Nefiracetam ameliorates associative learning impairment in the scopolamine-injected older rabbit

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Summary

Background: The cognition-enhancing drug, nefiracetam, is in Phase III clinical trials to treat memory impairment in Alzheimer's disease (AD). Nefiracetam ameliorates acquisition of delay eyeblink classical conditioning in older rabbits, a form of associative learning with striking behavioral and neurobiological similarities in rabbits and humans. In both species, delay eyeblink conditioning engages the septo-hippocampal cholinergic system and is disrupted when the cholinergic system is antagonized. Delay eyeblink classical conditioning is impaired in normal aging and severely disrupted in AD.

Material/Methods: To test further the efficacy of nefiracetam in an animal model that mimics some of the neurobiological and behavioral effects present in AD, we tested 56 older rabbits assigned to 7 treatment groups in the 750 ms delay eyeblink conditioning procedure. Older rabbits were injected with 1.5 mg/kg scopolamine to simulate disruption of the cholinergic system in AD. Three doses of nefiracetam (5, 10, or 15 mg/kg) were also injected in older rabbits receiving 1.5 mg/kg scopolamine. Control groups were treated with 1.5 mg/kg scopolamine + vehicle, vehicle alone, or explicitly unpaired presentations of conditioning stimuli and vehicle or 1.5 mg/kg scopolamine + 15 mg/kg nefiracetam.

Results: Rabbits injected with 1.5 mg/kg scopolamine alone were impaired, but a dose of 15 mg/kg nefiracetam reversed significantly the behavioral impairment.

Conclusion: Nefiracetam had ameliorating effects on a task impaired in AD in an animal model of AD: older rabbits with cholinergic system antagonism.

key words: eyeblink classical conditioning • cognition-enhancing • acetylcholine • Alzheimer's disease • Muscarinic acetylcholine receptors


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BACKGROUND

The brain acetylcholine neurotransmitter system is comprised of several distinct clusters of nuclei that have extensive projections to cortical and subcortical structures. Basal forebrain cholinergic nuclei are selectively destroyed in Alzheimer’s disease (AD). It has long been established that cholinergic neurotransmission plays a crucial role in learning and memory. Severe memory loss is the most prominent clinical symptom of AD, and this memory impairment is associated with impairment in brain acetylcholine levels. Disrupted cholinergic neurotransmission is not the single cause of memory loss in AD, but together with substantial neuronal loss in critical medial-temporal lobe memory circuits, cholinergic disruption contributes to impaired memory in AD [1].

There are two broad classes of acetylcholine receptors in the nervous system that differ in their primary agonists. Muscarinic acetylcholine receptors (mAChRs) respond to the agonist, muscarine. Nicotinic acetylcholine receptors (nAChRs) are activated by the agonist, nicotine, that acts like acetylcholine on the nAChR. Cholinergic afferents to the hippocampal formation are generated from a single source in the medial septal area [2]. Both mAChRs [3] and nAChRs [4] are present in the same target regions of these cholinergic afferents. It is likely that both muscarinic and nicotinic receptor types are activated in parallel. Cholinergic modulation of hippocampal function likely reflects a complex, dynamic combination of mAChR and nAChR activation, rather than an exclusive action of either type of receptor. We explored mAChR effects on associative learning by blocking mAChRs with scopolamine when a cognition-enhancing drug, nefiracetam, was administered.

DELAY EYEBLINK CLASSICAL CONDITIONING AND THE SEPTOHIPPOCAMPAL SYSTEM

Bartus, Flicker, and Dean [5] emphasized the critical necessity of animal models for the investigation of learning and memory in normal aging and AD, and they created logical criteria for developing animal models for pharmacological applications. Applying these criteria to the model system of eyelid classical conditioning in the rabbit demonstrated the strength of this approach to investigations of associative learning, aging, and AD [6]. A large research literature has amassed about the neural structures and systems that are involved in eyelid classical conditioning, and the neural circuitry and effects of normal aging are similar in all mammals that have been investigated, including humans. Although the neural circuitry essential for acquisition and retention of the conditioned eyelid response resides in the cerebellum, the hippocampus is engaged during basic associative learning represented by simple delay eyelid classical conditioning [7]. In the delay procedure, a neutral stimulus such as a tone conditioned stimulus (CS) is presented half a second before the onset of a corneal airpuff eyelid-eliciting unconditioned stimulus (US). The organism learns to blink to the tone CS before the onset of the airpuff US, and the learned response is called the conditioned response (CR).

