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The Ability of NMDA-Type Glutamate Receptor Blockers to Prevent the Development of Pentylenetetrazole Kindling and Morphological Changes to Pyramidal Neurons in the Mouse Hippocampus

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Experiments on mice addressed the link between convulsive syndrome and morphological changes in hippocampal neurons occurring on development of pentylenetetrazole (PTZ) kindling. Kindling was induced by i.p. PTZ (35 mg/kg) three times a week for one month. By the end of this period, 70% of the mice responded to administration of PTZ with severe clonic or clonic-tonic seizures. Hippocampal sections (stratum pyramidale, field CA1, Nissl staining) from convulsive mice showed large numbers of altered cells (24.7 ± 2.1%). Most of these were pyramidal neurons. These hyperchromic neurons had reduced body sizes, loss of turgor, wrinkling of the cell body, and deformation of dendritic processes. These dark-type changes were present in 2.3 ± 2.1% of neurons in the hippocampus of intact mice and mice resistant to the convulsogenic effect of PTZ (30% of the population). Immunohistochemical studies demonstrated normal expression of NeuN (Fox3) protein in all hippocampal cells, including dark hyperchromic neurons. This is evidence that neurons did not die en masse and were relatively viable. Prophylactic s.c. administration of NMDA receptor blockers (0.5 mg/kg memantine, 0.1 mg/kg IEM-1921, or 1 mg/kg IEM-1958) decreased the proportion of mice developing PTZ kindling from 70% to 40%. The proportion of altered neurons in the 60% of mice given NMDA blockers and not developing PTZ kindling or convulsions in the presence of blockers was 0.1 ± 0.06%, which was the same as in intact mice. Conversely, the hippocampus of mice demonstrating convulsions despite simultaneous administration of NMDA blockers showed 24.0 ± 5.6% hyperchromic neurons. These results provide evidence that pathologically altered neurons appeared after convulsive seizures in animals after PTZ kindling and that blockade of NMDA glutamate receptors could weaken both the development of convulsive syndrome and the concomitant morphological changes to hippocampal neurons.

Keywords: hippocampus, pentylenetetrazole, kindling, dark neurons, memantine (1-phenylcyclohexylamine), IEM-1921, NMDA blockers, epilepsy.

Glutamatergic synaptic transmission plays an important role in the genesis of a variety of pathological processes in the nervous system, particularly the development of convulsive states [39, 41, 42, 49, 56]. Convulsions can be evoked by the intense and/or long-lasting excitatory actions of transmitter on the postsynaptic glutamate receptors of neurons involved in the pathological process. Blockade of glutamate receptors can, correspondingly, prevent or cure convulsive states, while selective glutamate receptor blockers can be used as pharmacological tools for detailed studies of the pathogenesis of convulsive phenomena and for researching anticonvulsive drugs.

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Many models have now been developed for experimental reproduction of convulsive states induced by various means (administration of glutamate and its agonists, pentylenetetrazole (PTZ, corasol), pilocarpine, electrical stimulation of various parts of the brain, electric shock, etc.). These models have been used to evaluate the therapeutic and prophylactic effects of test anticonvulsive compounds. Experimental treatments can be used once at relatively high doses to elicit severe convulsive seizures, which can result in delayed lethal outcomes. The same treatment, but at threshold or subthreshold intensity, can also be used repeatedly with quite long intervals. This results in increases in the animal's sensitivity to the agent inducing convulsions (kindling). Kindling imitates the state of convulsive readiness seen in patients during the period preceding a successive epileptic seizure [22, 41, 42, 44]. PTZ is one of the most commonly used agents for modeling kindling. The convulsive action of this compound is based on its suppressing action on GABA release, as well as its postsynaptic action [21, 26, 31]. The excitatory synaptic mechanisms mediated by activation of glutamate receptors are disinhibited. Extensive data have been obtained on the ability of NMDA-type receptor antagonists to suppress the development of the convulsive manifestations of PTZ kindling in mice and rats [9, 29, 41, 42, 56]. Modeling of PTZ kindling also produces morphological and histochemical changes to neurons [10, 11, 14, 27, 58]. It is not entirely clear that convulsions are the direct cause of these changes and that these changes are always evidence of neuron degeneration and death. The aim of the present work was to study morphological changes in mouse hippocampal neurons occurring as a result of PTZ kindling and the possible correlations between the abilities of NMDA-type glutamate receptor blockers memantine, IEM-1921, and IEM-1958 to prevent both the convulsive manifestations of kindling and the accompanying morphological changes.

