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Research Article

Memantıne Improves Learning In Kindled Rats

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Summary

Hippocampal kindling which have been used to investigate temporal lobe epilepsy can also be used to show the relation between hippocampus and learning procedures. In this study we aimed to investigate the effects of memantine, a non-selective NMDA receptor antagonist, by using water maze parameters in kindled rats. Adult male Wistar rats were used in 5 experimental groups (n=8); Control group (C); Sham group (had a hippocampal electrode but have no kindling stimulus) (S); Memantine treated group (M); Memantine treated group with kindling stimulus (MKI) and Kindling stimulated group (KI). After memantine treatments and kindling protocols, an acquisition period of 12 day water maze protocol was done in which all groups performances show a decline of escape latency (EL) and path length (PL) which indicates that they were able to learn the platform location in a spatial way. EL performance of M, KI, MKI groups were better than the C group, also the PL performance of M, KI, MKI groups were better than the C group. After day 9 the difference between the groups were disappeared. 13th choice day performance which indicates spatial memory of C, M, MKI groups were not statistically different from each other but for the KI group 13th day EL and PL performances were worse than the others.
Our findings indicates that modifying the neuronal plasticity in kindled rats produced a profound decrease in learning and memory. Memantine improves the changing parameters of WM learning model in kindling.

Key words: Kindling, rat, seizure, memory, water maze, epilepsy

Memantin'in 'Kindled' Sıçanlarda Öğrenme Üzerine Olumlu Etkisi

Özet

Temporal lob epilepsy modeli olan hippokampal kindling modeli aynı zamanda hipokampus ile öğrenme süreçlerinin incelenmesinde de kullanılmaktadır. Biz bu çalışmamızda kindled yapılmış sıçanlarada su tankı parametrelerinini kullanarak selektif olmayan NMDA antagonisti memantinin etkilerini incelemeyi amaçladık. Erişkin wistar türü sıçanlar, her grupta 8 hayvan olacak şekilde, 5 gruba ayrıldı; Kontrol grubu (K); Sham grubu (hippocampal elektrod takıldı ancak kindling uyarısı almadı)(S); Memantine uygulanan grup (M); Memantine uygulanan ve kindling uyarısı alan grup (MKI) ve Kindling grubu (KI). Memantine tedavileri ve kindling protokollerinin tamamlanmasının ardından 12 günlük su tankı denemelerine geçildi bu denemelerde tüm grupların kaçış zamanı (escape latency (EL)) and kaçış mesafesi (path length (PL)) performanslarında platform yerini öğrendiklerini

gösteren azalma izlendi. M,KI ve MIK gruplarının EL ve PL performansları K grubundan anlamlı düzeyde farklıydı. 9. Günden itibaren gruplar arasındaki farklılık ortadan kalkmaktaydı. Karar günü olarak belirlenen 13. üncü gün K, M, MKI arsında anlamlı faklılık yokken, KI grubunun EL ve PL performansı bozulmuştu.
Sonuçlarımız kindling protokolü ile değiştirilen nöronal plastisitenin öğrenme ve bellek üzerine etkisini irdelerken, memantinin su tankı parametrelerinde olumlu değişiklik yaptığı gözlenmiştir.

Anahtar Kelimeler: Kindling, sıçan, nöbet, öğrenme ve bellek, su tankı, epilepsi

INTRODUCTION

Hippocampal kindling which have been used most extensively to investigate temporal lobe epilepsy can also be used to show the relation between hippocampus and learning procedures. There is increasing evidence that neurogenesis and synaptogenesis can appear not only in the mossy fiber pathway in the hippocampus but also in other limbic structures[25].

Memantine is a non-selective NMDA antagonist but it also activates the receptors under certain conditions so it has dual effects on NMDA receptors[2,8,36]. It is one of the major drug for dementia therapy. Not only the the positive effects of memantine on cognition but also neuroprotective effects are verified by many studies[2,7,33,35,36]. It has been shown that in water maze (WM) model of learning memantine improved acquisition performance (path length) and spatial accurancy during probe trial in a dose dependent manner and also increased the durability of synaptic plasticity[20,33,35].