Whereas outright removal of the hippocampus [8] or direct hippocampal infusions of scopolamine [9] do not impair delay eyelid conditioning, disruption of the septohippocampal system with scopolamine infusions prolongs the rate of acquisition of CRs in the delay procedure [10]. In rabbits, antagonism of mAChRs with scopolamine injections impairs acquisition of CRs, and this impairment occurs only when the septohippocampal system is intact [11]. The essential site of learning is in the cerebellum ipsilateral to the eye receiving the air puff, but disruption of the hippocampus can impair the cerebellar acquisition of CRs [12]. The septohippocampal cholinergic system modulates the rate of learning.

SEPTOHIPPOCAMPAL ACETYLCHOLINE SYSTEM AND HUMAN NEUROPATHOLOGY

Brain structures and systems of demonstrated involvement in eyelid classical conditioning in rabbits and humans are compromised during the progression of AD. For example, the septohippocampal circuitry is impaired early in AD [13], and pyramidal cells in the CA1 field of hippocampus are selectively lost [14]. Patients diagnosed with probable AD are severely impaired in eyelid conditioning beyond the impairment observed in normal aging [15–17]. It is our working hypothesis that disruption of the septohippocampal cholinergic system and selective loss of hippocampal pyramidal cells impairs acquisition of eyelid classical conditioning in AD beyond the impairment observed in normal aging.

The nootropic drug, nefiracetam (N-[(2,6-Dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetyl]amide) is a cyclic derivative of gamma-aminobutyric acid (GABA). Comparing the effect of 6 doses of nefiracetam injected subcutaneously 15 minutes before testing in the 750 ms delay eyelid conditioning paradigm, there was significantly better conditioning in rabbits treated with the 10 and 15 mg/kg doses. These doses did not cause sensitization, habituation or elevations in motor responding of the eyelid [18]. Nefiracetam also ameliorated the effect of scopolamine on eyelid conditioning in young rabbits [19]. Furthermore, nefiracetam is effective in older rabbits only when the hippocampus is intact [20]. These results support the position that nefiracetam ameliorates conditioning via the hippocampus.

A closer approximation of a human probable AD patient than either a normal older rabbit or a young rabbit injected with a cholinergic antagonist would be an older rabbit with disrupted cholinergic function. The older, scopolamine-injected rabbit model would have a disrupted septohippocampal cholinergic system resulting from the scopolamine injection. The present investigation was undertaken to explore whether nefiracetam ameliorates eyelid conditioning in scopolamine-injected older rabbits.

MATERIAL AND METHODS

Subjects

Experiments were completed by a total of 56 female retired breeder specific pathogen free (SPF) New
Zealand white rabbits. Rabbits' weight ranged between 3.0-6.2 kg with a mean of 4.0 (s.d.=0.5) kg. Their ages ranged from 24 to 58 months with a mean age of 31.8 (s.d. = 8.2) months. A one-way analysis of variance (ANOVA) comparing the ages of the various experimental groups revealed no age differences. Rabbits were individually housed in stainless steel cages and fed 17 grams of Purina high fiber rabbit chow per day and tap water. They were on a 12/12 hour light/dark cycle.

Apparatus

 Plexiglas customized rabbit restrainers were used for restraint during injection and behavioral training. Elastic eyelid retractors held the left eye open. A removable platform to hold the headstage was secured behind the ears and under the animal's muzzle. The headstage attached to this platform held a nozzle positioned 1 cm from the cornea through which the airpuff US was presented. Also on the headstage was a minitorque potentiometer for measurement of the nictitating membrane (NM)/eyelid blink response. The potentiometer was attached by a lever and a thread to a nylon suture loop in the NM. A computer programed with Form and assembly language and interfaced with the stimulus equipment controlled the presentation and duration of stimulus events, collected analog data, and extracted dependent variable measures of conditioning. A minitorque potentiometer on the headstage converted NM movements into electrical signals that were subjected to an analog-to-digital analysis. The digitized values were analyzed from RAM immediately after each training session and also routinely logged on to a floppy disk for permanent storage.