Methods

Studies were performed using white male rats (SHR) weighing 18–20 g from the Rappolovo supplier. Animals were kept in the animal house with free access to water and food. The experimental conditions were standardized and spread in the results was minimized by performing all experiments at the same time of day, from 11:00 to 14:00. PTZ (Sigma, USA) was given at the subthreshold dose of 35 mg/kg i.p. Single doses of PTZ did not elicit any changes in motor activity or behavior. The same dose was repeated with intervals of at least 48 h three times a week (for one month). The effects of sequential doses of PTZ were assessed in terms of increases in the proportion of animals developing convulsions (kindling). The nature and intensity of convulsive reactions were evaluated in points using a modified Racine scale [50]: 0 – no reaction; 1 – mild twitching of the facial musculature; 2 – individual convulsive waves propagating along the axis of the trunk; 3 – increased motor activity with severe myoclonic convulsions without loss of posture; 4 – generalized clonic-tonic convulsions during which the animal fell onto its side for short periods but remained able to recover posture; 5 – tonic convulsions with marked extension of the tail and limbs. During each experimental day, convulsion intensity was measured in four groups of animals (at least 10 in each group): a control group and three experimental groups.

Animals of the control group initially received s.c. physiological saline, followed 30 min later by a standard i.p. dose of PTZ (35 mg/kg). Mice of the experimental groups received an s.c. standard dose of one of the glutamate blockers of interest 30 min before PTZ. The volumes of all solutions given were 0.1 ml/20 g body weight. Throughout chronic experiments, mice of the three experimental groups received prophylactic doses of NMDA receptor blockers: the adamantane derivatives memantine and IEM-1958 or the phenylcyclohexyl derivative IEM-1921 (n = 51). The doses of NMDA receptor blockers were identified as optimal in a preliminary study of PTZ kindling in mice (n = 9). All mice remained alive throughout the observation period (more than one month).

On the day after the last dose of PTZ, mice were taken from each group for morphological studies. At the light microscope level, the Nissl method was used to study nervous tissue from the brains of 46 mice (SHR). The animals' brains were fixed by vital perfusion using transcardiac administration of fixative: 4% paraformaldehyde solution (pH 7.4), with subsequent postfixation with 10% neutral formalin in 0.1 M phosphate buffer (pH 7.4) [1]. Brains were then placed in 20% sucrose solution for one day. Sections of thickness 20 μm were cut on a Leica CM 1510S cryomicrotome. Nervous tissue from the medial part of the hippocampal field CA1 was studied using an NU2 microscope (Karl Zeiss, Germany). Images were digitized using an MTN-413 camera (AOZT Alfa Telekom, Russia). The program Videotest Master-Morfologiya (VideoTest, Russia) was used to compare total cell densities and the ratios of the numbers of normal and altered neurons in mouse hippocampal field CA1. The optical density of altered neurons was more than twice the optical density of other neurons. The mean numbers of cells in areas of 984.64 μm^2 in the study groups were compared using Student's t test and mean numbers of altered (hyperchromic) neurons were compared using the Mann–Whitney test run on the free statistics program Mac Anova 5.05 (University of Minnesota School of Statistics). An immunohistochemical method was used to analyze the numbers of NeuN-positive neurons in the cortical areas of the brain in mice after administration of PTZ. The distributions of the neuron marker Fox3 (NeuN), expressed only in living, normally functioning neurons [45], were analyzed using ab104224 primary antibodies (Abcam, diluted 1:1000) and ab90722 FITC-conjugated secondary antibodies (Abcam, 1:200). Animal studies were performed in compliance with the European Communities Council Directive #86/609 for the Care of

Laboratory Animals. Data are presented as mean ± error of the mean.

Results

Studies of possible correlations between the convulsive manifestations of PTZ kindling and structural changes to hippocampal neurons used five groups of mice: 1) intact mice; 2) control mice given PTZ to develop kindling; 3) mice given 0.3 mg/kg IEM-1921 + PTZ; 4) mice given 1 mg/kg IEM-1958 + PTZ; and 5) mice given 0.5 mg/kg memantine + PTZ.

PTZ kindling. In mice of the control group, not given an NMDA receptor blocker, the first dose of PTZ produced no convulsive reactions. Only mild twitching of the tips of the ears and whiskers was seen in a small number of the animals. Starting from the second dose, some mice (not more than 1–2 of every 10) showed weak and short-lived clonic convulsions. As the number of identical doses of PTZ increased, the proportion of mice demonstrating convulsive seizures increased to reach a maximum (70%) by dose 11–12 (Fig. 1). The latent periods of onset of seizures decreased and the nature of the seizures changed. Clonic convulsions acquired a tonic component, with limb and tail extension, and some animals lost posture. Thus, clonic-tonic convulsions were seen in 6–8 of every 10 animals in the control group (mean score 3.9 points), 2–4 mice being resistant to PTZ and not developing convulsions during the whole of the experiment (mean score 0 points). The total mean score for all 22 mice of the PTZ kindling control group was 2.7.

Structural changes to pyramidal neurons in hippocampal field CA1. *Intact mice.* Of each group of mice obtained from the supplier and not given pharmacological treatments, 2–3 mice were selected for studies of hippocampal architecture (group 1, total eight animals). Of the total number of neurons studied (75,647), 882 sections showed a total of only 0.15% altered neurons with dark cytoplasm, so-called "dark cells" (Fig. 2, *a*, *b*).