Kindled seizures produced a profound decrease in learning and memory accompanied by a selective and long-lasting decrease in hippocampal and striatal concentration of glutamate, glycine and alanine in the striatum[6]. There are very few studies regarding with the effects of memantine on kindling procedures. Memantine induces epileptiform discharges in high doses (20 mg/kg) and kindled rats are more sensitive to central nervous system stimulating effects of memantine than non-kindled rats, which could relate to an impairment of inhibitory processes and/or alterations in synaptic transmission mediated by excitatory amino acids in the kindled brain[1,2,3,4].

In this study we aimed to further investigate the effects of memantine on learning and memory by using Morris water maze parameters in kindled rats.

MATERIAL AND METHODS

Animals and experimental conditions

Male Wistar rats, (200-250 g), were used throughout the experiments after at least one week of acclimatization. They were housed in plastic cages under standard laboratory conditions (ambient temperature of 22 ± 1 °C, 12 hour light-dark cycle). Chow pellets and tap water was freely available. All experiments were done at the same period of time of the day. (between 9.00 a.m. and 12.00 a.m.) to minimize the influence of circadian rhythms on learning.

Five experimental groups, each consisting 8 rats, were prepared for water maze procedure. The groups were as follows: 1-Control group (C); 2-Sham group, animals had a hippocampal electrode but have no kindling stimulus (S); 3-Memantine treated group, with no kindling stimulus (M); 4-Memantine treated group with kindling stimulus (MKI) and 5-Kindling stimulated group (KI). All animal care and experimental procedures were carried out in accordance with Ethical Committee of Pamukkale University Medical School and Turkish law on animal welfare.

Memantine treatment

Memantine groups (M and MKI) were treated by 1mg/kg/day i.p. for 7 days after

a 20 mg/kg bolus dosage. M group was taken to the water maze (WM) procedure directly without kindling stimulation. MKI group had the kindling stimulation after the memantine protocol and then were taken to the WM procedure.

Surgery and kindling procedure

The rats were anesthetized with ketamine (100 mg/kg i.p.) and Rhompun (50 mg/kg i.p.) and received stereotaxic implantation of one bipolar electrode in the right ventral hippocampal CA1 area. The AP-4.8, L-5.0, V-6.7 stereotaxic coordinates for electrode implantation were used according to the brain atlas of Paxinos and Watson 31. All coordinates were measured from bregma. The electrode assembly was attached to the scull by dental acrylic cement. Scull screws served as to fix the dental cement.

After a post-operative period of 7 days, the stimulation of hippocampus was initiated. The afterdischarges (AD) were defined as spikes with a frequency of at least 1 Hz and amplitude at least twice greater than the pre-stimulation baseline present in the EEG recorded from the site of stimulation. Afterdischarges threshold (ADT) was determined after a series of stimulations at intervals of 3 min increasing in steps of about 20% of the previously applied current until an AD duration of at least 3 sec was evoked. After ADT determination a standard stimulus consisted of a 1s train of 50 Hz, 1 ms biphasic square-wave pulses, with pulse amplitude of 500 μA, and was delivered every 24 h, until at least 5 sequential fully kindled stage 5 was elicited. The seizure severity was assessed according to the Racine's system. In total rats received 15 stimuli.

The Water Maze apparatus, data collection and protocol

A circular pool (150 cm diameter, 60 cm height) was filled to a depth of 45 cm with water, at 22 ^{o}C. The visible platform was constructed of plexiglas (12 cm × 12 cm) and protruded 1.5 cm above the surface of the water. The hidden platform was also plexiglas (12 cm×12 cm), painted black, and submerged 1.5 cm below water level. The maze was located in a 4m × 3m room and extra maze cues included posters on the walls. The maze was divided into four virtual quadrants, Northwest (NW), Northeast (NE), Southwest (SW) and Southeast (SE).