Procedure

 Rabbits were placed in restrainers for two adaptation sessions before training or drug injections began. In the second adaptation session, rabbits were given a local ophthalmic anesthetic (proparacaine hydrochloride) in the left eye so that a 6-0 nylon suture loop could be placed in the temporal margin of the NM. Sterile saline was used to irrigate the eye, and eye ointment was applied if irritation occurred. For training in the paired condition, beginning two days after the second adaptation session, rabbits were injected subcutaneously with combinations of nefiracetam, scopolamine, or vehicle 15 minutes before training. Next they were placed into the restrainer, fitted with the headstage, and then positioned in a ventilated and sound-attenuated experimental chamber. An 850-ma, 85 dB sound pressure level, 1-kHz tone CS was followed 750 ms after its onset by a 100-ms, 3-psi corneal airpuff US. The CS and US coterminated. The inter-trial-interval was random, ranging from 10-20 seconds. For the explicitly unpaired condition, adaptation and drug injection were identical to the paired condition as was headstage apparatus and placement in the experimental chamber. Tones and airpuff of the exact magnitude and duration to those in the paired condition were presented in a explicitly unpaired, pseudo-random sequence (no more than 3 stimuli of one type presented in a row). There were 45 tones and 45 airpuff presented with a randomized inter-

trial-interval ranging from 10-20 seconds. Both the paired and unpaired conditioning sessions lasted 35 to 45 minutes. Rabbits were tested two to three at a time in individual chambers. Training was completed within one hour after drug administration while the compounds were attaining peak efficacy.

Rabbits were trained for a total of 20 90-trial sessions, making the total number of paired trial presentations 1,800. The total number of explicitly unpaired stimuli was also 1,800. Twenty sessions were required because previous research with older rabbits indicated that the average vehicle-treated rabbit achieved learning criterion during the 9th session [18]. We expected that prolonging acquisition in older rabbits with scopolamine might require more than the 15 training sessions we normally used for older rabbits.

After completion of the 20th training session, animals were deeply anesthetized with Nembutal and decapitated. Brains were removed rapidly and frozen for later analysis.

Behavioral Data Analyses

A CR was automatically scored by the computer system if the NM movement was 0.5 mm or greater and occurred in the period between 25 to 750 msec after onset of the tone CS. Amplitude measurements on this system were calibrated to be accurate to the nearest 0.1 mm, and latency measurements were accurate to the nearest millimeter. The UR was the reflexive blink to the corneal airpuff and was scored as any response of 0.5 mm or greater occurring in the interval between 751 to 1041 msec after CS onset. Response latency was the time in milliseconds between CS onset and the first response of 0.5 mm or greater. Amplitude of the UR was measured at the point of peak amplitude of the response.

Drugs

Nefiracetam was supplied by Daiichi Pharmaceutical Co, Ltd. Dosage levels of 10 and 15 mg/kg nefiracetam were effective in ameliorating impaired eyelid blink conditioning in older rabbits, whereas a 5 mg/kg dose did not cause significant amelioration of learning and was used for comparison [18]. For muscarinic cholinergic antagonism, scopolamine (Sigma) was administered at dosage levels of 1.5 mg/kg. These drugs were dissolved in sterile saline, administered 1 ml/kg, and injected subcutaneously in a shaved area on the back at the shoulders. Nefiracetam was dissolved in sterile saline and administered 5 ml/kg. Scopolamine was prepared in solution as needed, stored in a refrigerated, covered container, and injected prior to nefiracetam or vehicle. Nefiracetam was freshly prepared in solution each day and injected after scopolamine and 15 min before behavioral testing. Since the highest concentration possible of nefiracetam in solution is 5.0 mg/ml, nefiracetam was dissolved in solution in concentrations of 1.67, 3.33, and 5.0 mg/ml and the amount of solution was tripled in order to get dosage levels of 5, 10, and 15 mg/kg. The vehicle was sterile saline.
Research Design

There were 5 groups of rabbits (8 rabbits/group) receiving paired presentations of tone and air puff and 2 groups receiving explicitly unpaired presentations of stimuli. Animals received combinations of nefiracetam, scopolamine, or vehicle. Among the 5 paired groups, 1.5 mg/kg of scopolamine was injected to Groups 1–4 that also received the following doses of nefiracetam (in mg/kg): 0 (Group 1), 5 (Group 2), 10 (Group 3), 15 (Group 4). Group 5 received vehicle only. The groups receiving explicitly unpaired training received vehicle only (Group 6) or 1.5 mg/kg scopolamine and 15 mg/kg nefiracetam (Group 7). Nefiracetam, scopolamine, and vehicle were administered in separate injections given within 0.5 to 1 min of one another. Thus, the timing of drug injections in relation to the training was the same for nefiracetam, scopolamine, or vehicle. All drugs were administered 15 min before behavioral testing. The timing of drug administration resulted in drugs attaining peak efficacy during the training session within one hour after injection [18,22]. All volumes of total injected solutions were balanced among the groups of rabbits tested with scopolamine and nefiracetam by injecting extra vehicle into those rabbits not treated with nefiracetam.

