

Involvement of α -1-adrenergic receptors in central region of amygdala and the effects of cannabinoid agonist on inhibitory avoidance memory in male rats

A. Moshfegh¹ A.R. Yarabi² A. Tehranifard¹ K. Dastan³

Assistant Professor Department of Biology¹, General Practitioner², Instructor Department of Biology³, Islamic Azad University, Lahijan Branch, Lahijan, Iran.

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Abstract

Introduction: There are many similarities between memory impairment in patients suffering from Alzheimer and animals treated by Cannabinoids. The agonists of Cannabinoid receptors affect on a variety of memories and learnings. The present study aims to investigate the role of α -1-adrenergic receptors in central region of amygdala in state-dependent learning induced by WIN55,212-2 (cannabinoid agonist) in rats.

Methods: Cannulae placement was performed bilaterally in the central amygdala region of male rats. The rats were trained in the avoidance learning apparatus (step-down model). 24 hours after training, the memory was tested by measuring the lag time for stepping down the platform.

Results: Injection of WIN55, 212-2 intra central amygdala (dose-dependent, 0.25, 0.5 μ g/rat) post-training reduced lag time/latency for stepping down. Injection induced amnesia was reversed by pre-test administration of the same dose of WIN55, 212-2. It is called state-dependent learning. Pre-test intra-central injection of α -1-adrenoceptor agonist, Phenylephrine (0.5, 0.25 μ g/rat) improved post-training WIN55, 212-2 (0.5 μ g/rat) intra central injection induced retrieval impairment. But intra injection of Prazosin (0.5 μ g/rat) 2 minutes before injection of WIN55, 212-2 (0.5 μ g/rat) on the testing day inhibited WIN55, 212-2 state-dependent learning.

Conclusion: The results suggest that α -1-adrenergic receptors in central region of amygdala are involved in learning which dependent on the state induced by WIN55, 212-2.

Key words: Cannabinoids - α 1 - Adrenergic - Learning

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Introduction:

Pharmacological studies on memory are carried out hoping to investigate the behavioral

findings accompanied by drug action mechanism; in order to clarify the neurobiological principles of memory (1).

Drug-related state-dependent learning is the behavior which is either expressed or trained in the presence of a drug. In the testing session reminding the behavior is improved in presence of the same drug.

There are many models for assessing memory and learning in laboratory animals. Inhibitory avoidance learning model is widely applied in pharmacological studies to investigate long-term memory in which hippocampus and amygdala are involved (1,2).

Studies show that amygdalae play a significant role in many aspects of addiction to abused drugs (3,4,15). The central amygdala - the biggest part of the amygdalae group-exerts its role in the processes- dependent to reward and learning via hippocampus and accumbens nucleus (6,19).

Cannabinoids are compounds found in Cannabis plant and artificial analogues from derivatives of fatty acids particularly arachidonic acid. For thousands of years, hashish and marijuana - both derived from Indian Cannabis with scientific name of Cannabis sativa - have been used because of their pharmacologic effects and mimic mental states. Endocannabinoid system is involved in physiologic and pathophysiologic functions. Therefore, cannabinoids can be helpful in treating diseases both via reinforcement and activation of the system and via confronting and inhibition of the system (7). Some helpful solutions: include pain treatment, muscular multiple sclerosis, regulation of glutamate neurotransmitter and its function on memory, as neuro-protector in ischemia and brain trauma (via inhibition of glutamate release), regulation of dopamine neurotransmitter and its function in basal ganglia and its subsequent effect on movement disorders like Parkinson and function in depression (7,8).

Cannabinoids present their physiologic effects through interaction with cannabinoid receptors including CB1 and CB2. CB1 cannabinoid receptors are widely expressed in brain and some of peripheral tissues, while CB2 are mainly found in immune system (9). Recent studies show that such receptors are also found in the brain of mammals (10). CB1 receptors are extensively expressed in the regions of brain which are involved in memory and learning such as

amygdalae (11), hippocampus (12), cortex, basal ganglia and cerebellum (13).

Both CB1 and CB2 are G protein-coupled receptors. The receptors are paired with inhibitory G protein and their activation results in inhibition of adenylate cyclase enzyme and prevention of intracellular cyclic adenosine monophosphate (cAMP) secondary pick formation (7,8).

Moreover, CB1 receptors affect on various types of channels via $G_{i/o}$. Secondary peaks activated by CB1 receptors are not the same in different brain regions. As an example, CB1 receptors inhibit adenylate cyclase and Ca^{2+} (N-type) while increasing the activity of Mitogen-Activated Protein (MAP) Kinase and K^+ channels (A-type) (6,14).

Behavioral studies show that cannabinoids directly interact with some neuro-transmitting systems (15). There are evidences showing cannabinoids reduce release of several neuromediators throughout the brain (16). According to the studies on amygdalae and hippocampus cannabinoids reduce release of various neurotransmitters like Glutamate, Acetylcholine, GABA, Opioids and Noradrenaline (16,17).

Upward neurons in noradrenergic system originate from locus coeruleus. They then innervate different regions of brain including hippocampus and cortex. It has been shown that upward noradrenergic neurons - which innervate hippocampus and amygdala - are involved in behavioral compatibility, attention and facilitating the processing of new sensory stimuli (18,19).

Noradrenaline released from this type of neurons functions via two groups of G protein-coupled receptors: α and β receptors (20,21). Based on the type of ligand, kinetic and effects, α receptors are divided into α -1-adrenergic and α -2-adrenergic groups. It has been specified that α -1-adrenergic receptors are post-synaptic (22).

There are many documents reporting the involvement of noradrenaline and noradrenergic receptors in learning and memory (23). For example, infusion of noradrenaline into different regions of the brain including hippocampus (24) and amygdalae (25,26) reinforce memory formation. Moreover, the amount of noradrenaline increases in the brain after training. This has direct

relation with memory and retrieval. Although the mechanism under which norepinephrine influences the memory is not quietly clear, it seems that norepinephrine functions by adjusting transmission of glutamate messages in the synapse via activation of adrenergic G protein-coupled paired receptors (27).