Control mice with severe signs of PTZ kindling. A total of 14 animals were selected from this group for morphological study: of these, eight mice developed PTZ kindling and subthreshold doses of PTZ induced clonic or clonic-tonic convulsions. Another subgroup consisted of six mice which were resistant to PTZ and developed no convulsions. Hippocampal sections from mice developing convulsive seizures showed severe pathological changes (Fig. 2, *c*). The stratum pyramidale of hippocampal field CA1 contained large numbers of altered neurons (24.7 ± 2.1% of the 29,032 examined), most of which were pyramidal neurons. These cells stained intensely with basic aniline stains. The following changes were seen in these cells: loss of turgor, wrinkling, and shrinkage, with deformation of dendritic processes. Some cells acquired an extended, narrowed shape. In the literature, this type of neuron degeneration is termed "dark type" and the cells are called "dark neurons" [3, 23].

Studies of the subgroup of mice in which did not develop typical kindling despite systematic administration of PTZ showed that hyperchromic neurons in hippocampal sections were present in small numbers (2.3 ± 2.3% of the 14,805 examined) (Fig. 2). This can be evaluated as evidence for a cause-effect relationship between convulsive episodes and the appearance of hyperchromic neurons. To answer the question of whether hyperchromia is a sign of severe degenerative changes and/or neuron death, we carried out an immunohistochemical study of parallel brain sections from both subgroups of mice, which demonstrated normal NeuN (Fox3) expression in all hippocampal cells, including dark, hyperchromic cells (Fig. 2, *g*). There were no changes in the total number of cells in hippocampal sections from mice in which PTZ kindling induced seizures (85.6 ± 2.6 neurons in the section areas studied) compared with the same brain areas in intact mice (mean 83.7 ± 4.3 neurons) ($p < 0.05$). This is evidence that neurons did not die en masse and remained relatively viable.

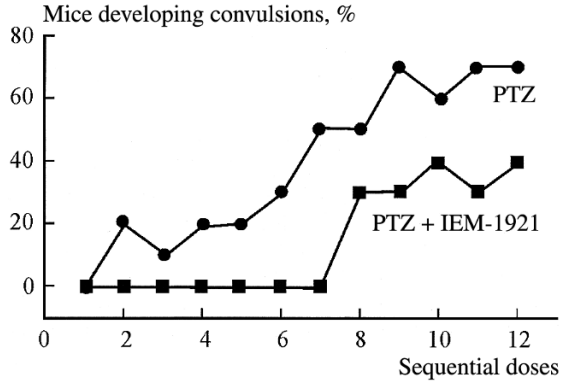

Fig. 1. Weakening of the development of PTZ kindling in mice exposed to the NMDA receptor blocker IEM-1921 (0.3 mg/kg). The ordinate shows the proportions of mice developing convulsions; the abscissa shows sequential dose numbers (three doses/week).

Effects of the NMDA receptor blocker IEM-1921 on the development of PTZ kindling and morphological changes to mouse brain neurons. Mice received 0.3 mg/kg IEM-1921 30 min before the standard subthreshold dose of PTZ (given by the same scheme as in control animals) for one month. This significantly slowed the development of PTZ kindling. A total of 14 of the 20 mice used in this experiment showed no convulsions after the first seven PTZ doses, convulsions gradually appearing from dose 8; stable PTZ kindling was achieved only by the end of the experiment (12 doses of PTZ + IEM-1921). The other six mice lacked signs of convulsions to the end of the experiment. The mean convulsion intensity score in the (PTZ + IEM-1921) group was 0.7, i.e., 3.9 times lower than in the control group.

The brains of six mice in which convulsions did not develop throughout the experiment were examined. Hippocampal field CA1 in these mice showed no pathological changes to neurons (0.1 ± 0.6% of the 55,271 cells studied) (Fig. 2, *d*). Conversely, others of this set of animals, also given the combination of PTZ + IEM-1921 (five mice) but