The experiments were recorded using a camera. The output of the camera was captured by the tracker (Noldus, Ethovision, NL) and analyzed with a PC using the Ethovision 3.1 software. The analyzed parameters for the rats were the escape latency to the platform (EL), the path length to reach the platform (PL) and the swim speed (V).

In a protocol that is modified from McDonald and White[19], the platform was in the same position (center of quadrant SE), either visible (days 1–4, 9,11) or hidden (days 5- 8, 10, 12) during the first 12 days and the rats were trained to find the platform. There were four trials each day with an intertrial interval of approximately 10 min for each rat. Each animal was handled each day before the first trial and then was released once from each of the four quadrants facing to the wall of the pool. The order of the release positions was varied systematically throughout the experiment as follows: day 1: NWES, day 2: WESN, day 3: ESNW, day 4: SNWE . . . , day 12: WESN, day 13: NWES. A trial ended when the rat climbed on the platform. If a rat had not found the platform after 60 s, it would be placed on the platform by the experimenter and left there for 15 s. On the 13th day, there were again four trials with the first-day release-position order (i.e. NWES), but the visible platform was transferred to another quadrant (NW). On this test day, time spent in the quadrant where the platform had been on days 1–12 (quadrant SE) was measured in addition to other parameters. This day is providing a choice for the rats between navigational cues (based on the

previous platform) and look-out cues (based on present platform location).

Data analysis

To analyze the performance of rats throughout acquisition, mean ELs and PLs to visible or hidden platform and Vs (average of four trials per day) were subjected to separate multifactorial ANOVAs. PostHoc Bonferronni tests were used for the difference between groups and days. On day 13, when the animals were presented with a choice, separate ANOVAs were performed for V, EL, and PL. The probability level interpreted as statistically significant was $P< 0.05$.

RESULTS

Repeated stimulation of rats of the kindling groups (KI and MKI) resulted in constantly increasing seizure severity and seizure duration and all the rats reached Racine's stage 5 at the end of the 15th stimulation.

All groups performances show a decline of EL and PL through the 12 days acquisition period which indicates that they were able to learn the platform location in a spatial way. Mean velocity of the groups were not statistically different which shows that the motor performances of the groups are the same [ANOVA, between factor, $F (44, 1680) = 1,194$, $P=0.18$]. There was not any statistical difference between the EL and PL's of sham and control groups. (data is not shown)

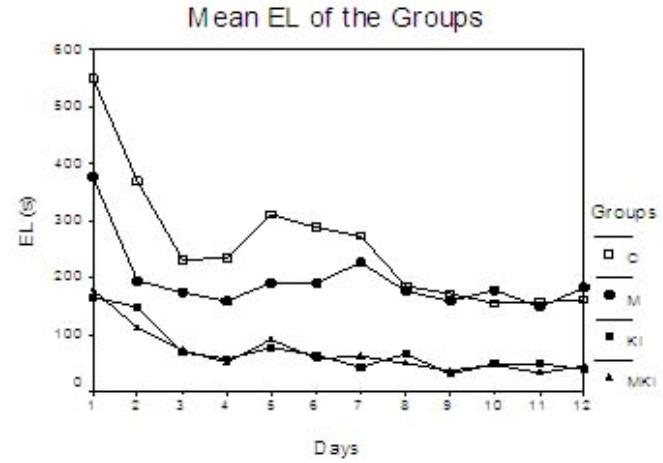
EL performance of M, KI, MKI groups were better than the C and S group [ANOVA, between factor, ($F (4, 1680) =105,989$, $p< 0.0001$)]. PL performance of M, KI, MKI groups were better than the C and S group, [ANOVA, between factor, ($F (4,1680) = 33.39$, $p< 0.0001$)]. After day 9 the differences between the groups were disappeared. (Figure 1)

13th choice day performance of C, S, M, MKI groups were not statistically different from each other but for the KI group 13th day EL and PL performances were worse than the others (39.29 ± 9.1, $p< 0.05$; 950.1 ± 237, $p< 0.05$ respectively) (Figure 2).