Earlier studies show that cannabinoids effect on the function of noradrenergic system (28,29). It has also been reported that destruction of locus coeruleus region significantly reduces catalytic effects of cannabinoids (30). Behavioral studies; moreover, suggest that there is a direct interaction between cannabinoids and neuro-transmitting systems like adrenergic system (23,31,34), nicotinic system glutamate system (32).

This study aims to investigate the effects of intra- central Amygdala bilateral injection of α -1-adrenergic receptor agonist and antagonist on the amnesia induced by WIN55, 212-2 and WIN55, 212-2 state-dependent learning by the use of passive avoidance learning model.

State-dependent learning is the phenomenon through which memory retrieval is most efficient when an individual is in the same sensation and physiologic state as they were at the time of memory formation (33-35).

Methods:

Animals

Male rats (Wistar, 200-250 gr) supplied by Pasteur Institute of Iran were used for the experiments in this study. The rats were provided with enough food and water while kept for the study. The room temperature where the rats kept was 22 ± 3 °C. They were divided into eight-member groups.

The inhibitory (passive) avoidance learning apparatus (Step-down model)

In this research, step-down inhibitory avoidance learning model was used. Rats had to step down a platform. The inhibitory (passive) avoidance learning apparatus (Step-down model) was a wooden box with dimensions: 40 X 30 X 40 cm. There were steel rods (0.3 cm in diameter, with 1 cm space between the rods) as the floor of the apparatus. There was a wooden cubic platform (12 X 10 X 7 cm) on the left corner of the floor on

the steel rod floor. The rods were connected to a stimulating device which transferred electrical shock to the rats under experiments via the rods. The advantage of using inhibitory avoidance learning model is that the induced learning and memory occur just by one experience – that is, electrical foot shock. In this model, the animal learns to avoid electrical foot shock by suppressing its innate tendency towards stepping down from the platform.

Drugs

Drugs used in this study were: WIN55, 212-2 or Cannabinoid agonist (Tocris Bioscience, USA), Phenylephrine as α -1-adrenergic and Prazosin as antagonist α -1-adrenergic (Sigma, USA). Just before experiments Phenylephrine and Prazosin were solved in sterile physiological saline (0.9%). WIN55, 212-2 was solved in a vehicle solution containing 90% sterile physiological saline and 10% Dimethyl sulfoxide. A drop of tween 80 oil was added to the solution.

The surgery procedure and cannula placement in the amygdala region

The rats were anesthetized by injection of Ketamine hydrochloride (50 mg/Kg) and Xylazine (4mg/Kg). After anesthesia the rats were placed in stereotaxic device. Then two guide cannulae (22G) were bilaterally placed in the central amygdala according to Paxinos and Watson (1997). The coordinates of the central amygdala was $V = -8.1$, $ML = \pm 4.2$, $AP = -2.2$ (36).

The inhibitory avoidance method for studying memory in rats is performed in two consecutive days. On the 1st day – the training day – the animals are trained in the apparatus. On the 2nd day – testing day – memory retrieval in the trained rats is tested.

Training phase

In the inhibitory avoidance method (step-down model), each animal is placed on the cubic wooden platform for assessing memory. Then the latency (lag time) on the platform before stepping down is recorded. Just after stepping down (four feet on the steel bars grid floor), an electrical shock (0.5 mA, for 3 seconds) is delivered. Then the rat is taken out from the apparatus and is administered post-training infusion.

Test phase or examining the memory (retrieval)

The testing session is performed 24 hours post-training. Pre-test injection is administered 5 minutes before the test. When there must be 2 drug injections, the 2nd administration is 2 minutes after the 1st one. To examine the memory, a rat is placed on the platform in the apparatus like the 1st day. There is no electrical shock on this day. The latency (lag time) of the rat on the platform for stepping down is considered as a criterion for testing the memory. The cut-off for stopping on the platform is 300 seconds.

Intracerebral injection

In injection phase, after removing the lock from the cannula guide, 0.5 microliter (19,37) drug is administered in each cannula in 60 seconds by a 27 G dentistry needle via 22G cannula guide.



Figure 1. Schematic adopted from Paxinos and Watson Atlas specifying the CeA region (A). Tissue section related to cannula placement in CeA region (B)

Histology

After euthanizing the animals with chloroform, methylene blue (1%, 0.5 μ l) was injected into each cannula. Then the brain was extracted and placed in Formalin (10%) for 1 week. Cannula entrance was sectioned with a surgical blade. The section was examined under a loop microscope. To study the prepared tissue sections Paxinos Atlas was used.

Statistical analysis

Memory score in each group was recorded as mean \pm S.E.M. To determine the significant difference between the groups under experiments, one-way analysis of variance (ANOVA) and

Tukey's test were used. The statistical application used in this was SPSS. To draw the charts MS-Excel was employed.

Drug treatments and experiments

1. Experiment No. 1: The effect of WIN55, 212-2 on passive avoidance memory

There were 5 groups of rats in this experiment. Group 1 was administered 1 μ l/rat saline intra-Central Amygdala (intra- CeA) immediately post-training. Group 2 was administered 1 μ l/rat vehicle intra- CeA. The other 3 groups were administered different doses (0.1, 0.25, 0.5 1 μ l/rat) of WIN55, 212-2 intra-CeA immediately post-training. On the test day, all the groups were administered 1 μ l/rat saline intra-CeA 5 minutes before test. (Figure 2)

2. Experiment No.2: The effect of intra-CeA injection of WIN55, 212-2 pre-test on destructed memory by WIN55, 212-2 on training day

There were 4 groups of rats in this experiment. All the groups were administered 0.5 μ l/rat WIN55, 212-2 immediately post-training. Group 1 was administered 0.5 μ l/rat saline intra-CeA 5 minutes pre-test. Other groups were administered different doses (0.1, 0.25, 0.5 μ l/rat) WIN55, 212-2 intra-CeA 5 minutes pre-test. The inhibitory avoidance memory of the groups was examined and measured. Each column shows Mean \pm S.E.M of the 8 rats in each group. (Figure 3)

3. Experiment No. 3: The effect of pre-test intra-CeA injection of Phenylephrine on destructed memory by WIN55, 212-2

There were 4 groups in this experiment. All the groups were administered 0.5 μ l/rat WIN55, 212-2 intra-CeA just post-training. Group 1 was administered 1 μ l/rat saline intra-CeA 5 minutes pre-test. Other groups were administered different doses (0.25, 0.5, 1 μ l/rat) of Phenylephrine intra-CeA 5 minutes pre-test. 24 hours post-training (on test day), the inhibitory avoidance in different animal groups were examined and measured. Each column shows Mean \pm S.E.M of the 8 rats in each group (Figure 4).