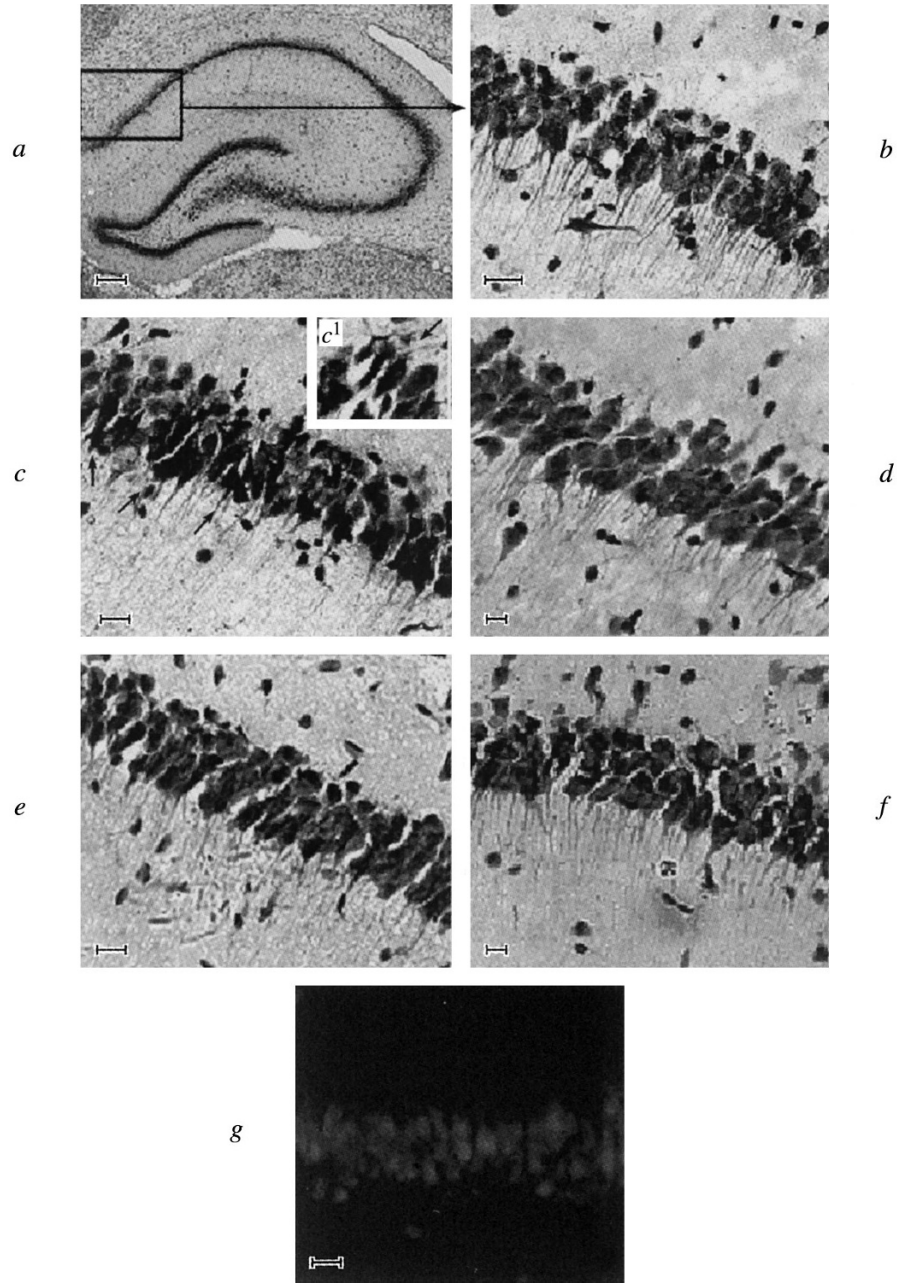

Fig. 2. Structural changes in nervous tissue in hippocampal field CA1 in mice after PTZ kindling. Scale bar: 20 μm. *a*) The rectangle shows the study area of hippocampal field CA1; *b*) intact mice, neurons with normal structural organization; *c*) at the end of PTZ kindling. Arrows show "dark-type" altered shrunken, hyperchromic pyramidal neurons with deformed dendritic process; c^1 – greater magnification (30 μm); *d*) effective prevention of the development of PTZ kindling on exposure to 0.3 mg/kg IEM-1921; *e*) ditto, exposure to 1 mg/kg IEM-1958; *f*) ditto, exposure to 0.5 mg/kg memantine; *g*) NeuN-positive neurons with decreased turgor in the pyramidal layer of hippocampal field CA1 in mice after PTZ kindling.

developing PTZ kindling with a significant delay, showed not only normal cells, but also neurons with wrinkled and extended bodies and deformed dendrites, i.e., dark-type alterations, in the stratum pyramidale of hippocampal field CA1. Altered neurons in these animals accounted 24.0 ± 5.6% of the total 14,213 pyramidal layer neurons studied in the medial part of hippocampal field CA1 (Fig. 2, *d*). Thus, there were statistically significant differences ($p < 0.01$) in the numbers of altered pyramidal neurons between groups of mice demonstrating convulsions and groups not developing convulsions (Fig. 3).

Effects of the NMDA receptor blocker IEM-1958 on the development of PTZ kindling and morphological changes to mouse brain neurons. Using an analogous scheme, another

