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## Neuroprotective and Cognition-Enhancing Properties of MK-801 Flexible Analogs Structure-Activity Relationships

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**ABSTRACT:** Neuroprotective and biobehavioral properties of a series of novel open chain MK-801 analogs, as well as their structure-activity relationships have been investigated. Three groups of compounds were synthesized: monobenzylamino, benzhydrylamino, and dibenzylamino (DBA) analogs of MK-801. It was revealed that DBA analogs exhibit pronounced glutamate-induced calcium uptake blocking properties and anti-NMDA activity. The hit compound of DBA series, NT-1505, was investigated for its ability to improve cognition functions in animal model of Alzheimer's disease type dementia, simulated by treating animals with cholinotoxin AF64A. The results from an active avoidance test and a Morris water maze test showed that experimental animals, treated additionally with NT-1505, exhibited much better learning ability and memory than the control group (AF64A treated) and close to that of the vehicle group of animals (treated with physiological solution). Study of NT-1505 influence on locomotor activity revealed that it is characterized by a spectrum of behavioral activity radically different from that of MK-801, and in contrast to the latter one does not produce any psychotomimetic side effects in the therapeutically significant dose interval. The computed docking of MK-801 and its flexible analogs on the NMDA receptor elucidated the crucial role of the hydrogen bond formed between these compounds and the asparagine residue for magnesium binding in the NMDA receptor. It was suggested that strong hydrophobic interaction between MK-801 and the hydrophobic pocket in the NMDA receptor-channel complex determines much higher irreversibility of this adduct compared to the intermediates formed between this site and Mg ions or flexible DBA derivatives, which might explain the absence of PCP-like side effects of the latter compounds.

**KEYWORDS:** Neuroprotection; MK-801; NMDA-receptor; Antagonists; Docking; Cognition enhancer.

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## INTRODUCTION

It is generally recognized that specific blockade of calcium ion influx via hyper-activated glutamate receptor-channel complexes (GluR), in particular NMDA subtype receptors, can provide efficient protection against diverse group of neurological disorders related to glutamate excitotoxicity, such as brain ischemia and Alzheimer's disease (AD). In the latter, it is considered that NMDA receptor antagonists may provide efficient protection of neurons against neurotoxic action of beta-amyloid peptide (A $\beta$ ), realized, in part, by potentiating the excitotoxic effects of endogenous glutamate.<sup>1</sup> On the other hand, it is assumed that glutamate receptor agonists (in particular, AMPA subtype receptor agonists) can exhibit strong cognitive enhancing effects due to activation of glutamatergic neurotransmission that is otherwise reduced in the course of AD.<sup>2</sup> Such dualism in the properties of glutamatergic compounds explains the interest to these substances as promising neuroprotectors and cognition enhancers. Previously it had already been revealed that the well known noncompetitive NMDA receptor antagonist, MK-801, shows strong neuroprotective properties in cell culture experiments. However, the therapeutic potential of MK-801 is diminished by side remarkable psychotomimelic effects associated with its high affinity interaction with the intrachannel binding site (ICS) of the NMDA receptor.<sup>3</sup> In contrast to MK-801, the low affinity open channel blockers, such as amantadine, memantine, or ARL 15896, often show better therapeutic indices because of their rapid blocking kinetics and strong voltage dependency.<sup>4,5</sup> The goal of the study reported here was to provide: (1) focused synthesis of flexible analogs of MK-801, (2) a computational estimate of the binding of MK-801 and its flexible analogs to the NMDA receptor by the molecular docking method, and (3) a study of neuroprotective and behavioral (in particular, cognition-enhancing) properties of series of novel open chain MK-801 analogs whose side effects were expected to be minimized due to less rigid interaction of ICS with flexible molecules relative to MK-801.

## METHODOLOGY OF DIRECTED SEARCH FOR NEUROPROTECTORS AND COGNITION ENHANCERS

To screen and study the action of potential neuroprotectors and cognition enhancers among wide spectrum of GluR ligands a battery of *in vitro* and *in vivo* tests and models was used in the present study. This set of tests includes:

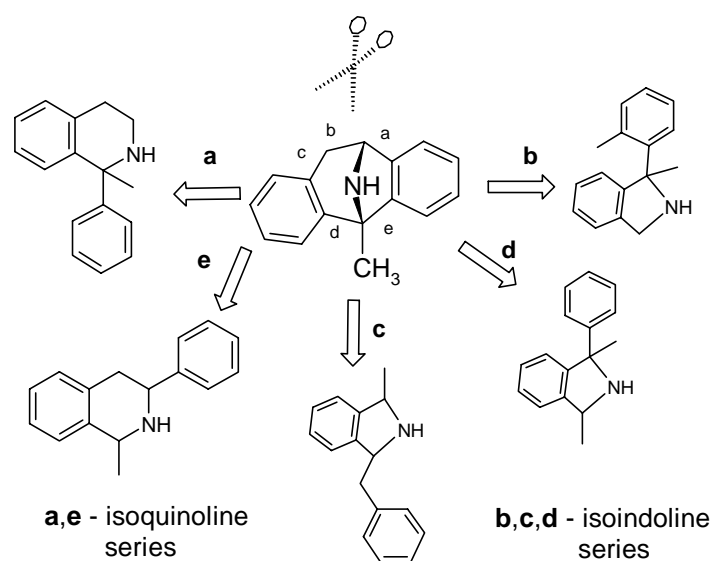
1. Inhibition of total and specific glutamate-stimulated calcium ion (Ca<sup>2+</sup>) uptake in rat brain synaptosomes;
2. Study of the ability of the compound to prevent the development of NMDA-induced convulsion and lethality in experimental animals — as a measure of anti-NMDA activity.
3. Animal behavioral study of the cognition-enhancing properties for the most promising compounds in an active avoidance test and in a water maze test, after artificial cholinergic neurodegeneration in rat brain induced by a specific synthetic cholinotoxin, ethylcholine aziridinium ion (AF64A).

As a first step the ability of each compound to inhibit glutamate-induced  $\text{Ca}^{2+}$  uptake (in rat brain synaptosomes) and to manifest anti-NMDA activity was estimated. This permitted us to limit the total number of potentially promising structures and to create a uniform data bases for QSAR analysis and docking. In the second stage of our research, the most promising compounds ("hit compounds") were studied for their neuroprotective and cognition enhancing properties in animal models of AD-type degeneration.