4. Experiment No. 4: The effect of intra-CeA Prazosin pre-test on WIN55, 212-2 state-dependent learning

There were 4 animal groups in this experiment. 0.5 μ l/rat WIN55, 212-2 was injected to the group members intra-CeA just post-training. In group 1, 0.5 μ l/rat WIN55, 212-2 was administered intra-CeA 5 minutes pre-test. Others were administered different doses (0.5, 1, 2 μ l/rat) of Prazosin along with 0.5 μ l/rat WIN55, 212-2 intra-CeA. 24 hours post-training (on test day) inhibitory avoidance memory of the animal groups were examined and measured. Each column shows mean \pm S.E.M. related to the 8 rats in each group. There were 8 animals in each group (Figure 5).

Results:

Experiment No. 1: The effect of WIN55, 212-2 on passive avoidance memory

One-way analysis of variance test showed that post-training WIN55, 212-2 (0.5 μ l/rat) injection – in comparison with animals administered saline and vehicle – caused destruction of memory on the test day [F (3, 35) = 26.35, p < 0.001]. Complement Tukey's test showed that post-training administration of 0.5 μ l/rat intra-CeA reduced latency (lag time) for stepping down the platform. In other words, it reduced memory in 24 hours. So it can be concluded that WIN55, 212-2 is able to induce amnesia (Figure 2).

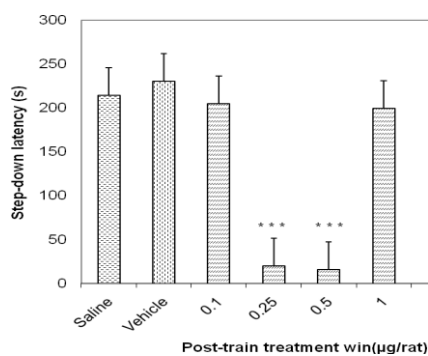


Figure 2. Effectiveness of post-training WIN55, 212-2 on inhibitory avoidance memory

*** P < 0.001 is a comparison of saline group before test with saline after training

Experiment No. 2: The effect of intra-CeA injection of WIN55, 212-2 pre-test on destroyed memory by WIN55, 212-2 on training day

To find out whether WIN55, 212-2 is able to cause state-dependent learning, the animals administered different doses of WIN55, 212-2 pre-test and 0.5 μ l post-training were examined. Complement Tukey's test showed that administration of 0.5 μ l WIN55, 212-2 pre-test was able to inhibit memory destruction due to post-training injection. It led to state-dependent learning [F (4, 38) = 28.44, P < 0.001]

It can be concluded that it is possible to use such animal models to examine the effects of adrenergic factors on amnesia due to WIN55, 212-2 and also WIN55, 212-2 state-dependent (Figure 3).

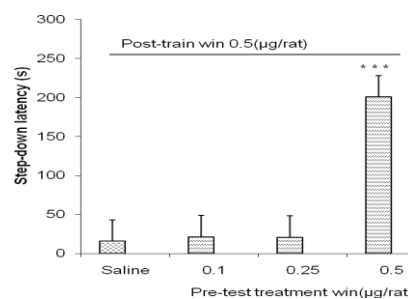


Figure 3. The effect of post-training and pre-test WIN55, 212-2 injection on inhibitory avoidance memory

*** P < 0.001 in comparison with saline pre-test/WIN55, 212-2 (0.5 μ l/rat) post-training

Experiment No. 3: The results of intra-CeA injection Phenylephrine pre-test on memory destroyed by WIN55, 212-2

This experiment was carried out to examine whether injection of different doses of Phenylephrine α -1-adrenergic effect on memory formation and learning or not.

One-way analysis of variance test showed that sole injection of Phenylephrine pre-test to the animals administered which receive saline on training day caused no significant change for stepping down the platform latency (or memory) in comparison with the control group (saline/saline) [F (3, 26) = 0.27, p > 0.05]. Moreover, one-way analysis of variance test revealed that administering 0.25 and 0.5 μ l/rat Phenylephrine pre-test by itself can improve amnesia caused by WIN55, 212-2 post-training. It can result in inhibition of amnesia due to WIN55, 212-2 [F (3, 28) = 34.74, p < 0.001]. Complement Tukey's

test showed that Phenylephrine (0.25, 0.5 μ l/rat) can reverse memory destructed by WIN55, 212-2 post-training (Figure 4).

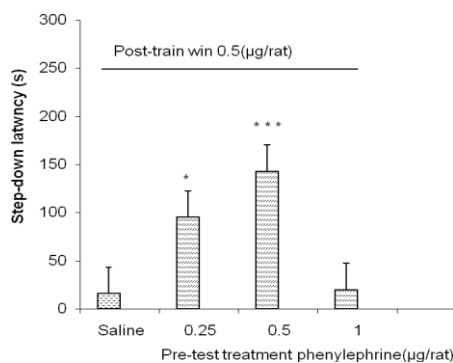


Figure 4: The effect of Phenylephrine on inhibitory avoidance memory and on avoidance memory destructed by WIN55, 212-2

*** $P < 0.001$, * $P < 0.05$ in comparison with saline pre-test/WIN55, 212-2 (0.5 μ g/rat) post-training.

Experiment No. 4: The effect of intra-CeA Prazosin pre-test on WIN55, 212-2 state-dependent learning

Prazosin is an antagonist for α -1 receptors. Blocking the action of such receptors induces several effects on central nervous system. Regarding the effects of Prazosin consumption on the processes of memory and learning, it has been suggested that it suppresses the processes. Considering the effects of Phenylephrine in the previous experiment, the effects of bilateral intra-CeA administration on WIN55, 212-2 state-dependent were examined.