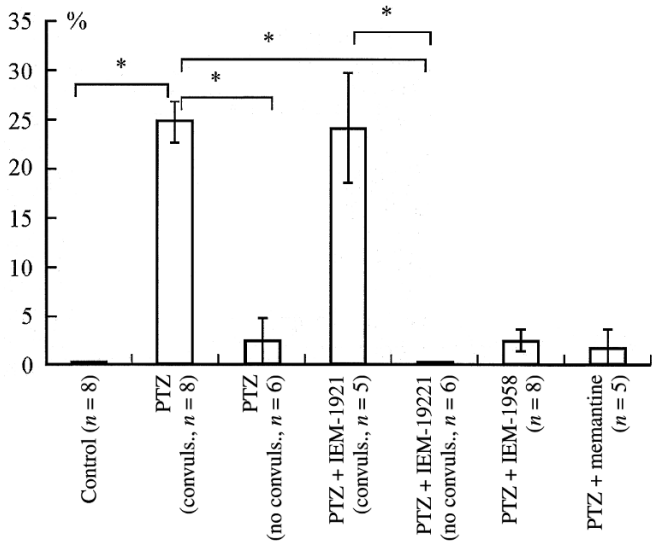

Fig. 3. Relationship between the number of altered pyramidal neurons to the total number of cells in the stratum pyramidale of the medial area of hippocampal field CA1 in mice, %. Data are presented as mean ± error of the mean. Statistically significant differences ($p \leq 0.05$) between the following groups are shown: intact mice (controls) and PTZ (with convulsions); PTZ (with convulsions) and PTZ (without convulsions); control and PTZ + IEM-1921 (with convulsions); PTZ (with convulsions) and PTZ + IEM-1958 (without convulsions); PTZ + IEM-1921 (without convulsions) and PTZ + IEM-1921 (with convulsions); PTZ (with convulsions) and PTZ + IEM-1958; PTZ (with convulsions) and memantine.

new NMDA-type glutamate receptor blocker, IEM-1958, was studied, this being an adamantane derivative. IEM-1958 was given at a dose of 1 mg/kg 30 min before the standard subthreshold dose of PTZ. Five of the eight animals in this series did not develop PTZ kindling before the experiment ended. In the remaining three mice, the first convulsive seizures, of level 2–3 points, appeared after the ninth dose, i.e., prophylactic administration of the agent was effective.

There were no pathological changes to neurons in hippocampal field CA1 on Nissl-stained brain preparations from mice given PTZ + IEM-1958 and not developing convulsive seizures (Fig. 2, *e*). In three mice with delayed onset of PTZ kindling, the proportion of altered hyperchromic neurons was increased in hippocampal field CA1, more in one hemisphere than the other. Comparison of the actions of IEM-1921 and IEM-1958 identified a difference in the responses of hippocampal field CA1 neurons: animals demonstrating convulsions and given IEM-1921 had large numbers of wrinkled altered neurons (24.0 ± 5.6%), while brain sections from mice developing convulsions despite IEM-1958 had fewer altered neurons (6.6 ± 0.9% of the total 6179 cells examined (Fig. 3).

Effects of the NMDA receptor blocker memantine on the development of PTZ kindling and morphological changes to mouse brain neurons. Memantine is the only NMDA-type glutamate receptor channel blocker approved for clinical use (mainly for the treatment of Alzheimer's disease), so it was used in the present study as a reference agent. Of eight mice given memantine at a dose of 0.5 mg/kg, five did not develop convulsions and convulsive seizures, in contrast to parallel controls, were not seen. On Nissl-stained sections, a small number of hyperchromic cells (1.7% of the total 3397 cells examined) was seen in hippocampal field CA1 in only one animal (Figs. 2, *f* and 3).

Discussion

The morphological consequences of severe forms of epilepsy in humans and seen in a variety of experimental models of convulsions in rodents (rats and mice) have been the subject of numerous investigations. However, the details of the pathogenetic pathways which lead from different types of convulsion to biochemical and structural processes in brain neurons require systematic elucidation. The main task of the present study was to identify the link between convulsive syndrome and morphological changes to hippocampal neurons arising during the development of kindling. Kindling provides a convenient experimental approach to this task, as the gradual increase in the pathological process allows its sequence and interactions to be followed. Our studies employed the PTZ kindling model, i.e., the state of increased convulsive readiness arising during systematic (with intervals of 2–3 days) subthreshold doses of the GABAergic synaptic transmission blocker PTZ [21, 25, 26]. PTZ kindling is used as a suitable model for assessing the activity of anticonvulsant substances [22, 40–42, 44]

and detecting convulsion-induced degenerative changes to brain neurons [30, 54, 57].

The first stage of this study was to investigate the relationship between the state of kindling and the probability of detecting pathologically altered neurons in Nissl-stained mouse brain sections. The criterion for these changes was the appearance of "dark neurons." In hippocampal sections from mice developing PTZ kindling, changes consisted mainly of intensely staining pyramidal neurons, whose characteristic features were a reduction in cell body size and shape, with loss of turgor and deformation of the processes. We measured the frequency of hyperchromic neurons in hippocampus of intact mice not subjected to any pharmacological treatments (0.2 ± 0.04%). In the 70% of control mice with clear signs of PTZ kindling, hyperchromic neurons accounted for 24.7 ± 2.1% of cells. About 30% of mice were resistant to PTZ and did not develop seizures throughout the chronic experiment. The frequency of hyperchromic neurons in the hippocampus of these mice was the same as that in the intact control group (2.3 ± 2.3%, $p < 0.5$). The overall results provided evidence that dark cells appeared as a result of convulsive seizures developing in the animals during the development of PTZ kindling.

Similar "dark-type" changes to neurons have been seen on morphological analysis of very diverse pathological processes in the brain – in ischemia [32, 37], hypoglycemia [15, 16], epilepsy [52], and excessive exposure to excitatory transmitters [35, 53]. However, current views on the nature of this phenomenon and even its cause-effect relationship with the pathology of individual neurons is controversial. Some investigators even assess the appearance of dark cells as an artifact evoked by abnormal conditions for storing and fixing tissues [34]. Nonetheless, use of the appropriate controls and identification in each case of the appropriate signs of a link between neuron hyperchromicity and the pathological action allows this criterion to be used for assessing the intensity of the process.

