces acquisition and retention memory deficit [16,19]. For example, blocking NMDA receptors in the amygdala impairs the consolidation of fear-conditioned memory [26,31], avoidance learning [7,8] as well as acquisition of olfactory discrimination tasks [24]. Similarly, infusion of AMPA receptor antagonist into the hippocampus during the acquisition period of the water maze task induces learning deficits [12,20]. These reports demonstrate that glutamate neurotransmitter and its receptors play an important role in encoding, consolidation and retrieval of different memory types.

Normally, a low level of glutamate is maintained in the extracellular compartment for an optimum signal-to-noise ratio in synaptic neurotransmission, which is primarily achieved through an interactive mechanism between neurons and glia [10,11]. Glutamate

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is removed from the synaptic cleft through glutamate exchangers and transporters, which are present on neurons and glia, respectively, but it is largely up-taken into glia by the glial glutamate transporter [21]. In the glia, up-taken glutamate is converted to glutamine by a glia specific enzyme glutamine synthetase. Glutamine is transported out from glia and is taken up by neurons, where it is hydrolyzed by glutaminase enzyme and converted back to glutamate. Glial glutamine thus plays an essential role for the maintenance of optimal glutamate level in the nerve terminal pool and remains a major glutamate source for neurons [10,21].

Altering brain glutamatergic neurotransmission and its receptor activity induces impairment in acquisition and performance of cognitive tasks [15]. Methionine sulfoximine (MSO) is a specific irreversible inhibitor of glial glutamine synthetase enzyme [22]. MSO injection lowers brain glutamine and glutamate level significantly [5] and has been used to study the effects of altered brain glutamate level under several experimental approaches including learning and memory [2,4–6]. For example, inhibiting glutamine synthetase enzyme activity with MSO prevents memory consolidation [6]. Interestingly, the amnesic effect of MSO was counteracted with further administration of glutamine and glutamate in the brain [6]. But, it has also been reported that chronic inhibition of glutamine synthetase did not impair a spatial learning task [2]. It could be possible that MSO may induce impairment of one memory type while other memories may remain intact. In this study, using an associative trace learning task [where subjects obtain skills about spatial recognition learning (to recognize the location of the stimuli) as well temporal learning (timing correlation between the presented paired stimuli)] [30], we found that MSO treated animals exhibited impairment in temporal memory, while, their recognition memory appeared unaltered.

2. Materials and methods

Male Wistar rats (250–300 g) were used in this study. Animals ($n = 11$) were obtained from the University's animal house facility. They were brought to our institutional animal house facility a week before the commencement of experiments. They were maintained on a 12:12 light–dark (L:D) cycle at 23–24 °C room temperature. Animals were given food and water ad libitum. All procedures were approved by the Institutional Animal Ethical Committee (IAEC) of Jawaharlal Nehru University, New Delhi, India.

2.1. Drug used

Three hours before the learning task on the training day, animals were injected 1 ml of either vehicle or MSO (Sigma–Aldrich, USA) intraperitoneally (i.p.). L-Methionine (500 mg/kg wt) was also injected along with MSO (72 mg/kg wt), as it has been used previously to prevent MSO induced seizures [28]. MSO was dissolved in sterile saline, while L-methionine was dissolved in 100 mM NaOH, which was further titrated to a pH 7.6 with HCl. A total 1 ml volume of L-methionine and MSO (8:1 ratio) were co-injected in the animals ($n = 5$) [28]. Similarly, in control animals ($n = 6$), 1 ml vehicle [NaOH (100 mM) titrated to a pH 7.6 with HCl and sterile saline (8:1 ratio)] was co-injected.

2.2. Associative-trace learning task

We used an associative learning task where animals learn about the timing between the different stimuli/cue presented (temporal memory) as well as the location where the stimuli are presented (spatial recognition memory) [30]. Animals were trained in a behavior chamber ($12'' \times 12'' \times 11''$), where light was used as a cue predictor for the fruit juice. During the training and testing periods, the fruit juice was given to the animal through a computer controlled liquid dispensing unit (Coulbourn Inc., USA). In the liquid dispensing unit, a liquid dipper, which was attached with a motorized lever lifted the juice from the juice tank to the juice dispensing chamber. The juice filled (100 μl) raised dipper was accessible to the animal through a small window of the behavior chamber, where the photo-beams were also installed. The total number of head entries into the liquid dispensing chamber to obtain fruit juice was registered by the computer using Graphic State Software, (Coulbourn Inc., USA) with the help of photo-beams.

The animal was habituated in the chamber for two consecutive days for 30 min between 3:00 and 3:30 PM. During the first day of habituation, the animal was kept in the behavior chamber for 30 min, while on the second day, mango fruit juice was also given through the bottle to develop fondness for the juice. On Day 3, fruit juice was kept in the juice tank and was presented to the animal 5–6 times by raising the dipper (experimenter manually raised the lever). The experimenter also guided the animal four–five times toward the dispensing chamber. Prior exposure and fondness for the fruit juice helped the animal to approach the dispensing unit. These procedures were adopted to just demonstrate to the animal the location of the liquid dipper before training. On the training day (Day 4), animals were randomly divided into two groups: MSO and vehicle control group. MSO or vehicle was injected in the animal of the respective group three hours (at 12 PM) before the training started.

For training, the animal was kept in the behavioral chamber (at 3 PM) for 10 min. Thereafter, the house light and juice were presented in a sequential manner using Graphic State software (Coulbourn Inc., USA). A total of 75 presentations of the house light and juice were dispensed in a total of 5 sessions (15 presentations per session with 5 min inter-session interval) over a period of 1 h 45 min. The presentation started with house light (HL) phase, where the light was turned-on for 15 s only, followed by 5 s trace interval (TI) phase, when house light was off and no stimulus was given. Next was the fruit-juice presentation (FJP) phase, during which a juice filled dipper was automatically raised into the liquid dispensing chamber for 20 s. Finally, in a subsequent inter-presentation interval (IPI) phase, no stimulus was given for next 20 s. The house light and fruit juice were presented in this sequential manner 15 times in one session and each session was repeated five times with a 5 min inter-session interval. Next day (Day 5), the animal was tested for his performance at the time matched hour of the training period (3:00 to 4:45 PM). For testing, we followed the same protocol as was used during training. At the end of the sessions, the animal was left in the behavior chamber for additional 5 min and was then taken back to the animal facility (Supplementary Fig. 1).

The number of head entries into the juice-dispensing unit was accounted as an outcome measure of learning. To determine the changes in recognition memory, average head poking into the juice dispensing chamber during all the sessions on training and testing days in MSO and vehicle treated animals were compared statistically. For studying the effect on temporal memory, the changes in the average head entries during HL, TI, FJP and IPI phases of all sessions on training and testing days between MSO and vehicle groups were statistically compared.

2.3. Data analysis

The changes in overall average head entries (recognition memory) and during different phases (temporal memory) on training and testing days were compared statistically between MSO and control groups using one-way repeated measure ANOVA (RM-ANOVA) followed by Tukey post hoc test. We also calculated the number of cumulative head entries for each phase in all sessions in control and MSO groups and compared them statistically (one-way