One-way analysis of variance test showed that administration of only Prazos pre-test led to no significant change in regard to latency for stepping down the platform (memory) in comparison with the control group (saline/saline) [$F(3,28) = 0.38$, $P > 0.05$].

Moreover, administration of 0.5 μ g/rat Prazosin pre-test along with WIN55, 212-2 (0.5 μ g/rat) to the animals injected WIN55, 212-2 (0.5 μ g/rat) post-training led to amnesia. In fact, it inhibited WIN55, 212-2 state-dependent learning. One-way analysis of variance test showed that administration of Prazosin (0.5 μ g/rat) pre-test reduced memory correction induced by WIN55, 212-2 pre-test in the rats administered WIN55, 212-2 (0.5 μ g/rat) post-training [$F(3, 28) = 8.93$,

$p < 0.001$]. Complement Tukey's test revealed that Prazosin (0.5 μ g/rat) can inhibit induced WIN55, 212-2 state-dependent learning (Figure 5).

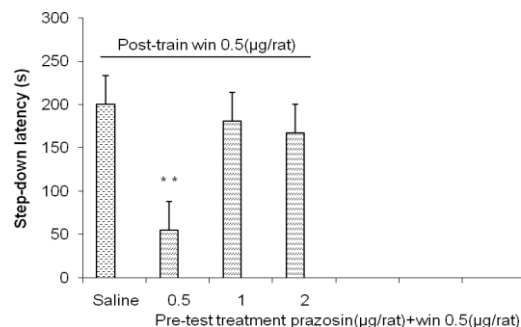


Figure 5: The effect of Prazosin on inhibitory avoidance memory and WIN55, 212-2 state-dependent learning

** $P < 0.01$ in comparison with WIN55, 212-2 (0.5 μ g/rat) + saline pre-test/ WIN55, 212-2 (0.5 μ g/rat) post-training

Conclusion:

Memory sometimes assessed by changes in the animal's behavior after learning reflects many processes including acquisition, encoding, consolidation, retrieval and performance (38).

Several pharmacologic studies reveal that CB1 receptor agonist destructs memory and learning (39). CB1 receptors are in the membrane of pre-synaptic axon terminals. In case of activation, they inhibit release of glutamate (40), acetylcholine (41) and noradrenaline (29) in the cells cultured in rat's hippocampus. Reduction of neuro-mediators release and inhibition of potential long term are due to inhibition of adenylate cyclase and Ca^{2+} channel type N following CB1 in the nervous system (19, 23).

WIN55, 212-2 probably reduces memory through one or several mechanisms: 1) The CB1 receptors in the presynaptic axon terminals of gabaergic lead to reduced release of GABA. This causes over-activation of neurons and neural interactions (42); 2) Reduced release of GABA in parallel with reduced release of Cholecystokinin (CCK) (42). A wide range of studies show that inhibition of cholecystokinin receptor leads to memory destruction (43); 3) Stimulating CB1 receptor results in balanced release of other neuro-

mediators in amygdalae (19, 23) and hippocampus – for instance Dopamine (44) and Acetylcholine. Release may either be increased or decreased (45); 4) Activation of CB1 receptor probably leads to memory destruction via inhibition of stimulation transfer (46); 5) Acute administration of cannabinoid drugs in hippocampus reduces the general activities of neurons (47,48); 6) long-term use of cannabinoids results in either toxic neuropathy or decreased number of synapses and cells (49). Specifying the actual role of the above mechanisms in memory destruction requires further both molecular and behavioral studies.

Injecting drug immediately post-training consolidates the drug effect further. It will be more effective on recall if the drug is administered pre-test as well (38). This study was supposed to investigate the effect of drugs on memory consolidation and recall. Based on this, 1) WIN55, 212-2 was administered post-training to examine its effect on information consolidation, 2) WIN55, 212-2 and α -adrenergic were individually or together administered pre-test to determine their effect on recall.

Our findings show that administration of non-elective cannabinoid receptors – WIN55, 212-2 – intra-CeA post-training leads to inhibitory avoidance memory destruction on the test day. The results confirm studies reporting CB1 receptor agonist causes induced amnesia (12, 19, 23, 34, 50). There are researches reporting CB1 receptor agonist influence different phases of memory processing like acquisition and consolidation (51). CB1 is extensively expressed in the regions of the brain involved in memory and recall such as amygdala, cortex, basal ganglia, hippocampus and cerebellum (13). Earlier studies have mostly investigated peripheral effects of cannabinoids (52,53). Hence, the main reason through which CB1 receptors cause the destruction of memory is not so clear.

It was observed that destructed memory with administration of WIN55, 212-2 intra-CeA post-training is completely inhibited by administration (intra-CeA) of the same dose and cause state-dependent learning. Our previous studies showed that intracerebroventricular (ICV) administration of WIN55, 212-2 on the test day improved the destructed memory by ICV administration of

WIN55, 212-2 on the test day (54). Similar response is also observed for Morphine (55), Lithium (56) and Histamine (57). It shows that WIN55, 212-2 produces a state in memory in which the animal is able to learn and recall a particular response. It is called state-dependent learning (34).

State-dependent learning is a phenomenon caused by drugs which mimic mental states in human (58,59). In this phenomenon, retrieval of newly acquired information is possible whenever the animal is in the same state which it was at the time of information encoding (60). Such identical conditions is established by administration of the drug on the training day as well as on post day (61). In the last 30 years, this type of learning has been reported in different animal species and even humans for many drugs including stimulants of central nervous system, sedatives (tranquilizers), Opioids and hallucinogenic drugs.

Various studies have reported Interaction between opioids and cannabinoids. They showed that pre-test administration of morphine is able to reverse memory destructed by WIN55, 212-2 on the training day (54). There are also reports showing α -1-adrenergic receptor are involved in emergence of morphine withdrawal symptoms (62). These studies show that receptors of α -1 antagonist (Prazosin) systemic symptoms of opioids withdrawal. The effects of cannabinoids are similar to opioids from many aspects. Cannabinoids like opioids have analgesic and anti-inflammatory effects, suppress of immune system as well as amnesia induction.