The next convincing argument supporting this relationship is provided by the ability of the three NMDA-type glutamate receptor blockers – memantine, IEM-1921, and IEM-1958 – to prevent the development not only of the convulsive component of PTZ kindling, but also the appearance of hyperchromic neurons in the hippocampus. The molecular mechanism of the interaction of these blockers with glutamate-activated NMDA receptors has been studied in detail [17–19]. Adamantane derivatives (memantine and IEM-1958) and a phenylcyclohexyl derivative (IEM-1921) produce voltage-dependent blockade of the open state of the NMDA receptor channel [12, 17–20], competing with magnesium ions [36, 46]. These blockers are characterized by high selectivity in relation to open NMDA-type glutamate receptor channels [18]. These characteristics of the molecular mechanism of blockade make them a good tool for studying the involvement of NMDA receptors in various physiological and pathological processes.

We have previously reported a comparison of the anticonvulsant activities of memantine and IEM-1921 using several models in experiments in mice: acute convulsive states induced by intraventricular administration of NMDA or kainate [7], i.p. administration of PTZ [6] or arecoline [8], PTZ kindling [9], and in experiments on Krushinskii–Molodkina rats, which are genetically predisposed to audiogenic seizures [2, 4, 5]. In the present study, equieffective doses in the range ED_{40}–ED_{55} were used to address any possible correlation between the anticonvulsant and neuroprotective actions of noncompetitive NMDA receptor blockers.

The results of our experiments identified such a correlation. In the control group, systematic administration of PTZ induced a state of kindling in 70% of the mice, which was apparent as the occurrence of convulsive responses. Morphological studies of hippocampal field CA1 sections from these mice demonstrated a significant number of dark cells. Preventive administration of NMDA receptor blockers decreased the proportion of mice developing PTZ kindling, on average from 70% to 40%. The content of altered neurons in 60% of the mice of the experimental groups in which PTZ kindling did not develop was 0.1 ± 0.06%, i.e., much the same as that in intact mice. This 60% could include mice resistant to PTZ in the initial state rather than as a result of the prophylactic actions of the blockers. However, the proportion of these mice in the control group was no more than 40%. In addition, the appearance of convulsive seizures in the experimental groups of mice was significantly delayed as compared with controls. Even after PTZ dose No. 7, none of the mice developed convulsions, while these occurred in 50% of control animals at this stage (Fig. 1). These results suggest that blockade of NMDA receptors can not only slow and weaken the development of PTZ kindling and, thus, convulsive reactions, but can also prevent the appearance of dark neurons in the hippocampus.

As regards the cause-effect relationship between convulsions and the phenomenon of dark neurons, contradictory data have been reported. First is the question of whether dark cells appear in the rat brain after single doses of 70–80 mg/kg of PTZ, eliciting convulsions [13], or whether dark cells can only be seen as a result of repeated dosage with PTZ [14], as there are reports of the brains of animals with severe convulsive manifestations and the visible absence of the same [10]. According to the results obtained here, PTZ kindling in mice, preceding convulsive syndrome, is an indispensable condition for detecting dark cells in the hippocampus. The divergence in the data may be based on differences in the study systems (rats, mice), assessments of convulsion intensity, and criteria for morphological changes.

It is important to know whether or not these changes in neuron shape and affinity for aniline stains are obligatory evidence for cell death. This can be tested using a set of neuron markers [43]. The present studies included analysis of the distribution of neuron marker Fox3 (NeuN), which is expressed only in living, normally functioning neurons [38, 45];

expression of this marker in all hippocampal cells was found, including in dark, hyperchromic neurons (Fig. 2, c^1). This indicates that dark cells may be the precursors for the further development of the neuron pathology obligatorily required for death of the cell. Little is known of the further fate of dark cells after the occurrence of a pathological focus in the brain; such data are required for a better understanding of their nature [28, 35, 47]. Results from complex studies of the processes of the degeneration of mature neurons and the proliferation of young neurons in nervous tissue growth areas have been reported recently [14, 24, 33, 48, 55]. Studies in this direction may throw light on the general problem of the cellular mechanisms of epilepsy. Further information on the role of glutamate in the sequence of processes leading to damage to brain tissue induced by convulsive syndrome is also required, especially regarding whether or not glutamate increases the release of calcium ions in neurons via the opening of glutamate receptor ion channels.