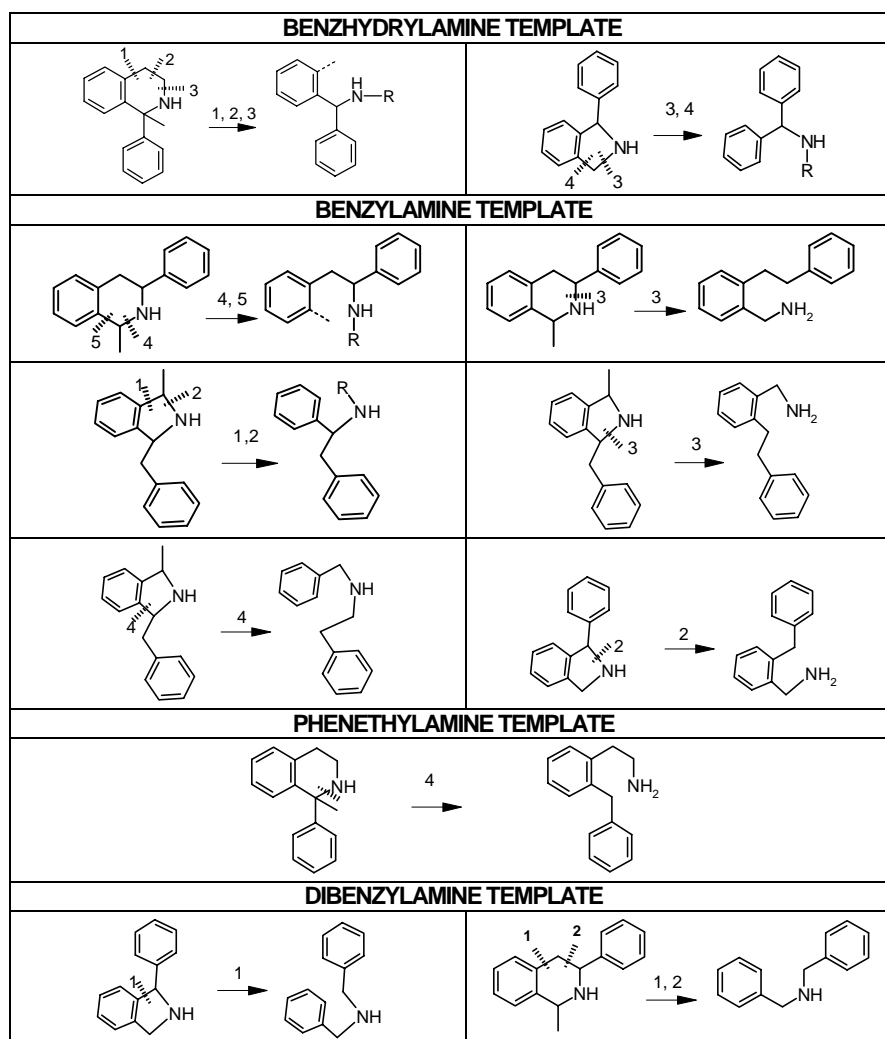
### TOTALLY OPEN CHAIN ANALOGS OF MK-801 STRATEGY FOR TEMPLATE GENERATION AND SYNTHESIS

Disclosure of the MK-801 bicycle leads to the generation of two types of compounds: (1) open chain analogs as a result of breaking one cycle (see FIGURE 1): and (2) totally open chain analogs as a result of breaking two cycles (see FIGURE 2). Open chain analogs include two groups of compounds: the isoquinoline series and the isoindoline series (FIG. 1). The molecular shapes of these molecules are very similar to MK-801, and some of the compounds display properties of high affinity open channel blockers and associated side effects, such as isoquinoline FR-115427.<sup>6</sup>

Four templates result from the further opening of isoquinoline and/or isoindoline cycles to form totally open chain types of MK-801 analogs: monobenzylamine, benzhydrylamine, phenethylamine, and dibenzylamine series (FIG. 2). These compounds are very similar, but essentially more flexible molecules, relative to MK-801. Focusing libraries contained 100-200 substances were synthesized in each series, forming a total of about 600 compounds. The functional variation<sup>7</sup> in each series included changes "around" the template (introduction of substituents to aryl, and/or alkyl, and/or nitrogen, and bioisosteric substitution of aryls in the template) but not involving a change of template (e.g., transformation of the template into novel cycles).



**FIGURE 1.** Template generation for isoquinoline and isoindoline series.



**FIGURE 2.** Opening of isoquinoline and/or isoindoline cycles leads to the generation of total open-chain templates (benzhydrylamines, benzylamines, and dibenzylamines).

## MOLECULAR DOCKING

### *Homology Modeling*

Amino acid sequences of human GLUR1 (Swiss Protein Data Bank accession number P42261), GLUR2 (P42262), GLUR3 (P42263), GLUR4 (P48058), GLUR5 (P39086), GLUR6 (Q13002), GLUR7 (Q13003), KA1 (Q16099), KA2 (Q16478), NR2A (Q12879), NR2B (Q13224), NR2C (Q14957), NR2D (O15399), and NR1 (P35437) subunits were multiply aligned with the amino acid sequence of the K<sup>+</sup> channel from *Streptomyces lividans*<sup>8</sup> (KcsA K<sup>+</sup> channel, Brookhaven Protein Data Bank<sup>9</sup> accession number 1BL8) using the program Clustal X.<sup>10-12</sup> The BLOSUM 30 homology matrix was used to evaluate amino acid similarity. This sequence alignment was used as the basis for homology modeling using the Biopolymer module in SYBYL 6.5.<sup>13</sup>

The X-ray structure of the KcsA K<sup>+</sup> channel was used as a template for building a NR1/NR2B/NR1/NR2B tetramer of the NMDA receptor. Chains A and C from the K<sup>+</sup> channel were "mutated" to NR2B subunits, whereas chains B and D were mutated to NR1 subunits in accord with the following procedure. All aligned non-identical amino acids were mutated to each other by changing their side chains while keeping main chain atoms unchanged and fixed at their original positions in space. Internal torsion angle values in side chains of new amino acids were chosen to be those most appropriate for a given amino acid in a given secondary structure state (which remains unchanged by mutating residues). All insertions and deletions were handled by defining "loops" containing all residues being inserted and several neighboring atoms to the positions of insertion or deletion were followed by searching for them in the Brookhaven Protein Data Bank. Subsequently, that search results were visually inspected, and main chain atoms of those *loops* for which (1) the geometry of the main chain atoms neighboring the deletion or insertion positions underwent smaller changes, (2) the amino acid sequence possessed the strongest homology to the required sequence, and (3) no unfavorable spatial hindrance between atoms belonging to the *loop* and to the remaining part of the protein could be expected, were inserted in the protein model and followed by adding appropriate side chains in accord with the aforementioned procedure. Next, all proline residues and side chains of all other residues were "fixed" by finding new values for several torsion angles so as to remove the spatial overlap between atoms. This was followed by adding hydrogen atoms that were capable of forming hydrogen bonds. The protein was then subjected to 5,000 steps of constrained energy minimization, with all main chain atoms fixed in space using the Tripos force field, as implemented in the SYBYL package. This was followed by 5,000 steps of constrained minimization without fixing the main chain atoms, but keeping the mutual spatial arrangement of several oxygen atoms at the narrowest part of the pore unchanged so as to preserve the requirements for effective ion conduction.

