

Use of Colchicine in Cortical Area 1 of the Hippocampus Impairs Transmission of Non-Motivational Information by the Pyramidal Cells

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ABSTRACT

Colchicine, a potent neurotoxin derived from plants, has been recently introduced as a degenerative toxin of small pyramidal cells in the cortical area 1 of the hippocampus (CA1). In this study, the effect of the alkaloid in CA1 on the behaviors in the conditioning task was measured. Injections of colchicine (1,5 µg/rat, intra-CA1) was performed in the male Wistar rats, while the animals were settled and cannulated in a stereotaxic apparatus. In the control group solely injection of saline (1 µl/rat, intra-CA1) was used. One week later, all the animals passed the saline conditioning task using a three-day schedule of an unbiased paradigm. They were administered saline (1 ml/kg, s.c.) twice a day throughout the conditioning phase. To evaluate the possible effects of cell injury by the toxin on the pyramidal cells, both the motivational signals while in the conditioning box and the non-motivational locomotive signs of the treated and control rats were measured. Based on the present study the alkaloid caused no change in the score of place conditioning, but affected both the sniffing and grooming behaviors in the rearing or compartment entering in the rats. According to the findings, the intra-CA1 injection of colchicine may impair the neuronal transmission of non-motivational information by the pyramidal cells in the dorsal hippocampus.

1. Introduction

Traditional lesion-producing methods by injecting toxic analogs of presumed neurotransmitters have received condemnation due to the lack of lesion selectivity in specific cell populations. This fact causes a serious problem whenever one attempts to ascribe the effects on the neurophysiology and neuronal transmission to the removal of a specific cell population. Thus, considerable interest has been generated in recent years by drugs that show a selective neurotoxic effect between cell populations.

Colchicine is a plant alkaloid (Alali, et al., 2005), which is known as a potent inhibitor of physiological processes. The alkaloid specifically binds to the receptor site of tubulin (Lockwood, 1979) and blocks mitosis (Mundy and Tilson, 1990).

Goldschmidt and Steward (1980) have previously demonstrated colchicine as a substance that preferentially destroys granulocytes in the dentate gyrus. Since Correia and Lobert (2001), we believe that the colchicine toxin affects other cellular processes rather tubulin subunits. Similar findings have been provided by Porkhodadad et al (2011). In this experiment, the possible damage to pyramidal cells in the cortical area 1 (CA1) of the hippocampus was evaluated by the saline conditioning task.

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This research plan is important because neuronal functions are dependent on an intact cytoskeleton and cytoskeletal alterations are thought to be associated with neurological disorders (Selkoe, 1999). This phenomenon is common in cases of neurodegenerative pathology (Uchida, et al., 2004). Neural lesions also may impair learning and spatial memory (McNaughton, et al., 1989), because sub-regions of the hippocampus seem to have a role in the function of spatial and working memory processes (Okada and Okaichi, 2009).

2. Experimental Procedure

2.1. Animals

The animals were male Wistar rats (Pasteur Institute of Iran, Tehran, Iran) weighing between 250-350g at the start of the experiments. Animals were housed in standard cages in a controlled colony room (temperature 21 ± 3 °C). They were maintained under a 12:12 h light/dark cycle with water and food provided ad libitum. Six to eight animals were used in each experiment and all experiments were approved by the local ethics committee at Shahed University (Document No: 282/Nov 21, 2012).

2.2. Drugs

The drug colchicine (Merck Co., Germany) used in the present study was prepared freshly in sterile 0.9% NaCl solution. A mixture level of 5:2 of ketamine (100 mg/kg) and xylazine (20 mg/kg) purchased from the Veterinary Organization of Iran were intraperitoneally injected to anesthetize the experiment animals. Vehicle injections were of the appropriate concentration of 0.9% physiological saline.