It is good to note that both cannabinoid and opioid receptors are in pre-synaptic membrane and lead to reduction of neuro-mediators release; both have similar effects and overlapping in many regions of the brain. For example, inhibition of adenylate cyclase, inhibition of Ca^{2+} channel, activation of K^+ channel. On the other hand, cannabinoid and opioid responses are respectively inhibited by cannabinoid and opioid antagonists (63). Therefore, it is possible that similar systems are involved for both cannabinoids and opioids.

There are similarities between cannabinoid and opioid systems (64). Earlier studies show that morphine establishes state-dependent learning (61, 65) and morphine induced state-dependent

learning interacts with neuro-transmitting systems like dopamine (66), histamine (67), acetylcholine (68), glutamate (69), GABA (70), cannabinoids (71) and nitric oxide (65). Previous studies have also shown that α -2-adrenergic drugs are involved in state-dependent learning induced by morphine in inhibitory avoidance learning (72). On the other hand, it has been shown that pre-test morphine administration is able to reverse the memory destroyed by cannabinoids (71). According to the above evidences and other findings (34), it is possible that the effects of cannabinoids to be mediated via adrenergic receptors.

Upward neurons in noradrenergic system originate from locus coeruleus. They then innervate different regions of brain including hippocampus and cortex. It has been shown that upward noradrenergic neurons - which innervate hippocampus and amygdala - are involved in behavioral compatibility, attention and facilitating the processing of new sensory stimuli (18,19).

Therefore, we investigated the effects of pre-test α -1-adrenergic receptors antagonist on inhibitory avoidance memory destroyed by WIN55, 212-2 and also the effects of the α -1-adrenergic on state-dependent learning induced by WIN55, 212-2.

The memory destroyed by WIN55, 212-2 post-training administration. In other words, the results confirm studies reporting the involvement of adrenergic system (73) and α -adrenergic receptors (66) in regulation of memory. Lots of researches show that administration of adrenergic agonists like epinephrine (74), amphetamine (75) and phenylephrine (76) improve memory in the models which have defects in memory. Moreover, phenylephrine as α -1-adrenoceptor improves avoidance memory via α -1 post-synaptic receptors (77).

Earlier studies have also shown that α -1-adrenergic agonist - phenylephrine - improves memory retrieval (78). Pharmacologic researches show that α -1-adrenergic receptors are mostly post-synaptic (76). α_1 receptors stimulate polyphosphoinositide hydrolysis resulting in development of Inositol 1,4,5-trisphosphate (IP_3) and Diacylglycerols (DAG). G_q -from G protein family - pairs α_1 receptors with phospholipase C. IP_3 stimulates the release of Calcium stored in cell

reservoirs. This increases the cytoplasmic concentration of free Calcium and activation of a variety of Calcium-dependent protein kinases. The activation of the receptors can increase intracellular (cytoplasmic membrane) flow of Calcium. IP_3 is consecutively phosphorylated which ultimately develops free inositol. DAG activates protein kinase C which changes the activity of many signaling pathways. Moreover, α_1 receptors activate signal transduction pathways.

Studies show that α -1-adrenergic influence that storage process of memory by effecting on the function of β -adrenergic receptors (79, 80). Although the mechanism through which adrenergic effects on memory process is not quite clear, it seems that this is related to the ability of the system for adjusting the transmission of glutamate signals in the synapse. The adjustment occurs through coupling of G-proteins with adrenergic receptors (79).

In this study, we also investigated the effect of pre-test administration of α -1 antagonist receptors (Prazosin in presence and absence of WIN55, 212-2) on memory.

The results show that Prazosin reduces improvement of memory induced by WIN55, 212-2 on the test day in the animals which were administered WIN55, 212-2 (0.5 μ g/rat) post-training and pre-test.

In fact, Prazosin significantly inhibits WIN55, 212-2 state-dependent learning. The results may imply that WIN55, 212-2 state-dependent learning is mediated in Amygdalae via alpha adrenergic receptors. Reduction of memory by Prazosin in our study confirms earlier studies reporting Prazosin diminishing memory acquisition (80,81). Other studies report that administration of Prazosin to stria terminalis (pre or post-training) leads to destruction of acquisition or spatial memory retrieval while administration of norepinephrine to this region improves acquisition or memory retrieval. Simultaneous administration of Prazosin attenuates the reinforcing effect of norepinephrine (77). A research has shown that ICV administration of Prazosin post-training leads to memory reduction in rats (82).

At the end, there are two effects raising this possibility that WIN55, 212-2 state-dependent learning is related to the activation of alpha

adrenergic receptors in the central amygdala region: 1) reinforcing effects of α -1-adrenergic receptor agonists on memory when accompanied by WIN55, 212-2, and 2) the destructive effects of α -1-adrenergic receptor agonists on memory when accompanied by WIN55, 212-2. Further experiments are required for clarification of the actual interaction mechanism between WIN55, 212-2 and α -1-adrenergic receptors.

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References:

- Izquierdo I, McGaugh JL. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. *Behav Pharmacol.* 2000;11:517-534.
- Izquierdo I, Medina JH. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiol Learn Mem.* 1997;68:285-316.
- Ferry B, Roozendaal B, McGaugh JL. Involvement of alpha1-adrenoceptors in the basolateral amygdala in modulation of memory storage. *Eur J Pharmacol.* 1999;372:9-16.
- Ferry B, Roozendaal B, McGaugh JL. Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between beta- and alpha1-adrenoceptors. *J Neuro Sci.* 1999;19:5119-523.
- Gregory J. Quirk, Ekaterina Likhtik, Joe Guillaume Pelletier, and Denis Paré. Stimulation of Medial Prefrontal Cortex Decreases the Responsiveness of Central Amygdala Output Neurons. *Neuroscience.* 2003;23: 8800-8807.
- Lazzaro SC, Hou M, Cunha C, LeDoux JE, Cain CK. Antagonism of lateral amygdala alpha1-adrenergic receptors facilitates fear conditioning and long-term potentiation. *Learn Mem.* 2010;17:489-493.
- Riedel G, Davies SN. Cannabinoid function in learning, memory and plasticity. *Handb Exp Pharmacol.* 2005;168:445-477.
- Goonawardena AV, Robert L, Hampson RE, Riedel G. Cannabinoid and cholinergic systems interact during performance of a short-term memory task in the rat. *Learn Mem.* 2010;17:502-511.
- Pertwee RG. Pharmacological actions of cannabinoids. *Handb Exp Pharmacol.* 2005;168:1-51.
- Onaivi ES. Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology.* 2006;54:231-246.
- Katona I, Rancz EA, Acsády L, Ledent C, Mackie K, et al. Distribution of CB1 Cannabinoid Receptors in the Amygdala and their Role in the Control of GABAergic Transmission. *Neuroscience.* 2001;21:9506-9518.
- Nasehi M, Sahebgharani M, Haeri-Rohani A, Zarrindast MR. Effect of cannabinoids infused into the dorsal hippocampus upon memory formation in 3-days apomorphine-treated rats. *Neurobiol Learn Mem.* 2009;92:391-399.
- Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. *Science.* 2002;296:678-682.
- Robinson L, Goonawardena AV, Pertwee RG, Hampson RE, Riedel G. The synthetic cannabinoid HU210 induces spatial memory deficits and suppresses hippocampal firing rate in rats. *Br J Pharmacol.* 2007;151:688-700.
- Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, et al. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc Natl Acad Sci U S A.* 2005;102:18620-18625.
- Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci.* 2001;22:565-572.
- Al-Hayani A, Davies SN. Effect of cannabinoids on synaptic transmission in the rat hippocampal slice is temperature-dependent. *Eur J Pharmacol.* 2002;442:47-54.
- Aura J, Riekkinen P Jr. Blockade of NMDA receptors located at the dorsomedial prefrontal cortex impairs spatial working memory in rats. *Neuroreport.* 1999;10:243-248.
- Ghiasvand M, Rezayof A, Zarrindast MR, Ahmadi S. Activation of cannabinoid CB1 receptors in the central amygdala impairs inhibitory avoidance

- memory consolidation via NMDA receptors. *Neurobiol Learn Mem.* 2011;96:333-338.
20. Sirviö J, MacDonald E. Central alpha-1-adrenoceptors: their role in the modulation of attention and memory formation. *Pharmacol Ther.* 1999;83:49-65.
 21. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev.* 2003;42:33-84.
 22. Stuchlík A, Petrášek T, Vales K. Effect of alpha (1)-adrenergic antagonist prazosin on behavioral alterations induced by MK-801 in a spatial memory task in Long-Evans rats. *Physiol Res.* 2009;58:733-740.
 23. Zarrindast MR, Ghiasvand M, Rezayof A, Ahmadi S. The amnesic effect of intra-central amygdala administration of a cannabinoid CB1 receptor agonist, WIN55,212-2, is mediated by a beta-1 noradrenergic system in rat. *Neuroscience.* 2012;14:212:77-85.
 24. Izquierdo LA, Vianna M, Barros DM, Mello e Souza T, Ardenghi P, Sant'Anna MK, et al. Short- and long-term memory are differentially affected by metabolic inhibitors given into hippocampus and entorhinal cortex. *Neurobiol Learn Mem.* 2000;73:141-149.
 25. Lazzaro SC, Hou M, Cunha C, Ledoux JE, Cain CK. Antagonism of lateral amygdala alpha-1-adrenergic receptors facilitates fear conditioning and long-term potentiation. *Learn Mem.* 2010;17:489-493.
 26. Clayton EC, Williams CL. Adrenergic activation of the nucleus tractus solitarius potentiates amygdala norepinephrine release and enhances retention performance in emotionally arousing and spatial memory tasks. *Behav Brain Res.* 2000;112:151-158.
 27. Scheiderer CL, Dobrunz LE, McMahon LL. Novel form of long-term synaptic depression in rat hippocampus induced by activation of alpha 1 adrenergic receptors. *J Neurophysiol.* 2004;91:1071-1077.
 28. Hernández-Tristan R, Arévalo C, Canals S, Leret ML. The effects of acute treatment with delta9-THC on exploratory behaviour and memory in the rat. *J Physiol Biochem.* 2000;56:17-24.
 29. Schlicker E, Timm J, Zentner J, Göthert M. Cannabinoid CB1 receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn Schmiedebergs Arch Pharmacol.* 1997;356:583-589.
 30. Kataoka Y, Ohta H, Fujiwara M, Oishi R, Ueki S. Noradrenergic involvement in catalepsy induced by delta 9-tetrahydrocannabinol. *Neuropharmacology.* 1987;26:55-60.
 31. Reyes BA, Rosario JC, Piana PM, Van Bockstaele EJ. Cannabinoid modulation of cortical adrenergic receptors and transporters. *J Neurosci Res.* 2009;87:3671-3678.
 32. Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, et al. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc Natl Acad Sci U S A.* 2005;102:18620-18625.
 33. Izquierdo I, Dias RD. Memory as a state dependent phenomenon: role of ACTH and epinephrine. *Behav Neural Biol.* 1983;38:144-149.
 34. Piri M, Zarrindast MR. Modulation of WIN55,212-2 state-dependent memory by α 2-adrenergic receptors of the dorsal hippocampus. *Arch Iran Med.* 2011;14:389-395.
 35. Overton DA. Basic mechanisms of state-dependent learning. *Psychopharmacol Bull.* 1978;14:67-68.
 36. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1997.
 37. Azami NS, Pri M, Oryan S, Jahanshahi M, Babapour V. Involvement of dorsal hippocampal α -adrenergic receptors in the effect of scopolamine on memory retrieval in inhibitory avoidance task. *Neurobiol Learn Mem.* 2010;93:455-462.
 38. Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol.* 2001;11:180-187.
 39. Davies SN, Pertwee RG, Riedel G. Functions of cannabinoid receptors in the hippocampus. *Neuropharmacology.* 2002;42:993-1007.
 40. Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid Receptor Agonists Inhibit Glutamatergic Synaptic Transmission in Rat Hippocampal Cultures. *J Neurosci.* 1996;16:4322-4334.
 41. Gifford AN, Samiian L, Gatley SJ, Ashby CR Jr. Examination of the effect of the cannabinoid receptor agonist, CP 55,940, on electrically evoked