### *Docking Ligands*

All ligands were manually docked to the putative binding silo followed by 500 steps of energy minimization for the entire protein-ligand complex. Interactive docking was carried out by means of the Sybyl 6.5 molecular-modeling package running on a SGI Octane workstation.

### *Sequence Alignment*

In order to avoid chance effects and increase validity of the alignment used for predicting the three-dimensional structure of the NMDA receptor channel, we included all members of the ionotropic glutamate receptor family subunits into multiple alignment of amino acid residues, along with the amino acid sequence of the K<sup>+</sup>-channel. Since all sequences have different numbering for the residues, we introduced a uniform numbering for them, the rows are labeled in the multiple alignment diagram with the abbreviation C.N. The mere existence of such multiple alignment implies that the general architecture of channel pores in subunits

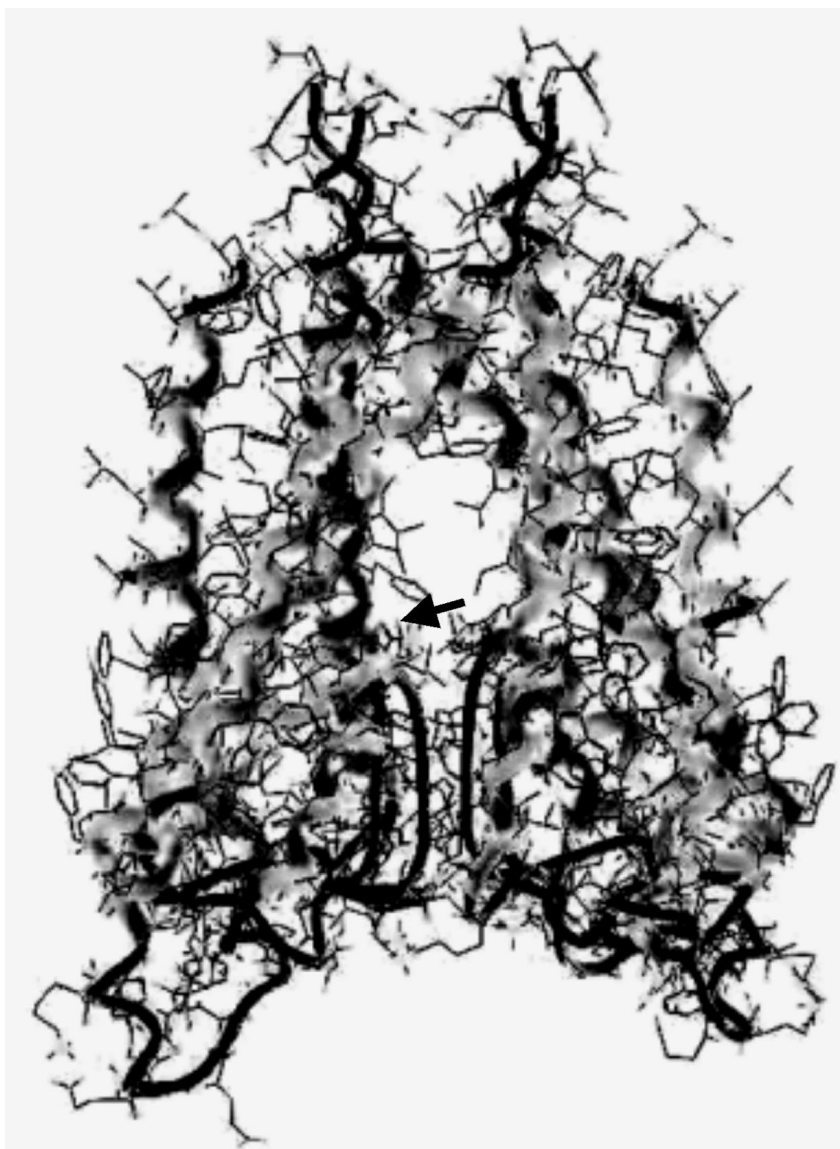
of all ionotropic receptors can actually be expected to be the same. Thus, the part in each sequence that is generally associated with transmembrane segment M1 corresponds to C.N. 3-21, the segment M2 has C.N. 51-71, and the amino acids in transmembrane segment M3 have C.N. 82-100. Four "fingerprint" amino acid residues (that correspond to the ion selectivity filter of the KcsA K<sup>+</sup> channel) in the nearest part of the channel pore have C.N. 67-70. The most important among them is generally believed to be the amino acid with C.N. 67 (Q/R/N site). For the case of AMPA receptors this residue can undergo RNA editing,<sup>14</sup> which controls the Ca<sup>2+</sup> permeability of these receptors. In the NMDA receptor, this amino acid in a NR2 subunit (Asn614 in NR2A, Asn615 in NR2B, Asn612 in NR2C, and Asn642 in NR2D) has been shown to take part in the voltage-dependent Mg<sup>2+</sup> blockage of the receptor channel.<sup>15</sup> Homologous residue Asn616 in NR1 has been shown to control the Ca<sup>2+</sup> permeability<sup>15</sup> of the NMDA receptor channel, as well as being involved in the voltage-dependent blockage by compounds believed to bind to the PCP blocking site in the receptor pore.<sup>16</sup> This residue, along with Trp611 and Ala645 in the NR1 subunit are supposed to form the PCP binding site.<sup>16</sup>

### *Architecture of the NMDA Receptor Channel*

A three-dimensional model of the NMDA receptor channel, built in accordance with the procedure considered above, is depicted in FIGURE 3. The receptor channel in this model is a heterotetramer with two-fold symmetry about a central pore. Each of its subunits consists of two transmembrane  $\alpha$ -helices, segments M1 and M3, connected by a pore region, segment M2, that consists of a turret, pore helix, and selectivity filter. Four subunits are arranged in the heterotetramer such that one transmembrane helix (segment M3) faces the central pore and the other (segment M1) faces the lipid membrane. The transmembrane helices M3 are tilted with respect to the membrane, so that the subunits open like the petals of a flower. The M3 helices are attached to the pore region near the intracellular surface of the membrane. This region contains the selectivity filter of the channel.