2.3. Surgery and Colchicine Injection

The animals were anesthetized and placed in a stereotaxic apparatus, with the incisor bar set at approximately 3.3 mm below horizontal zero to achieve a flat skull position. An incision was made to expose the rat's skull. Two holes were drilled in the skull at stereotaxic coordinates: AP-3.8 mm posterior to bregma, and $L\pm 1.8$ to ± 2.2 mm according to the atlas of Paxinos and Watson (2005). Two 21-gauge guide cannulae were inserted into the holes. In the animals receiving bilateral injections of colchicine into the cortical area 1 (CA1) of the hippocampus, the guide cannulae were lowered 2 mm below bregma through the holes drilled at the above-mentioned coordinates.

The colchicine (1,5 µg/rat) was administered intra-CA1 of the hippocampus while the cannulated rats were

still immobilized by the stereotaxic apparatus. The guide cannulae were anchored with a jeweler's screw, and the incision was closed with dental cement. After that, the injection cannula that extended 1 mm beyond the guide cannulae was inserted into the guide cannula, through which the alkaloid (1,5 µg/rat) was gently injected into the site. The injection cannula was then left in place for another 60 seconds to facilitate the diffusion of the drug. One week after recovery, the rats passed the saline place conditioning using an unbiased procedure.

2.4. Place Conditioning Apparatus and Saline Conditioning Task

A two-compartment conditioned place preference apparatus (30x60x30 cm) was used in the experiments. Place conditioning was conducted using an unbiased procedure, of which the design and the apparatus were previously described (Karami, et al., 2002).

2.5. Place Conditioning Paradigm

The experiment consisted of the three following phases:

Pre-conditioning (Familiarization): On day 1 (before the conditioning phase), animals received one habituation session. They were placed in the middle line of the apparatus to move freely in the entire apparatus for 10 minutes. In this phase, the removable wall was raised 12cm above the floor. The time spent by rats in each compartment was recorded by an Ethovision system (model LVC-DV323ec of an Auto iris LG) located 120 cm above the apparatus. The behavior recorded by the system was then analyzed by an observer who had no knowledge of the treatments. None of the groups displayed a significant preference for one of the compartments, confirming that this procedure is unbiased.

Conditioning: This phase was started one day after the familiarization. The conditioning phase consisted of 6 saline pairings; the animals were simply injected saline (1 ml/kg, s.c.) twice a day with a 6 h interval. Saline administration in the conditioning phase was carried out during the light phase of a 12 h light/dark cycle (e.g. at 09.00 am and at 15.00 pm). This protocol was performed similarly in the control group (1 µl/rat saline given intra-CA1) as well as in the experimental groups (1,5 µg/rat colchicine given intra-CA1).

Testing (Post-conditioning): Test sessions were carried out on day 5, one day after the last conditioning session, in an injection saline-free state. Each animal was tested only once. For testing, the removable wall was raised 12 cm above the floor and each animal was allowed free ac-

cess to both compartments of the apparatus for 10 min. The time spent in the compartments on the day of testing was subtracted from the time measured during the familiarization phase and the testing phases and the result was expressed as mean \pm S.E.M. The count of behavioral signs as expressed by the change in the number of signs per 10 min in the apparatus was also measured.

2.6. Histological Verification

After completion of the experiments, the animals were decapitated after an overdose of chloroform. The brains were removed and fixed in a 10% formalin solution for 48 h before sectioning. Sections were taken through the brain areas of the cannulae placements, and the cannulae placements were verified using the atlas of Paxinos and Watson (2005). Data from rats with injection sites located outside the CA1 area were excluded from the analysis.

2.7. Cresyl Violet Stain

Thin (10-15 μ m) brain sections provided by paraffin blocks were placed in 50%, 75%, and 95% ethanol for 1 min each and in 100% ethanol for 15 min. The sections were then rehydrated in 95%, 75%, and 50% ethanol and water for 1 min each and administered with cresyl violet solution (Merck Co., Germany) for 2 min. Subsequently, the sections were placed in 0.1% acetic acid in 75% etha-

nol for 30 sec, rinsed in water, dehydrated, and mounted in Entellan (Merck Co., Germany).