- transmitter release from rat brain slices. *Eur J Pharmacol.* 1997;324:187-192.
42. Harro J, Orelund L. Cholecystokinin receptors and memory: a radial maze study. *Pharmacol Biochem Behav.* 1993;44:509-17.
 43. Acquas E, Pisanu A, Marrocu P, Di Chiara G. Cannabinoid CB₁ receptor agonists increase rat cortical and hippocampal acetylcholine release in vivo. *Eur J Pharmacol.* 2000;40:179-185.
 44. Nava F, Carta G, Battasi AM, Gassa GL. D₂ dopamine receptors enable Δ^9 -tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. *Br J Pharmacol.* 2000;130:1201-1210.
 45. Kathmann M, Weber B, Schillicker E. Cannabinoid CB₁ receptor-mediated inhibition of acetylcholine release in the brain of NMRI, CD-1 and C57BL/6J mice. *Naunyn-Schmiedeberg's Arch Pharmacol.* 2001;363:50-56.
 46. Pontieri FE, Conti G, Zocchi A, Fieschi C, Orzi F. Metabolic mapping of the effects of WIN 55212-2 intravenous administration in the rat. *Neuropsychopharmacology.* 1999;21:773-776.
 47. Bloom AS, Tershner S, Fuller SA, Stein EA. Cannabinoid-induced alterations in regional cerebral blood flow in the rat. *Pharmacol Biochem Behav.* 1997;57:625-631.
 48. Chan GC, Hinds TR, Impey S, Storm DR. Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *J Neurosci.* 1998;18:5322-5332.
 49. Lawston J, Borella A, Robinson JK, Whitaker-Azmitia PM. Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55,212-2. *Brain Res.* 2000;877:407-410.
 50. Sullivan JM. Cellular and Molecular Mechanisms Underlying Learning and Memory Impairments Produced by Cannabinoids. *Learn Mem.* 2000;7:132-139.
 51. Kobilov T, Hazvi S, Dudai Y. Role of cortical cannabinoid CB₁ receptor in conditioned taste aversion memory. *Eur J Neurosci.* 2007;25:3417-3421.
 52. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, et al. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A.* 1990;87:1932-1936.
 53. Pettit DA, Harrison MP, Olson JM, Spencer RF, Cabral GA. Immunohistochemical localization of the neural cannabinoid receptor in rat brain. *J Neurosci Res.* 1998;51:391-402.
 54. Zarrindast MR, Kangarlu-Haghighi K, Khalilzadeh A, Fazli-Tabaei S. Influence of intracerebroventricular administration of cannabinergic drugs on morphine state-dependent memory in the step-down passive avoidance test. *Behav Pharmacol.* 2006;17:231-237.
 55. Zarrindast MR, Rezayof A. Morphine State-dependent Learning: Sensitization and Interactions with Dopamine Receptors. *Eur J Pharmacol.* 2004;497:197-204.
 56. Zarrindast M, Madadi F, Ahmadi SJ. Repeated administrations of dopamine receptor agents affect lithium-induced state-dependent learning in mice. *Psychopharmacol.* 2009;23:645-651.
 57. Zarrindast MR, Fazli-Tabaei S, Khalilzadeh A, Farahmanfar M, Yahyavi SH. Cross state-dependent retrieval between histamine and lithium. *in Physiol Behav.* 2005;86:154-163.
 58. Overton DA. Historical context of state dependent learning and discriminative drug effects. *Behav Pharmacol.* 1991:253-264.
 59. Overton DA. Comparison of the degree of discriminability of various drugs using the T-maze drug discrimination paradigm. *Psychopharmacology.* 1982;76:385-395.
 60. Shulz DE, Sosnik R, Ego V, Haidarliu S, Ahissar E. A neuronal analogue of state-dependent learning. *Nature.* 2000;403:549-553.
 61. Zarrindast MR, Rezayof A. Morphine state-dependent learning: sensitization and interactions with dopamine receptors. *Eur J Pharmacol.* 2004;497:197-204.
 62. van der Laan JW. Effects of alpha 2-agonists on morphine withdrawal behaviour: potentiation of jumping mediated by alpha 2-receptors. *Naunyn Schmiedebergs Arch Pharmacol.* 1985;329:293-298.
 63. Fattore L, Deiana S, Spano SM, Cossu G, Fadda P, Scherma M, et al. Endocannabinoid system and opioid addiction: behavioural aspects. *Pharmacol Biochem Behav.* 2005;81:343-359.
 64. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993;365:61-65.
 65. Zarrindast MR, Askari E, Khalilzadeh A, Nouraei N. Morphine state-dependent learning sensitization