The selectivity filter is formed by four amino acids with C.N. 67-70. Sixteen main chain carbonyl oxygen atoms of these residues face the pore and take part in stabilizing dehydrated cations moving through it. In addition to the main chain atoms, the oxygen atoms of the asparagine side chains (C.N. 67) located near the entrance to the narrow pore formed by the selectivity filter amino acids may play an important role in dehydrating and stabilizing metal cations (since it controls the Mg<sup>2+</sup> blockage for the case of a NR2 subunit of the NMDA receptor and Ca<sup>2+</sup> permeability for the cases of the AMPA and the NR1 subunit of the NMDA receptor).

Two important features of the cavity inside the channel should be considered in connection with their possible pharmacological implications. First, the walls of the pore are formed by hydrophobic amino acids. And second, there is a narrow hydrophobic pocket between segments M2 and M3 just above the selectivity filter. The shape of this pocket is nearly complementary to the shape of the most potent phencyclidine binding site ligands, such as PCP and MK-801, and in the vicinity of this pocket there is the important Asn616 residue that controls the Ca<sup>2+</sup> permeability of the channel. The space location of this amino acid permits easy formation of a strong hydrogen bond with PCP, MK-801, and their analogs. Thus, all important



**FIGURE 3.** General view of the NMDA receptor channel modeled by homology (the arrow indicates the approximate location of MK-801-binding site).

features of the phencyclidine binding site pharmacophore, two hydrophobic areas (one formed by the pocket and one by the surface of the M3 helix facing the central part of the pore) arranged nearly perpendicularly to each other and the hydrogen bond acceptor part located near the corner of this triangle, are present here. We suggest that this part of the channel contains the phencyclidine binding site.

## MATERIALS AND METHODS

### *Estimation of Calcium-Blocking Activity*

Interaction between compounds and glutamate-dependent  $\text{Ca}^{2+}$  uptake system was studied on newborn (8-11 days old) rat brain synaptosomal  $\text{P}_2$ -fraction. Specific  $\text{Ca}^{2+}$  uptake was measured in the presence of the compound tested as a variation between  $^{45}\text{Ca}^{2+}$  content in glutamate stimulated synaptosomes and  $^{45}\text{Ca}^{2+}$  content in unstimulated synaptosomes according to the equation:

$$K = [(\text{Ca}4 - \text{Ca}3)/(\text{Ca}2 - \text{Ca}1)] \times 100\%$$

Where  $\text{Ca}1$  is the  $\text{Ca}^{2+}$  influx in a blank experiment (without glutamate and tested compounds);  $\text{Ca}2$  is the  $\text{Ca}^{2+}$  influx in the presence of glutamate only (glutamate-induced  $\text{Ca}^{2+}$  influx);  $\text{Ca}3$  is the  $\text{Ca}^{2+}$  influx in the presence of tested compound (without glutamate);  $\text{Ca}4$  is the  $\text{Ca}^{2+}$  influx in the presence of both glutamate and the tested compound.

### *Estimation of Anti-NMDA Activity*

Adult non-pedigree male mice weighing 20-24 g were used. The compounds tested were administered i.p. 30 min prior to administration of NMDA. NMDA was i.c.v. injected in doses of 0.1  $\mu\text{g}$  in 1.0  $\mu\text{l}$  of distilled water (pH = 7.0); this induced clonic seizures in 100% of mice. The anti-NMDA activity was determined as a dose of the compound under i.p. administration needed to prevent NMDA-induced seizures in 50% of mice.  $\text{ED}_{50}$  values were calculated from the linear portions of sigmoidal curves. The  $\chi$ -test was used to compare  $\text{ED}_{50}$  values for different treatments.

### *Animal Model of AD Type Dementia*

Neuronal atrophy related to the loss of cholinergic markers in some areas of human brain is known to be the most typical pathological feature of AD.<sup>17</sup> It was shown previously that i.c.v. injection of AF64A leads to degeneration of cholinergic neurons in the brain of experimental animals and reproduces some specific features of chronic cholinergic hypofunction, along with learning and memory impairments analogous to those observed in AD.<sup>18-19</sup> In our research we used a cholinotoxin-induced animal model of AD type neurodegeneration to study cognition-enhancing properties of the compounds in a Morris water maze test and in an active avoidance test.

### *Experimental Design*

#### *Compound Injections*

The experiments were performed on male Wistar rats (200-230 g weight). AF64A was prepared from commercial samples of AF64 (RBI) according to a method described elsewhere<sup>20</sup> and diluted with artificial cerebrospinal fluid (CSF). Before the surgery, rats were anesthetized with ether. Freshly prepared AF64A solution in a dose of 3 nmol/3  $\mu\text{l}$  was injected into each of lateral cerebral ventricles of experimental rats. Control rats received 3  $\mu\text{l}$  CSF. One day after the surgery AF64A injected rats received orally 2 mg/kg of Aricept (Pfizer, USA) or Memantine (RBI, Memantine HC1) or NT1505 as a suspension in 1% starch. The compounds were

injected over a period of 23 days. Ten days after the first injections, animal training in the pool was started. During the training the compounds were injected 1-1.5 hours after the daily learning trials (to study their effect on long-term memory).

#### *Morris Water Maze Test*

Experiments were performed in a circular black swimming pool 180 cm in diameter. The surface of a removable platform (10 cm) was located 2-3 cm below the water level, so that the platform was invisible to the swimming rat. All experiments were detected by means of a video camera connected with a computer and S-VHS recorder.