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REFERENCES

1. D. S. Vasil'ev, N. L. Tumanova, and I. A. Zhuravin, "Structural changes in the nervous tissue of the neocortex in ontogenesis in rats after hypoxia at different stages of embryogenesis," *Zh. Evolyuts. Biokhim. Fiziol.*, **44**, No. 3, 258–267 (2008).
2. S. I. Vataev, E. P. Zhabko, N. Ya. Lukomskaya, et al., "Effects of memantine on convulsive reactions and the organization of sleep in Krushinskii–Molodkina rats," *Ros. Fiziol. Zh.*, **95**, No. 8,802–812 (2009).
3. Yu. M. Zhabotinskii, *Normal and Pathological Neuron Morphology*, Meditsina, Leningrad (1965).
4. K. Kh. Kim, A. V. Zaitsev, V. V. Lavrent'eva, et al., "Effects of ionotropic glutamate receptor blockers on pentylenetetrazole convulsions in Krushinskii–Molodkina rats," *Ros. Fiziol. Zh.*, **98**, No. 12, 1520–1529 (2012).
5. N. Ya. Lukomskaya, S. I. Vataev, E. P. Zhabko, and L. G. Magazanik, "Effects of ionotropic glutamate receptor channel blockers on audiogenic convulsions in Krushinskii–Molodkina rats," *Ros. Fiziol. Zh.*, **98**, No. 4, 449–460 (2012).
6. N. Ya. Lukomskaya, N. I. Rukoyatkina, L. V. Gorbunova, et al., "Studies of the role of NMDA and AMPA glutamate receptors in the mechanisms of corasol convulsions in mice, " *Ros. Fiziol. Zh.*, **89**, No. 3, 292–301 (2003).
7. N. Ya. Lukomskaya, N. I. Rukoyatkina, L. V. Gorbunova, et al., "Comparison of the anticonvulsive activities of organic mono- and dications with their ability to inhibit NMDA and AMPA glutamate receptors," *Ros. Fiziol. Zh.*, **88**, No. 9, 1161–1171 (2002).
8. N. Ya. Lukomskaya, V. V. Lavrent'eva, L. A. Starshinova, et al., "Involvement of ionotropic glutamate receptors in the occurrence of arecoline tremor in mice," *Ros. Fiziol. Zh.*, **93**, No. 3, 275–282 (2007).
9. N. Ya. Lukomskaya, V. V. Lavrent'eva, L. A. Starshinova, et al., "Effects of ionotropic glutamate receptor channel blockers on the development of pentylenetetrazole kindling in mice," *Ros. Fiziol. Zh.*, **91**, No. 11, 1241–1251 (2005).
10. T. V. Pavlova, A. A. Yakovlev, M. Yu. Stepanichev, and N. V. Gulyaeva, "Pentylenetetrazole kindling in rats: is neurodegeneration linked with signs of convulsive activity?" *Ros. Fiziol. Zh.*, **91**, No. 7, 764–765 (2005).
11. M. S. Popova, V. A. Aniol, N. A. Lazareva, et al., "Neurodegenerative changes in the hippocampal fields in rats during the development of pentylenetetrazole kindling," *Morfologiya*, **142**, No. 4, 25–30 (2012).
12. D. B. Tikhonov and L. G. Magazanik, "Different mechanisms of blockade of ion channels – a pathway to the design of new pharmacological agents," *Ros. Fiziol. Zh.*, **96**, No. 7, 726–739 (2010).
13. M. M. Ahmed, M. Arif, T. Chikuma, and T. Kato, "Pentylenetetrazole-induced seizures affect the levels of prolyl oligopeptidase, thimet oligopeptidase and glial proteins in rat brain regions, and attenuation by MK-801 pretreatment," *Neurochem. Int.*, **47**, No. 4, 248–259 (2005).
14. V. A. Aniol, M. Y. Stepanichev, N. A. Lazareva, and N. V. Gulyaeva, "An early decrease in cell proliferation after pentylenetetrazole-induced seizures," *Epilepsy Behav.*, **22**, No. 3, 433–441 (2011).
15. R. N. Auer, H. Kalimo, Y. Olsson, and B. K. Siesjo, "The temporal evolution of hypoglycemic brain damage. I. Light- and electron-microscopic findings in the rat cerebral cortex," *Acta Neuropathol.*, **67**, No. 1–2, 13–24 (1985).
16. R. N. Auer, H. Kalimo, Y. Olsson, and B. K. Siesjo, "The temporal evolution of hypoglycemic brain damage. II. Light- and electron-microscopic findings in the hippocampal gyrus and subiculum of the rat," *Acta Neuropathol.*, **67**, No. 1–2, 25–36 (1985).
17. K. V. Bolshakov, D. B. Tikhonov, V. E. Gmiro, and L. G. Magazanik, "Different arrangement of hydrophobic and nucleophilic components of channel binding sites in N-methyl-D-aspartate and AMPA receptors of rat brain is revealed by channel blockade," *Neurosci. Lett.*, **291**, No. 2, 101–104 (2000).
18. K. V. Bolshakov, V. E. Gmiro, D. B. Tikhonov, and L. G. Magazanik, "Determinants of trapping block of N-methyl-D-aspartate receptor channels," *J. Neurochem.*, **87**, No. 1, 56–65 (2003).
19. K. V. Bolshakov, K. H. Kim, N. N. Potapjeva, et al., "Design of antagonists for NMDA and AMPA receptors," *Neuropharmacology*, **49**, No. 2, 144–155 (2005).
20. H. S. Chen, J. W. Pellegrini, S. K. Aggarwal, et al., "Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity," *J. Neurosci.*, **12**, No. 11, 4427–4436 (1992).
21. M. G. Corda, M. Orlandi, D. Lecca, and O. Giorgi, "Decrease in GABAergic function induced by pentylenetetrazole kindling in rats: antagonism by MK-801," *J. Pharmacol. Exp. Ther.*, **262**, No. 2, 792–800 (1992).
22. D. A. Coulter, D. C. McIntyre, and W. Loscher, "Animal models of limbic epilepsies: what can they tell us?" *Brain Pathol.*, **12**, No. 2, 240–256 (2002).
23. A. Csordas, M. Mazlo, and F. Gallyas, "Recovery versus death of 'dark' (compacted) neurons in non-impaired parenchymal environment: light and electron microscopic observations," *Acta Neuropathol.*, **106**, No. 1, 37–49 (2003).
24. S. C. Danzer, "Depression, stress, epilepsy and adult neurogenesis," *Exp. Neurol.*, **233**, No. 1, 22–32 (2012).
25. G. De Sarro, E. Palma, N. Costa, et al., "Effects of compounds acting on GABA(B) receptors in the pentylenetetrazole kindling model of epilepsy in mice," *Neuropharmacology*, **39**, No. 11, 2147–2161 (2000).
26. A. Dhir, "Pentylenetetrazole (PTZ) kindling model of epilepsy," *Curr. Protoc. Neurosci.*, Chapter 9, Unit 9.37 (2012).
27. H. Franke and H. Kittner, "Morphological alterations of neurons and astrocytes and changes in emotional behavior in pentylenetetrazole-