RESULTS

Paired conditioning in scopolamine- and nefiracetam-treated older rabbits

A 5 (Group) by 20 (Training session) repeated measures analysis of variance (ANOVA) was conducted on the dependent variable percentage of CRs in the 5 groups receiving paired delay eyelink conditioning, scopolamine and nefiracetam treatment, and 20 sessions of testing. This analysis revealed a significant effect of Group, F (4,34) = 3.22, p = 0.024, a significant effect of Training session, F (19,646) = 17.95, p < 0.0001, and a significant interaction between Group and Training session, F (76,646) = 2.40, p < 0.0001 (Figure 1). Post hoc analysis of the significant Group effect using the Tukey Honestly Significant Difference (HSD) test indicated that percentage of CRs over the 20 sessions in the vehicle-treated rabbits was significantly greater than in rabbits treated with scopolamine + vehicle, scopolamine + 5 mg/kg nefiracetam, and scopolamine + 10 mg/kg nefiracetam. However, percentage of CRs in rabbits treated with scopolamine + 15 mg/kg nefiracetam was not different from rabbits treated with vehicle. The 15 mg/kg dose of nefiracetam reversed the effect of scopolamine so that this group performed like vehicle-treated rabbits. Post hoc analysis of the significant interaction effect using the Tukey HSD test indicated that the group injected with vehicle showed a significantly higher percentage of CRs than the group injected with scopolamine + vehicle, scopolamine + 5 mg/kg nefiracetam, and scopolamine + 10 mg/kg nefiracetam in Training sessions 13, 15, 18, 19, and 20.

A 5 (Group) by 20 (Training session) repeated measures ANOVA of the dependent measure of CR amplitude revealed a somewhat comparable pattern of significant effects. There was a significant effect of Group, F (4,34) = 4.17, p = 0.007, a significant effect of Training session, F (19,646) = 6.25, p < 0.0001, and a significant interaction between Group and Training session, F (76,646) = 2.44, p < 0.0001. Post hoc analysis of the significant Group effect using the Tukey HSD test indicated that CR amplitude over the 20 sessions in the vehicle-treated rabbits was significantly greater than in all other groups of rabbits. Post hoc analysis of the signifi-
Figure 2. Left: Mean average session response latency over 20 training sessions for 5 groups of older rabbits injected with combinations of 1.5 mg/kg scopolamine and nefiracetam (0, 5, 10, or 15 mg/kg) or sterile saline vehicle alone. Asterisks indicate significant difference from vehicle group. Error bars are standard error of the mean. Right: Response latency in each of 20 60-trial training sessions in 5 groups of older rabbits injected with combinations of 1.5 mg/kg scopolamine and nefiracetam (0, 5, 10, or 15 mg/kg) or sterile saline vehicle alone. Rabbits were tested in the 750 ms delay eyelink classical conditioning procedure. An asterisk indicates sessions in which the groups were significantly different.

significant interaction effect using the Tukey HSD test indicated that the group injected with vehicle showed a significantly higher CR amplitude than the other groups in Training sessions 13, 14, 15, 18, 19, and 20.