The training procedures aimed at finding the hidden platform were started 10 days after the first injections, with all animals receiving two escape trials a day from two different positions. On the day 11, the daily training was interrupted and a retention test was performed only on days 14 and 26. On days 5, 11, 14, and 26 after commencing the training, the movements of the rats during the test were recorded and then analyzed with the original computerized system "Behavioral Vision".<sup>21,22</sup> Four parameters were analyzed: *escape latency* (the time in seconds required for the rat to find the hidden platform), *depression index* (characterizing immobility of rats in the pool and calculated as a ratio between time that the rat spent with a speed less than a threshold value to the time of swimming with the higher speed; the threshold was set experimentally at 10 cm/s), *straightness index* (characterizing the track directionality and calculated as a ratio between trajectory length and distance from the track start to the platform center, i.e., deviation of the trajectory from the straight path-vector from start position to platform), and *strategy index* (calculated as trajectory length within the non-entering zone, as a percentage of the full trajectory of animal during the trial).

#### *Statistical Analysis*

The results were analyzed by ANOVA followed by post hoc LSD comparisons using program package STATISTICA ver. 5.0. The level of significance was preset at  $p < 0.05$ . Data are presented as mean  $\pm$  S.E.M.

#### *Active Avoidance Test*

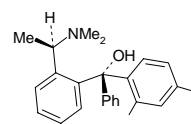
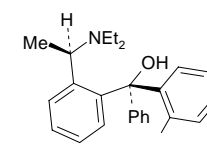
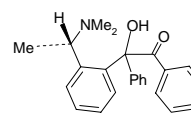
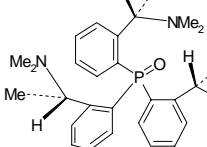
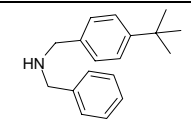
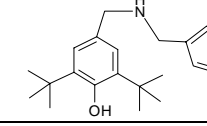
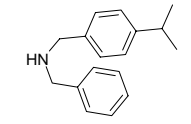
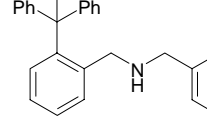
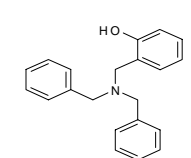
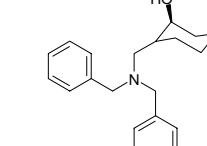
The test procedure is described elsewhere.<sup>23</sup> Briefly it is as follows: two weeks after the injection of AF64A or CSF, active avoidance training was conducted in a two-chamber shuttle box. Each chamber was 30 cm long and 20 cm wide, with a guillotine door (7 x 7 cm) between them, and a grid floor of steel rods 1 cm apart. A 12-W light bulb was fixed in the ceiling on each side. Before training, the animals were given a five-minute familiarization period in the darkened shuttle box with the central door open. The conditioned stimulus was a five-second light followed by the unconditioned stimulus, a 1 mA shock was delivered to the grid in the lit chamber. During the presentation of both stimuli, the door was open. After the rat either escaped or avoided the shock by crossing through to the other chamber, the door closed and a new trial sequence began. The intertrial interval was 30-60 sec. The time taken to escape or to avoid the shock (response latency) was measured. The avoidance during the conditioned stimulus (5 sec) was

considered a conditioned avoidance response (CAR). Training procedure consisted of 35 trials. Data from the last 15 trials (learning test) were collected and analyzed in five-trial blocks, as percentage of CAR summarized over each block. From this was calculated the mean percentage of CAR in three blocks. Twenty-five further trials were given 24 h later (retention test). In this case, data were analyzed as the mean percentage of CAR in first two blocks of five trials. Statistical significance was tested using an unpaired Student's *t*-test.  $p < 0.05$  was taken as indicative of statistical significance.

## RESULTS AND DISCUSSION

The results obtained at the first stage of investigation revealed the group of compounds that manifested strong anti-NMDA and calcium-blocking activity. Examples

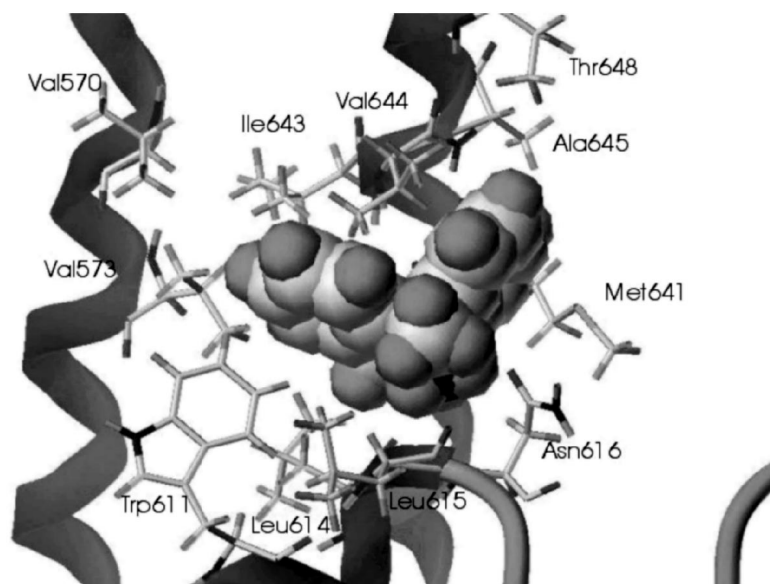
**TABLE 1. Calcium-blocking property ( $IC_{50}$ ) and anti-NMDA activity ( $ED_{50}$ ) of several totally open chain analogs of MK-801 in the monobenzylamine and dibenzylamine series.**

Structure	$IC_{50}$ ( $\mu$ M)	$ED_{50}$ (mg/kg)	Structure	$IC_{50}$ ( $\mu$ M)	$ED_{50}$ (mg/kg)
<b>NT-1505</b>	<b>15</b>	<b>10</b>	<b>LS-4206</b>	<b>11</b>	<b>30</b>
<b>NT-3101</b>	<b>6</b>	<b>16</b>	<b>NT-5001</b>	<b>18</b>	<b>35</b>
	<b>16</b>	<b>100</b>		<b>11</b>	<b>65</b>
	<b>100</b>	<b>&gt;100</b>		<b>100</b>	<b>55</b>
	<b>20</b>	<b>25</b>		<b>100</b>	<b>62</b>
	<b>31</b>	<b>52</b>		<b>100</b>	<b>70</b>
	<b>56</b>	<b>10</b>		<b>107</b>	<b>43</b>

of anti-calcium and anti-NMDA activities are presented in TABLE 1. The most interesting and promising results have been obtained in a series of newly synthesized monobenzylamine and dibenzylamine derivatives. Structure-activity analysis in these series of compounds revealed that their biological effects depend appreciably on the substituents in the benzene rings. The introduction of a bulky *ortho*-group into the each benzene rings leads to the increase of inhibition effect. The bioisosteric substitution of the benzene ring on the heteroaryl moiety (furyl, pyridyl, etc.) leads mainly to the appreciable stimulation of uptake. The hit compound in both series (NT-1505 and NT-3101) demonstrated a strong calcium-blocking property ( $IC_{50} = 15.0$  and  $6.0 \mu M$ , respectively) and anti-NMDA activity ( $ED_{50} = 10$  and  $16$  mg/kg). According to these results the most promising group of compounds, in particular, compound NT-1505, were selected for an expanded biobehavioral study. In parallel, the comparative docking study of NT-1505 and MK-801 binding with the NMDA receptor was performed.