DISCUSSION

The hippocampus is known as a heterogeneous structure which has been suggested that it plays a critical role in both learning and memory and epileptogenezis. Hippocampal kindling (HK) which have been used as a most useful model of temporal lobe epilepsy can also be used to show the relation between hippocampus and learning procedures and represents not only an experimental model for the study of epilepsy but also epilepsy related secondary alterations on the behavioral level with special regard to memory deficits. Clinical findings have shown that different type of epileptic seizures resulted in different types of learning and/or memory impairments[5,15,30]. Correspondent with clinical findings, a close correlation between the method of kindling induction and the resulting deficit has been shown in previous studies such as amygdale kindling led to diminished brightness discrimination learning, and after chemical kindling using pentylenetetrazole shuttle-box performance in rats are worsened, also some authors suggest that epileptiform activity in the hippocampus acutely impairs performance in tasks sensitive to spatial learning and memory deficits and suggest that both new learning and demonstration of an established place response are susceptible to such disruption[4,14,23].

Subsequently some researchers showed that different parts of the hippocampus might have different learning and memory functions[3,13,21]. Mc Donald et all suggests a double dissociation of learning and memory function between the ventral and dorsal hippocampus. As they mentioned the formation of an incidental inhibitory association was dependent on ventral but not dorsal hippocampal circuitry, and the opposite dependence was found for the spatial component of a tactile / spatial discrimination[1,6,12,16,21,22,29].



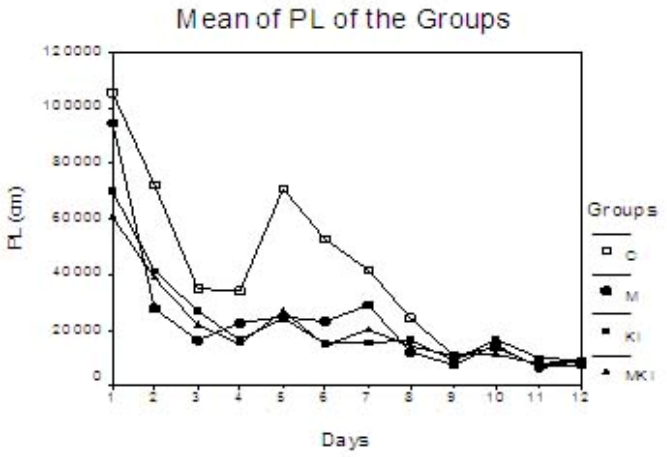
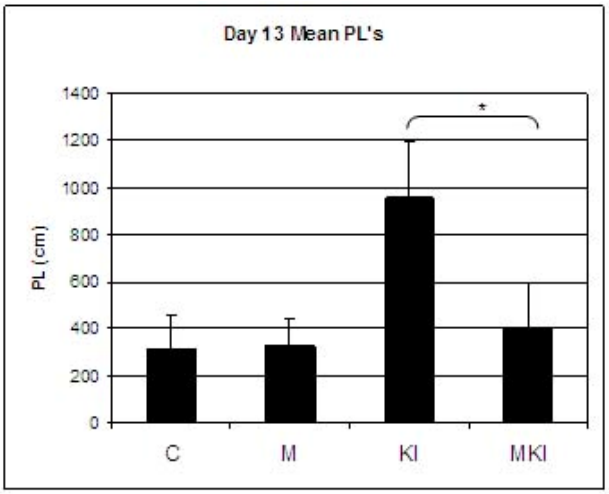


Figure 1: *The mean daily EL (escape latency), PL (path length) of the groups (control:C, memantine :M, Kindled: KI, kindled + memantine: MKI) respectively. In days 1-4, 9 and 11 the platform is visible, and in days 5-8, 10 and 12 the platform is hidden.*