As conditioning occurs, the dependent measure of response latency shifts from the UR period (response after US onset longer than 750 ms) to the CR period (response preceding US onset shorter than 750 ms). A 5 (Group) by 20 (Training sessions) repeated measures ANOVA of the dependent measure of response latency revealed this shift in latency along with a significant difference between the treatment groups (Figure 2). There was a significant effect of Group, F(4,54)=3.22, p=0.025, a significant effect of Training session, F(19,646)=17.03, p<0.0001, and a significant interaction between Group and Training session, F(76,646)=2.03, p<0.0001. Post hoc analysis of the significant Group effect using the Tukey HSD test indicated that response latency over the 20 sessions in the vehicle-treated rabbits was significantly shorter than in rabbits treated with scopolamine + vehicle and scopolamine + 10 mg/kg nefiracetam. However, response latency in rabbits treated with scopolamine + 5 mg/kg nefiracetam and scopolamine + 15 mg/kg nefiracetam was not different from rabbits treated with vehicle. The response latency measure indicated that both the 5 and 15 mg/kg dose of nefiracetam reversed the effect of scopolamine so that these groups performed like vehicle-treated rabbits. As indicated in Figure 2, rabbits treated with scopolamine + 5 mg/kg nefiracetam had faster response latencies earlier in training (e.g. Training sessions 3, 4, 6, 8), whereas rabbits treated with scopolamine and 15 mg/kg nefiracetam had faster response latencies later in training (e.g. Training sessions 17–20). Post hoc analysis of the significant interaction effect using the Tukey HSD test indicated that the group injected with vehicle showed a significantly shorter response latency than the group injected with scopolamine + vehicle, scopolamine + 5 mg/kg nefiracetam, or scopolamine + 10 mg/kg nefiracetam in Training sessions 13, 14, 15, 19, and 20.

The amplitude of the UR provides a measure of the strength of the reflexive NM response to the US. A 5 (Group) by 20 (Training sessions) repeated measures ANOVA of the dependent measure of UR amplitude was carried out. There was a significant effect of Group, F(4,54)=3.44, p=0.018, and a significant effect of Training session, F(19,646)=8.81, p<0.0001. The interaction between Group and Training session did not attain statistical significance. Post hoc analysis of the significant Group effect using the Tukey HSD test indicated that UR amplitude over the 20 sessions in the vehicle-treated rabbits was significantly greater than in rabbits treated with scopolamine + 10 mg/kg nefiracetam (Figure 3). In the case of the significant Training session effect, as is typical in delay eyelink conditioning, UR amplitude was lower in the first few training sessions and then became stable over subsequent sessions.

Unpaired conditioning

Unpaired conditioning provides a way to test the effects of nefiracetam and scopolamine on sensory and motor systems to determine whether sensitization or habituation affected performance. Animals receiving explicitly unpaired stimulus presentations were Group 6 (sterile saline vehicle) and Group 7 (1.5 mg/kg scopolamine and
(Training sessions) repeated measures ANOVA was conducted comparing the dependent measure of responses in the CR period. The Group, Training sessions, and interaction effect were not significant. Scopolamine combined with nefiracetam had no effect on the rate of reflexive blinking to the tone. The drugs did not sensitize responding to the tone CS, nor did habituation occur more readily in drug-treated animals.

UR amplitude was measured on airpuff-only unpaired trials. A 2 (Group) by 20 (Training sessions) repeated measures ANOVA was conducted comparing the 2 groups tested with unpaired conditioning on the dependent measure of UR amplitude. The effect of Group was significant, F(1,14)=4.98, p=0.042. The group treated with vehicle had significantly lower UR amplitude than the group treated with scopolamine and 15 mg/kg nefiracetam (Figure 3). The effect of Training sessions was also significant, F(19,266)=4.80, p<0.0001. UR amplitude was lower in the first few training sessions and then became stable over subsequent sessions.

**DISCUSSION**

Scopolamine injections result in a significant impairment in older rabbits' ability to acquire CRs in the 750 ms delay eyelink classical conditioning paradigm. The statistically significant interaction effects between percentage of CRs and response latency occurred as the result of differences between scopolamine- and vehicle-treated older rabbits. This significant impairment caused by injections of 1.5 mg/kg scopolamine was ameliorated by injections of 15 mg/kg nefiracetam. Older rabbits injected with 1.5 mg/kg scopolamine and 15 mg/kg nefiracetam had CR percentages and response latencies similar to vehicle-treated older rabbits.

Whereas 15 mg/kg nefiracetam ameliorated the effect of scopolamine and resulted in older rabbits performing as well as vehicle-treated rabbits, it did not further ameliorate the age-related deficit. Previously, we demonstrated that the 10 mg/kg dose of nefiracetam enabled older rabbits to acquire CRs in the 750 ms delay paradigm as rapidly as young rabbits [18]. Data in the present study indicated that the beneficial effects of nefiracetam were limited to reversing the effects of 1.5 mg/kg scopolamine and bringing scopolamine-injected older rabbits to a performance level comparable to vehicle-injected older rabbits.

It is in the repeated measures analyses with the dependent measures of percentage of CRs, CR amplitude, and response latency that the impairing effects of 1.5 mg/kg scopolamine on older rabbits are most evident. The session by session data (Figures 1 and 2) demonstrate that over 20 sessions scopolamine-treated rabbits were clearly impaired even when they received nefiracetam. The gap between the vehicle-treated group and the other four scopolamine-treated groups is evident in later trials.