### *Binding of Ligands to the Channel*

The three-dimensional structure of the complex between MK-801 and the putative PCP-binding site of the NMDA receptor is depicted in FIGURE 4. MK-801 forms two hydrogen bonds with the receptor, one with Asn616, and the other with the main chain carbonyl oxygen atom belonging to Leu615. In addition, MK-801 binds with one of its benzene rings deeply into the hydrophobic narrow pocket and the other sticks to the hydrophobic M3 helix. The side-chain amide group of Asn 616 also forms two hydrogen bonds: one between its oxygen atom and protonated nitrogen of MK-801, and one between its amide nitrogen and the oxygen atoms belonging to the main chain of the same residue. As a result, the oxygen atom belonging to the side chain



**FIGURE 4.** Predicted binding mode of MK-801 to the putative phencyclidine site inside the NMDA receptor channel.

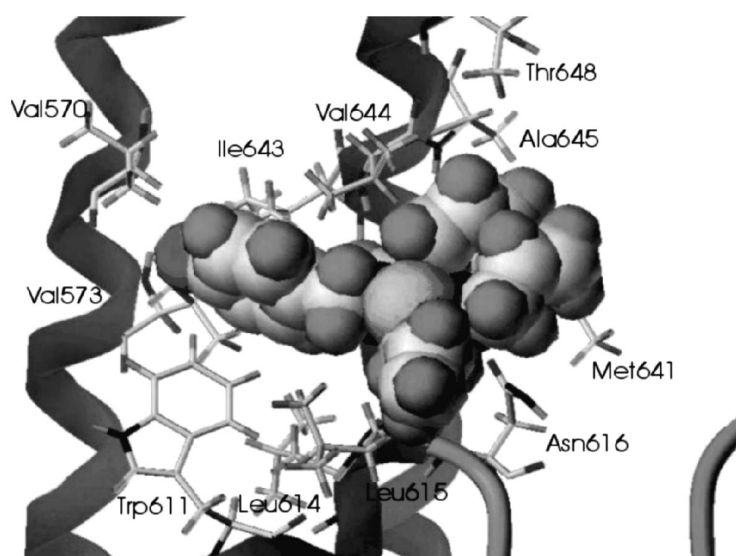
of Asn616 and the oxygen atom belonging to its main chain can no further stabilize cations inside the narrow pore formed by the selectivity filter amino acids.

The flexible MK-801 analog NT-1505 was docked to the putative PCP binding site in a conformation that mimics the spatial structure of MK-801, NT-1505 appeared to be able to bind to the same hydrophobic pocket as MK-801 in the same mode (see FIGURE 5). However, it binds to the receptor with only one hydrogen bond, the opposite site is more exposed to the channel pore, and the binding involves freezing of torsion degrees of freedom. Thus, one can suppose more favorable condition for dissociation of NT-1505 upon channel closure.

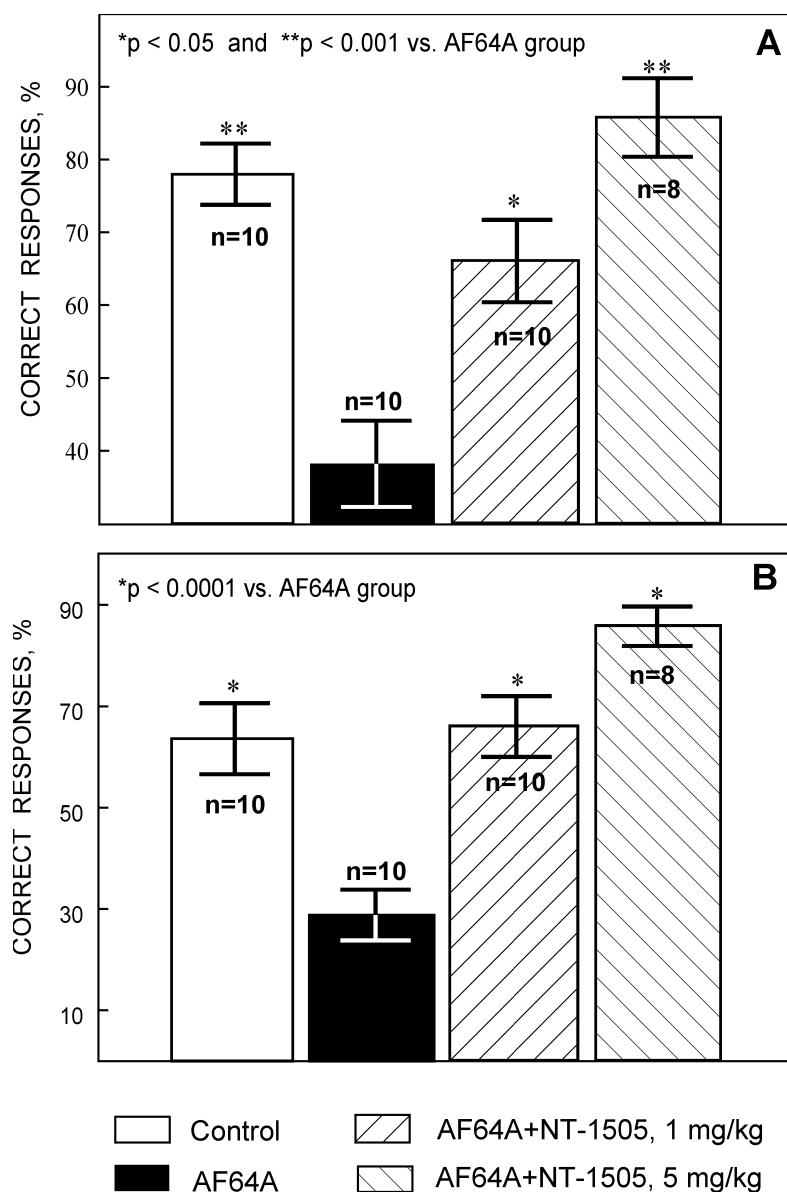
### *Behavioral Properties of NT-1505*

A preliminary examination of the cognition-enhancing properties of each compound and its ability to improve learning and memory from a toxin-induced animal model of dementia, studied in the active avoidance test. The results presented in FIGURE 6 indicate that rats treated with cholinotoxin AF64A demonstrate significant reduction in learning ability and pronounced memory impairment when compared to the vehicle group of animals treated with CSF (approximately 50% from the control level of CAR). Oral administration of NT1505 causes the recovery of the cognitive functions that deteriorate after the injection of neurotoxin AF64A, both in the learning test (the learning trials follow training on the same day) and in the retention test (the trials were performed the day after the training set). The compound produced statistically significant memory-enhancing effects at all doses, in the interval 0.5 to 5.0 mg/kg (orally) that lasted for at least 10 days after the learning trial.