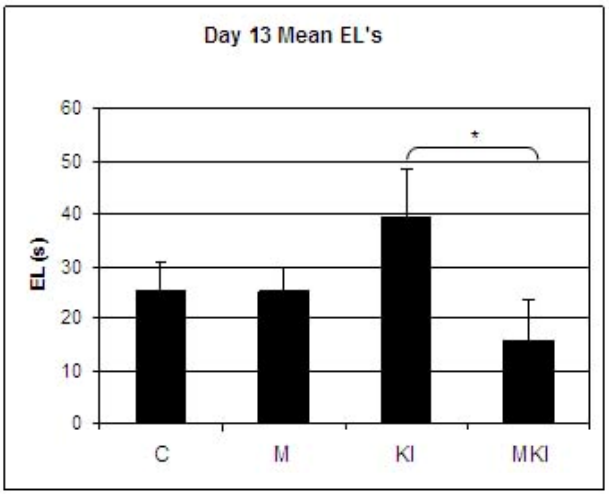


Figure 2: *Day 13 is the choice day of the animals. Memantine treated kindled group's (MKI) performance in both EL and PL is similar with the C and M groups. KI group has slower EL and longer PL than the other groups in 4 trials. The difference between KI and MKI groups is very important in terms of memantine's positive protective effect on "choice day". (* $p < 0.05$)*

Our study confirmed previous studies that, stimulating the ventral hippocampal neurons with under threshold stimuli improves performance since the first day of the study and the difference disappears from the 9th day. The main distinctive point from the other studies is the WM performance has been done with the kindled animals not shortly after the stimulus.

Most of the studies in the literature are acute models (1-2 h after or immediate kindling stimulations). We designed our WM trials 24 h after the last kindling stimulation (after the 15th stimulation) in order to see the late effects of kindling. So we concluded that time duration between the stage 5 seizure and WM trials is a very important factor for learning and memory function of kindling epilepsy model while commenting on WM learning parameters.

In many studies it has been shown that activation of NMDA receptors in hippocampus modulates and improves learning[18,28], NMDA receptor antagonists produce a number of different effects in vivo. Antagonists acting at distinct modulator sites of the NMDA receptor complex have different pharmacological and behavioral profiles[11,28]. Memantine, a non-selective NMDA receptor antagonist, has a dual affect on glutamergic neurons and besides this affect it also activates the NMDA receptors. Drever et al. investigated the actions of memantine on hippocampal function and signaling. They found out that in behavioral experiments using the water maze, memantine reversed scopolamine-induced learning deficits in mice and the data suggest actions of memantine beyond NMDA receptor antagonism, including stimulating effects on cholinergic signaling via muscarinic receptors[10]. Another study concluded that a stable dose of memantine improves cognition and exhibited a potential anxiolytic response in normal mice[24].

Place learning in a water maze (WM) is a widely used cognitive test in and can be modified to discriminate between different strategies employed to solve the problem rodents[9,17,26,27,32]. The WM studies reported that memantine has a cognitive improvement affect on different hippocampal pathologies[7,33,34]. Here in our study, the rat's ability to find the platform escape latency (EL) and path length (PL) was assessed for 12days, with the platform, always in the same place, but visible on days 1–4, 9 and 11, and hidden on the remaining days. The visible condition requires a “look-out” strategy for learning, whereas the hidden condition requires a spatial, “navigational” cognitive style. All of the rats acquired both the visible and hidden tasks similarly as mentioned before. After 12 days acquisition, on Day 13, the rats were challenged by a new cognitive task (platform position shifted and the platform made visible). Although only the platform in the new place provided escape, initially the rats did not have that information and therefore the first trial of the shift task can be assumed to present a choice for the rats. Here the C, M and MKI groups showed no difference but the KI group of rats had failed to find the new platform position. Memantine group has a better performance on EL and PL since the first day of the study. We emphasize that the difference between KI and MKI groups is very important in terms of memantine's positive effect on “choice day” inspite of the deleterious affect of kindling.

Our findings indicates that memantine has an evident effect on learning and improves the parameters of WM learning model in kindled rats. Further studies are needed to support this hypothesis.

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