2.8. Statistical Analysis

The behavioral data were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison tests. P-values under 0.05 were considered significant. To analyze the histological data, the stained brain slices of the animals were examined by the Image Tool program (UTHSCSA ImageTool, version 2.03). After spatial and density calibrations with the intact (only anesthetized) samples, we made comparisons between the treated rats' brain sections and the calibrated samples to provide the data \pm SEM at the significant levels.

3. Results

3.1. Cell Damage Induced by Colchicine in the Area of CA1 of Wistar Rats

The brain sections were analyzed with a qualitative tool (Image tool program) and showed a significant difference ($p < 0.05$). The colchicine (1,5 μ g/rat) was effective in inducing of lesion in the CA1 layer (Fig. 1) when compared with the intact (only anesthetized) one.

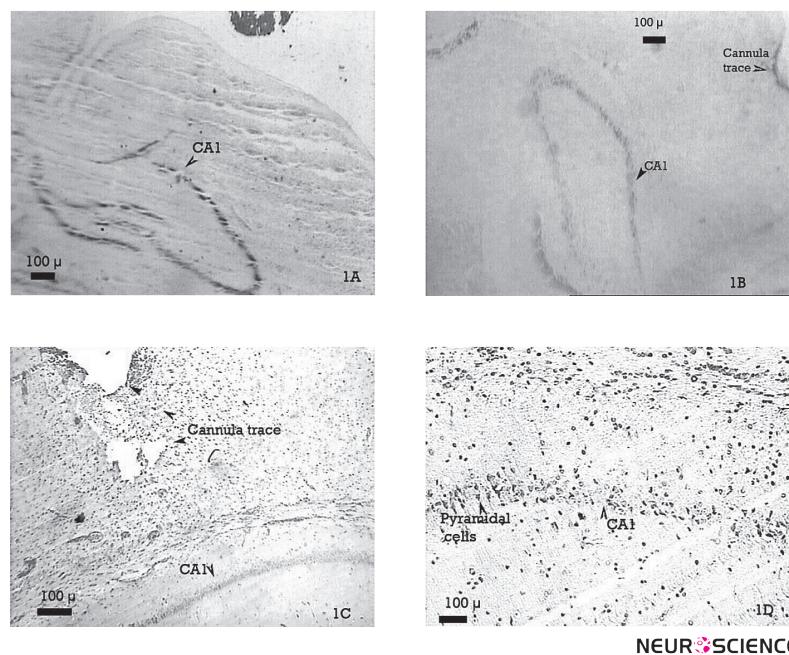
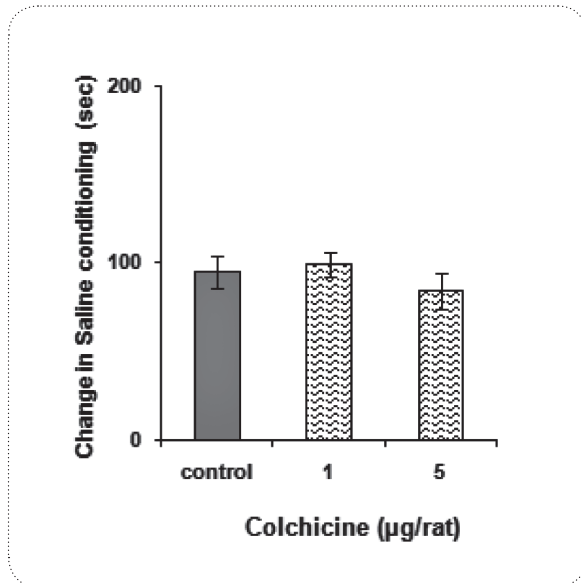


Figure 1. The histological verifications of the effect of colchicine lesion (1,5 μ g/rat, intra-CA1) in Wistar rats. The injection of colchicine was done only once. After receiving the drug each animal was allowed to recover. The animal then passed the behavioral tests. The rats' brains were then collected in formalin to allow for histological confirmation. Based on the Image Tool program the damaging effect was clear in the rats' brains that received colchicine (Fig. 1B-1D, from small to large magnification). As shown in figured 1B-1D, colchicine was effective to induce cell damage in contrast to the intact (anesthetized) sample (Fig. 1A).