A comparative long-term study of cognition enhancing properties of compound NT-1505 and widely used anti-Alzheimer medicines, Aricept and Memantine, was



**FIGURE 5.** Predicted binding mode of NT-1505 to the putative phencyclidine site inside the NMDA receptor channel.



**FIGURE 6.** Cognition-enhancing effect of NT1505 (*n* is the number of animals). A. Learning test, B. Retention test (the day after the learning trials).

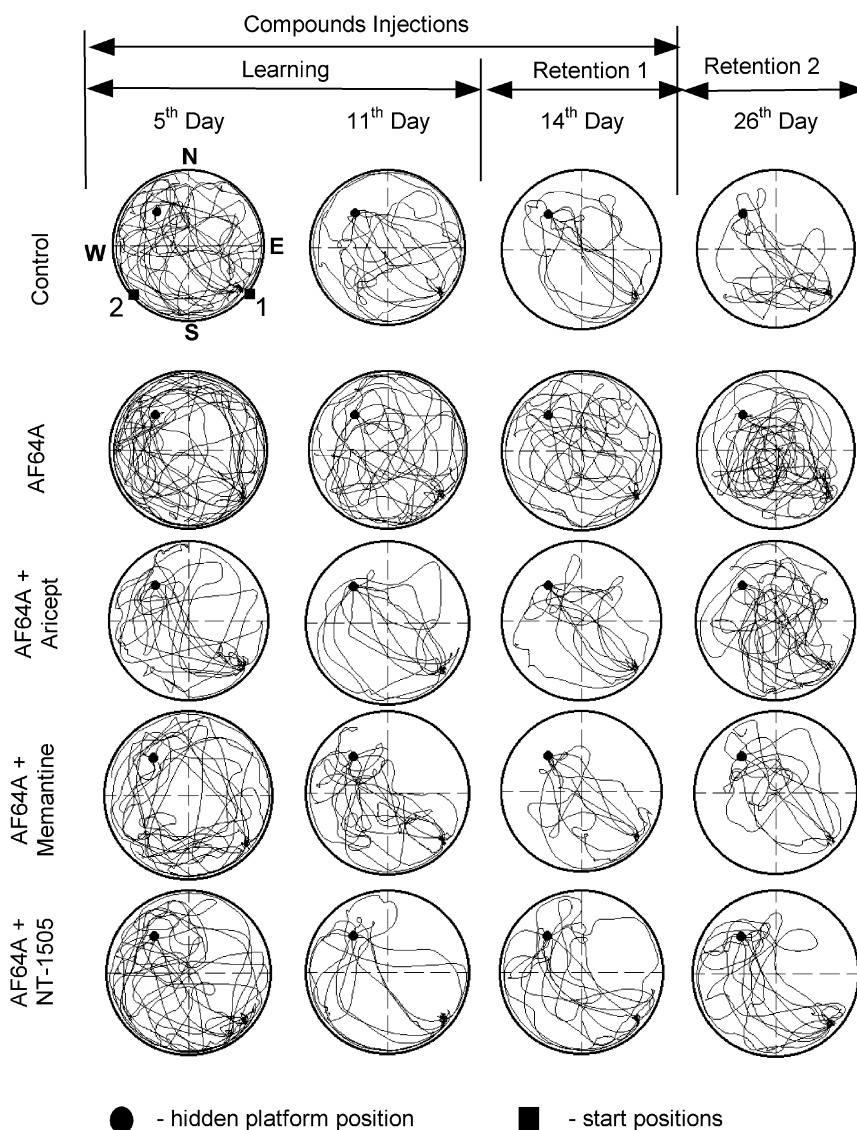
performed in a variant of the Morris water maze test in rats treated with toxin AF64A.

The superposition of swimming trajectories (start position 1) of all groups of rats in search of the hidden platform in the Morris water pool is presented in FIGURE 7. The results of computer estimated indices of escape latency and track directionality (straightness index) are presented in FIGURE 8.

Despite rather large mean escape latency observed on day 5 of the training, Aricept significantly ( $p = 0.048$ ) decreased the time and improved the trajectory in the

search of the platform, Memantine demonstrated a tendency to decrease the search time in comparison with AF64A-treated rats.