Most studies of scopolamine effects on delay eyelink conditioning in young rabbits used a dose of 1.5 mg/kg scopolamine [11,19,21,22]. Young rabbits in our labora-
tory treated with 1.5 mg/kg scopolamine were 4-month-old young females weighing an average of 2.2 kg [19]. The daily 1.5 mg/kg scopolamine injection was 5.3 mg. Older female retired breeder rabbits’ weight averaged 4.0 kg. The average daily 1.5 mg/kg scopolamine injection was 6.0 mg. The difference in brain size between young and older rabbits is minimal, the metabolism of the older rabbits is slowed, yet the average daily injections for older rabbits contained about twice as much scopolamine due to the older rabbits’ greater body weight. Lower doses of cholinergic antagonists may be more appropriate for older animals.

UR amplitude in the paired groups was similar in all of the scopolamine-injected groups with the exception of the group receiving scopolamine + 10 mg/kg nefircetam. In the explicitly unpaired groups, vehicle-treated rabbits had significantly lower UR amplitude. The amplitude of the NM response is a control for potential motor effects of the drugs. UR amplitude of 10 mg/kg nefiracetam alone was also reduced [18], suggesting that the nefiracetam rather than the scopolamine resulted in reduced UR amplitude. However, UR amplitude in the scopolamine + 15 mg/kg nefiracetam was not different from vehicle in the unpaired group and was higher than vehicle in the explicitly unpaired group. Although it is preferable that the UR amplitude measure is similar in all groups, it is not possible that the UR amplitude differences in this study account for the results. In previous work with nefiracetam [18], the highest percentage of CRs was obtained in the groups that had UR amplitude significantly lower than vehicle-treated rabbits (5, 10, and 15 mg/kg nefiracetam alone groups). Higher magnitude of motor responding does not explain the ameliorating and scopolamine-reversing effects of 15 mg/kg nefiracetam in this data.

Implications of the scopolamine-injected older rocket model

We proposed that patients with probable AD would be impaired on eyeblink conditioning based on behavioral evidence available from older rabbits and scopolamine-injected young rabbits. This hypothesis was supported [16], independently replicated in another laboratory [15], and replicated and extended in our laboratory with new samples of patients and normal control participants [17,23]. In the later study, we also demonstrated that in some cases eyeblink conditioning was effective in differentiating cerebrovascular dementia from probable AD. In addition to probable AD patients, the result was observed in adults with Down’s syndrome over the age of 55 who inevitably develop AD (called DS/AD) [24,25].

Because rabbits with disrupted hippocampal cholinergic systems have delayed acquisition of CRs but eventually acquire them [11], we predicted that if probable AD and DS/AD patients were given enough training trials, they would eventually produce CRs. Probable AD and DS/AD patients tested on eyeblink conditioning for five consecutive days eventually learned [26]. Another study testing probable AD patients in paired tone and corneal airpuff presentations in the 400 ms delay paradigm for 4 consecutive 70-trial sessions reported similar results [27].

Evidence has accumulated to suggest that drug-related modification of CR acquisition occurs via the hippocampus. Rabbits treated with scopolamine showed impairment in acquisition of eyeblink conditioning only when the hippocampus was intact [11]. Older rabbits treated with nefiracetam showed amelioration of learning deficits only when the hippocampus was intact [20]. The present study was an additional preclinical test of the potential efficacy of nefiracetam in probable AD using the animal model from which the predictions for AD were derived: the scopolamine-injected older rabbit.

Conclusions

At a dose of 15 mg/kg, the drug nefiracetam’s promotion of acetylcholine neurotransmission was sufficient to reverse the cholinergic disruption of scopolamine. As the scopolamine-injected older rabbit is a model for AD, nefiracetam may be effective at ameliorating the learning deficits associated with impaired acetylcholine neurotransmission in AD. However, a dose of 15 mg/kg nefiracetam only reversed the impairing effects of 1.5 mg/kg scopolamine. The 15 mg/kg dose did not improve learning beyond the diminished levels associated with vehicle-treated older rabbits. Additionally, the impaired performance observed in later training sessions indicates that a daily scopolamine dosage of 1.5 mg/kg may be excessive for older rabbits, which are larger and metabolically slower.

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