3.2. Effect of injection of Colchicine Intra-CA1 on Saline Conditioning

Bilateral injection of colchicine (1,5 µg/rat, intra-CA1), a plant derived alkaloid, before (1 week) starting the process of saline place conditioning, showed no significant difference in conditioning score of the the rats compared to the control group ($p > 0.05$) (Fig. 2).

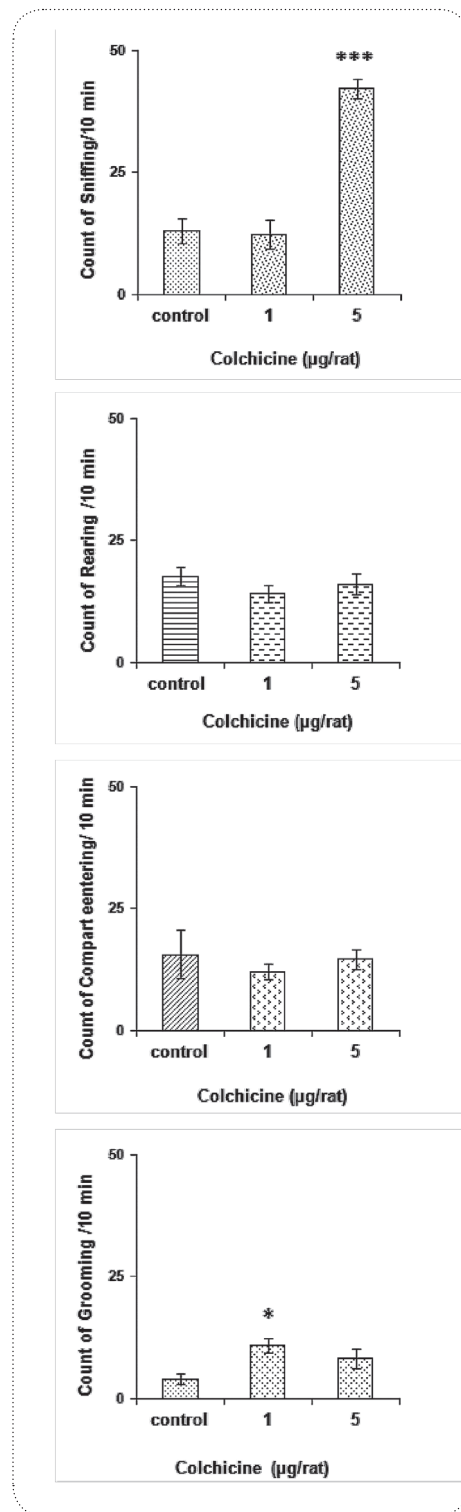


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Figure 2. Response to colchicine or saline (control), intra-CA1, in the saline place conditioning task. Each animal, after having recovered, was injected with saline (1 ml/kg, s.c.) twice per day throughout the conditioning phase. The animals were tested in the injection-free state on the day of testing to evaluate a simple type of learning notifying a link between the environment and the saline. Data are expressed as the score of changes in place preference and expressed as mean \pm S.E.M. No difference between the drug-administered group and the vehicle was observed.

3.3. Effect of Injection of Colchicine Intra-CA1 on Behavioral Signs

Fig. 3 shows the effect of colchicine injection (1,5 µg/rat, intra-CA1) on behavioral signs in Wistar rats. Administration of the neurotoxin showed a significant effect on sniffing ($F_{2,15}=23.414$; $p < 0.0001$) and grooming ($F_{2,15}=2.825$; $p < 0.05$). However, no significant effect on wet dog shaking (WDS) or rearing was recorded.