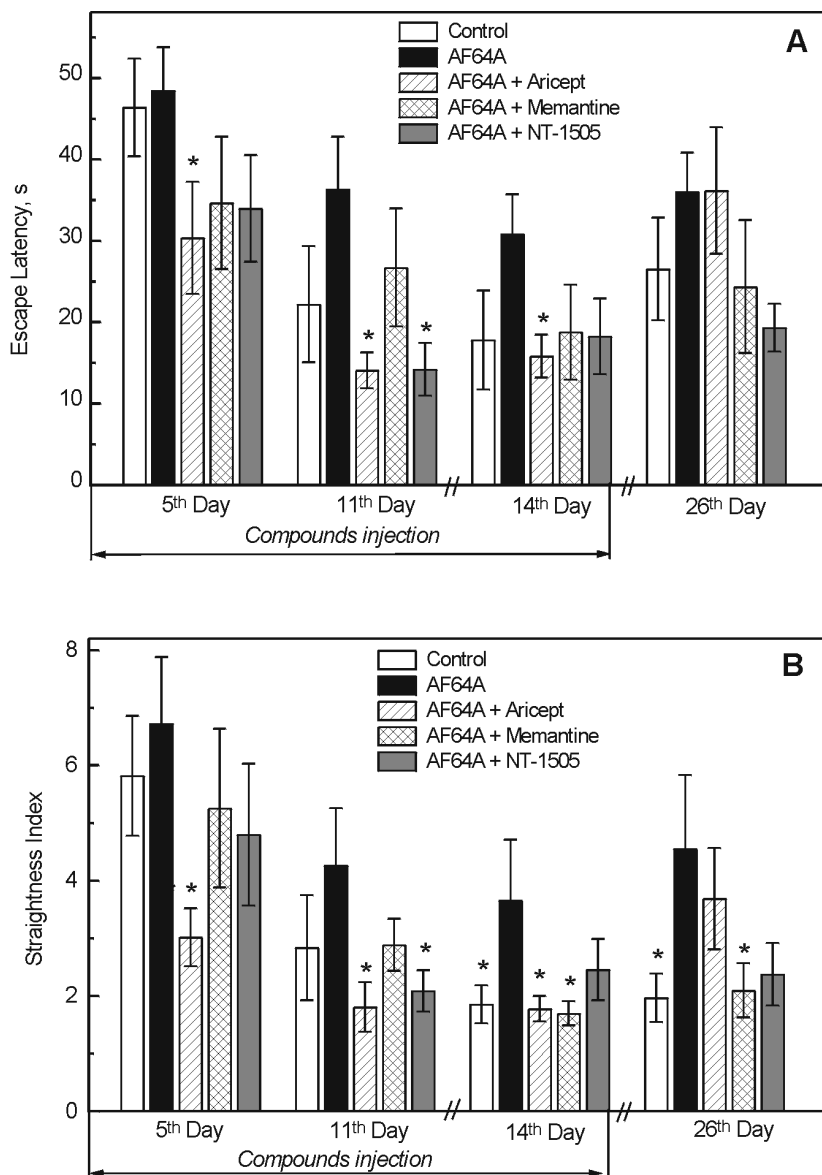
On day 11 of training, the difference between control and AF64A-injected groups in the escape latency was almost statistically significant ( $p = 0.077$ ). The AF64A treated rats receiving Aricept and NT-1505 escaped to the platform in almost the same time and this was significantly different from the AF64A group. Two-day interruption of the training with continued injections of the compounds led to a small increase in the search time for all the rats; however, for the rats receiving Aricept, this was significantly ( $p = 0.035$ ) less than that of AF64A-injected group.



**FIGURE 7.** Swimming trajectories of rats groups receiving different compounds.

The best values of the straightness index on day 14 was obtained for Aricept and Memantine. The rats receiving Memantine and NT-1505 had rather good values for the strategy index on day 14 (data not shown).

An eleven day break (day 26) in the training procedure after completing the compound injections led to a considerable deterioration in the spatial orientation of rats receiving Aricept,



**FIGURE 8.** Escape latency (A) and track directionality relative to platform position (B). Start position 1. Control,  $n = 10$ ; AF64A,  $n = 10$ ; AF64A+Aricept,  $n = 10$ ; AF64A+Memantine,  $n = 8$  (days 5 and 26),  $n = 9$  (days 11 and 14);  $n$  is the number of animals;  $*p < 0.05$ , versus AF64A group (ANOVA followed by post hoc comparisons).

and a slight decrease for the other groups, except for NT-1505-injected rats for which the ability to find the platform remained almost the same as on day 14. The most statistically significant differences among the groups, relative to AF64A-injected rats, were obtained for the straightness index.

The depression was slightly pronounced during all experiments. However, on day 26, some rats receiving Memantine and control rats swam reluctantly, demonstrating stress or less motivation (data not shown) that could lead to an increase in the escape latency.

Summarizing the results obtained at this stage, it was noted that Aricept, Memantine, and NT-1505 demonstrated statistically significant improvement in the learning ability of the experimental animals so that it became equal to the results for the control group, or even exceeded it. A significant imperfection of Aricept is its symptomatic effect, namely, cancellation of injections (results obtained on day 26) leads to a decrease in the cognitive functions of rats down to the level of the untreated group. Memantine and NT-1505 demonstrated a prolonged effect, observed after completing their injections, that allowed us to suppose that they influence regenerative and/or compensatory processes in brain. All the above data permit us to conclude that NT-1505 improves learning of rats with partial chronic deprivation of cholinergic functions in the task of finding a hidden platform, and demonstrates a long-term effect on their retention ability after finishing training and injection of the compound. The efficiency of its influence on spatial learning in the chronic experiment is almost the same as for Aricept. However, NT-1505 causes a more prolonged effect on memory functions than Aricept, which let us assume its effect on regenerative and/or compensatory processes in brain.

To reveal possible similarities and differences between NT-1505 and MK-801, a comparative study of the effects of the compounds at various doses on locomotor activity of rats was performed. It was found that MK-801 in doses of 0.1 mg/kg and higher produces a significant psychogenic effect in animals manifesting in stereotypic behavior, ataxia, and hyperactivity (see TABLE 2). In contrast to MK-801, agent NT-1505 did not potentiate stereotypy, ataxia, or behavioral activity; that is, it did not exhibit any side effects typical

**TABLE 2. Side effects of MK-801 and NT-1505**

MK-801				NT-1505			
ED <sub>50</sub> (Anti-NMDA) = 0.2 mg/kg				ED <sub>50</sub> (Anti-NMDA) = 10 mg/kg			
Dose, mg/kg	Stereotypic Movement	Ataxy	Activity	Dose, mg/kg	Stereotypic Movement	Ataxy	Activity
0.1	NO	NO	↑	0.5	NO	NO	NO
0.2	↑	↑	↑↑	1	NO	NO	NO
0.4	↑	↑↑	↑↑↑	5	NO	NO	NO
0.8	↑↑↑	↑↑↑	↑↑↑	10	NO	NO	NO
1.0	↑↑↑↑	↑↑↑↑	↑↑↑↑	50	NO	NO	↓

of MK-801. It is important to note that the absence of MK-801-like side effects is observed over wide interval of doses, from 0.5 to 50 mg/kg, which completely covers the interval of its neurological activity (according to previously published results, NT-1505 shows anti-NMDA activity in doses 10-50 mg/kg and exhibits cognition-enhancing properties at doses of 0.5-5 mg/kg). The results obtained permit us to conclude that NT-1505 possesses strong cognition-enhancing properties, manifested in a toxin-induced animal model of AD-type degeneration and characterized by a spectrum of behavioral activity radically different from that of MK-801. In particular, it does not produce any psychotomimetic side effects over the therapeutically significant dose interval.

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