- and interaction with nitric oxide. *Pharmacology*. 2006;78:66-71.
66. Zarrindast MR, Bananej M, Khalilzadeh A, Fazli-Tabaei S, Haeri-Rohani A, Rezayof A. Influence of intracerebroventricular administration of dopaminergic drugs on morphine state-dependent memory in the step-down passive avoidance test. *Neurobiol Learn Mem*. 2006;86:286-292.
 67. Khalilzadeh A, Zarrindast MR, Djahanguiri B. Effects of intracerebroventricular administration of ultra low doses of histaminergic drugs on morphine state-dependent memory of passive avoidance in mice. *Behav Brain Res*. 2006;166:184-187.
 68. Jafari MR, Zarrindast MR, Djahanguiri B. Influence of cholinergic system modulators on morphine state-dependent memory of passive avoidance in mice. *Physiol Behav*. 2006;88:146-151.
 69. Ahmadi S, Zarrindast MR, Nouri M, Haeri-Rohani A, Rezayof A. N-Methyl-D-aspartate receptors in the ventral tegmental area are involved in retrieval of inhibitory avoidance memory by nicotine. *Neurobiol Learn Mem*. 2007;88:352-358.
 70. Zarrindast MR, Noorbakhshnia M, Motamedi F, Haeri-Rohani A, Rezayof A. Effect of the GABAergic system on memory formation and state-dependent learning induced by morphine in rats. *Pharmacol*. 2006;76:93-100.
 71. Zarrindast MR, Kangarlu-Haghighi K, Khalilzadeh A, Fazli-Tabaei S. Influence of intracerebroventricular administration of cannabinergic drugs on morphine state-dependent memory in the step-down passive avoidance test. *Behav Pharmacol*. 2006;17:231-237.
 72. Hodayoun H, Khavandgar S, Zarrindast MR. Morphine state-dependent learning: interactions with α 2-adrenoceptors and acute stress. *Behav Pharmacol*. 2003;14:41-48.
 73. Kobayashi K, Kobayashi T. Genetic evidence for noradrenergic control of long-term memory consolidation. *Brain Dev*. 2001;23:16-23.
 74. Introini-Collison B, Castellano C, McGaugh JL. Interaction of GABAergic and β -noradrenergic drugs in the regulation of memory storage. *Behav Neural Biol*. 1994;61:150-155.
 75. Martinez JL, Vasquez BJ, Rigter H, Messing RB, Jensen RA, Liang KC, et al. Attenuation of amphetamine-induced enhancement of learning by adrenal demedullation. *Brain Res*. 1980;195:433-443.
 76. Chung YY, Bae CS. Ontogeny of Adrenergic Receptors in the Rat Amygdala. *Korean J Anat*. 1998;31:447-463.
 77. Chen HC, Chen DY, Chen CC, Liang KC. Pre- and post-training infusion of prazosin into the bed nucleus of the stria terminalis impaired acquisition and retention in a Morris water maze task. *Chin J Physiol*. 2004;47:49-59.
 78. Coull JT. Pharmacological manipulations of the α 2-noradrenergic system. Effects on cognition. *Drugs Aging*. 1994;5:116-126.
 79. Ferry B, Roozendaal B, McGaugh JL. Involvement of α 1-adrenoceptors in the basolateral amygdala in modulation of memory storage. *Eur J Pharmacol*. 1999;372:9-16.
 80. Ferry B, Roozendaal B, McGaugh JL. Basolateral amygdala noradrenergic influences on memory storage are mediated by an interaction between β - and α 1-adrenoceptors. *J Neurosci*. 1999;19:5119-5123.
 81. Obersztyn M, Kostowski W. Noradrenergic agonists and antagonists: effects on avoidance behaviour in rats. *Acta Physiol Pol*. 1983;34:401-407.
 82. Zarrindast MR, Khodjastehfar E, Oryan S, Torkaman-Boutorabi A. Baclofen-impairment of memory retention in rats: possible interaction with adrenoceptor mechanism(s). *Eur J Pharmacol*. 2001;411:283-288.

تداخل گیرنده‌های آلفا-۱ آدرنرژیک ناحیه آمیگدال مرکزی با اثرات آنگونیست کانابینوئید بر روی حافظه اجتنابی مهاری در موش‌های صحرائی نر

اعظم مشفق^۱ احمدرضا یاربی^۲ اکرم تهرانی فرد^۱ کتایون داستان^۳

^۱ استادیار، گروه زیست‌شناسی، ^۲ پزشک عمومی، ^۳ مربی، گروه زیست‌شناسی، دانشگاه آزاد اسلامی، واحد لاهیجان، لاهیجان، ایران.

مجله پزشکی هرمزگان سال هجدهم شماره پنجم ۹۳ صفحات ۳۶۹-۳۵۷

چکیده

مقدمه: شباهت‌های زیادی بین نقص حافظه ایجاد شده در بیماران مبتلا به آلزایمر و حیوانات تیمار شده با کانابینوئیدها وجود دارد. آگونیست‌های گیرنده‌های کانابینوئیدی، انواع متنوع حافظه و یادگیری را تحت تأثیر قرار می‌دهند. مطالعه حاضر به منظور بررسی نقش گیرنده‌های آلفا-۱-آدرنرژیک آمیگدال مرکزی در یادگیری وابسته به وضعیت القاء شده با WIN55,212-2 (آگونیست کانابینوئید) در موش‌های بزرگ آزمایشگاهی انجام گرفته است.

روش کار: کانول گذاری دو طرفه در ناحیه آمیگدال مرکزی رت‌های نر انجام شد. موش‌ها در دستگاه یادگیری اجتنابی مدل step-down آموزش دیدند. تست حافظه ۲۴ ساعت بعد از آموزش به صورت اندازه‌گیری زمان تأخیر در پایین آمدن از سکو انجام شد.

نتایج: تزریق پس از آموزش WIN55,212-2 (۰/۲۵، ۰/۵ μg/rat) به داخل آمیگدال مرکزی به صورت وابسته به روز، زمان تأخیر در پایین آمدن از سکو را کاهش داد. فراموشی القاء شده با تزریق بعد از آموزش WIN55,212-2 (۰/۵ μg/rat) با تزریق همان مقدار WIN55,212-2 قبل از آزمون اصلاح می‌گردد که به این حالت یادگیری وابسته به وضعیت گفته می‌شود. تزریق پیش از آزمون فنیل افرین (۰/۲۵، ۰/۵ μg/rat) به ناحیه آمیگدال مرکزی توانست حافظه تخریب شده با WIN55,212-2 (۰/۵ μg/rat) روز آموزش را اصلاح نماید، در صورتی که تزریق درون مغزی پرازوسین (۰/۵ μg/rat) دو دقیقه قبل از تزریق WIN55,212-2 (۰/۵ μg/rat) در روز آزمون، یادگیری وابسته به وضعیت WIN55,212-2 را مهار می‌نماید.

نتیجه‌گیری: این نتایج پیشنهاد می‌نماید که احتمالاً گیرنده‌های آلفا-۱ آدرنرژیک آمیگدال مرکزی در یادگیری وابسته به وضعیت القاء شده توسط WIN55,212-2 دخیل می‌باشد.

کلیدواژه‌ها: کانابینوئیدها - آلفا-۱ آدرنرژیک - یادگیری

نویسنده مسئول:

دکتر اعظم مشفق

گروه زیست شناسی دانشگاه آزاد

اسلامی، واحد لاهیجان

لاهیجان - ایران

تلفن: +۹۸ ۹۱۱۳۳۳۰۲۱۴

پست الکترونیکی:

moshfeghazam@gmail.com

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