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Figure 3. The behavioral signs in male Wistar rats that received colchicine or saline (control) intra-CA1. The colchicine (1,5 µg/rat) or saline (1 ml/kg, µl/rat) was given one week before starting the unbiased conditioning paradigm. The animals were then injected saline (1 ml/kg, s.c.), twice daily for 3 days. Data are expressed as counts of behavioral signs per 10 min \pm SEM. Post hoc analysis by Tukey showed the differences (* $p < 0.05$, *** $p < 0.001$) to the control.

4. Discussion

This research was designed to survey the effect of a potent plant neurotoxin, colchicine, on small pyramidal cells in the cortical area 1 (CA1) of the hippocampus and on the behavior in a conditioning task as well. According to this study the toxin showed degenerative effects on the pyramidal cells and produced a significant change in sniffing and grooming.

Colchicine is an alkaloid which is extracted from *Colchicum autumnale* L. This toxin binds to tubulin dimers which results in the formation of a tubulin–colchicine complex that acts primarily to prevent microtubule assembly (Panda, et al., 1995). In animal cells colchicine is usually considered as a lethal agent even at the lowest concentrations (Eigsti and Dustin, 1995; Rieder and Palazzo, 1992).

This work showed a damaging effect of colchicine on the small pyramidal cell population in the rat CA1 area. Supporting finding provides that the toxin colchicine irreversibly damages dendrites by disrupting their microtubular supporting network (Giuditta, et al., 2008), an effect which is caused by the selective toxic effect of colchicine on dentate granule cells (Walsh, et al., 1986). Furthermore, some other studies using doses of colchicine similar to those that were used in the present study have reported only reversible effects (Gajate, et al., 2000). To assess the neurotoxic effect of colchicine in the CA1 on learning programs, sniffing, rearing, grooming and compartment entering were analyzed for all rats within the saline conditioning task and the results provided some significant effects.

After place conditioning testing the experiment animals, those suffering from the lesion made by colchicine (1,5 µg/rat) in the CA1 area, exhibited different types of sniffing and grooming behaviors in comparison to the control saline (intra-CA1) treated group. It is proposed that this alkaloid inhibits the rapid axonal transport (Hanson and Edstrom, 1978) and produces long-lasting morphological changes in neurons and glia (Csillik, et al., 1977). It has also been suggested that the axonal membrane (Dziegielewska, et al., 1976) as well as dendrites (Partida-Sanchez, et al., 2000) are the site of action of colchicine, because these sites are rich in microtubules and contain specific receptors (Kumar, et al., 2009). A further important characteristic is that the CA1 neurons are responsible for simple learning processes like conditioning. In order to make clear responses to conditioned stimuli its neurons need complete entity and safe existence (Arushanyan and Beier, 2008). Thus, the neuronal

responses are changed and exhibited a different type of sniffing and grooming in regard to the non-treated ones. Damage to CA1 pyramidal cells causes deficits in aspects of learning as present data show. It should be noted that the degree of learning impairment has been investigated by other researchers (Dillon, et al., 2008) and is proportional to the extent of damage within the cortical area. So, the colchicine treated groups were impaired in information processing such as odor sensing. This may underlie fine properties of the CA1 area.

Odor sampling by sniffing behavior in this paradigm has raised the idea that sniffing plays a critical role in odor information processing by shaping spatial and temporal patterns of afferent input to the olfactory bulb and through the patterns of higher level neural activity as well (Wesson, et al., 2008). Also, in a survey of the novelty task in rats with the CA1 lesions, an impairment in place conditioning was recorded (Okada and Okaichi, 2009; Zheng, et al., 2004).

Thus, the effects reported in the present study may be the consequence of an impaired neuronal transmission in pyramidal cells of the hippocampus, a process which consequently may be related to the observed data. Based on the findings in this study the small pyramidal cells can be pointed out as another target for the action of colchicine, while the investigators have suggested granular cells as the main candidate for destruction by the alkaloid.

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