kindled rats," *Pharmacol. Biochem. Behav.*, **70**, No. 2–3, 291–303 (2001).
28. F. Gallyas, A. Csordas, A. Schwarca, and M. Mazlo, "'Dark' (compacted) neurons may not die through the necrotic pathway," *Exp. Brain Res.*, **160**, No. 4, 473–486 (2005).
29. M. Ghasemi and S. C. Schachter, "The NMDA receptor complex as a therapeutic target in epilepsy: a review," *Epilepsy Behav.*, **22**, No. 4, 617–640 (2011).
30. G. L. Holmes, "Seizure-induced neuronal injury: animal data," *Neurology*, **59**, No. 9, Suppl. 5, S3–S6 (2002).
31. R. Q. Huang, C. L. Bell-Horner, M. I. Dibas, et al., "Pentylenetetrazole-induced inhibition of recombinant gamma-aminobutyric acid type A (GABA(A)) receptors: mechanism and site of action," *J. Pharmacol. Exp. Ther.*, **298**, No. 3, 986–995 (2001).
32. L. W. Jenkins, J. T. Povlishock, W. Lewelt, et al., "The role of postischemic recirculation in the development of ischemic neuronal injury following complete cerebral ischemia," *Acta Neuropathol.*, **55**, No. 3, 205–220 (1981).
33. W. Jiang, K. Wolfe, L. Xiao, et al., "Ionotropic glutamate receptor antagonists inhibit the proliferation of granule cell precursors in the adult brain after seizures induced by pentylenetetrazole," *Brain Res.*, **1020**, No. 1–2, 154–160 (2004).
34. B. S. Jortner, "The return of the dark neuron. A histological artifact complicating contemporary neurotoxicologic evaluation," *Neurotoxicology*, **27**, No. 4, 628–634 (2006).
35. Z. S. Kherani and R. N. Auer, "Pharmacologic analysis of the mechanism of dark neuron production in cerebral cortex," *Acta Neuropathol.*, **116**, No. 4, 447–452 (2008).
36. S. E. Kotermanski and J. W. Johnson, "Mg^{2+} imparts NMDA receptor subtype selectivity to the Alzheimer's drug memantine," *J. Neurosci.*, **29**, No. 9, 2774–2779 (2009).
37. E. Kovesdi, J. Pal, and F. Gallyas, "The fate of 'dark' neurons produced by transient focal cerebral ischemia in a non-necrotic and non-excitotoxic environment: neurobiological aspects," *Brain Res.*, **1147**, 272–283 (2007).
38. A. M. Lavezzi, M. F. Corna, and L. Matturri, "Neuronal nuclear antigen (NeuN): a useful marker of neuronal immaturity in sudden unexplained perinatal death," *J. Neurol. Sci.*, **329**, No. 1–2, 45–50 (2013).
39. S. A. Lipton, "Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond," *Nat. Rev. Drug Discov.*, **5**, No. 2, 160–170 (2006).
40. W. Loscher, "Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy," *Progr. Neurobiol.*, **54**, No. 6, 721–741 (1998).
41. W. Loscher, "Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy," *Epilepsy Res.*, **50**, No. 1–2, 105–123 (2002).
42. W. Loscher, "Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs," *Seizure*, **20**, No. 5, 359–368 (2011).
43. S. S. Magavi and J. D. Macklis, "Immunocytochemical analysis of neuronal differentiation," *Methods Mol. Biol.*, **438**, 345–352 (2008).
44. K. Morimoto, M. Fahnestock, and R. J. Racine, "Kindling and status epilepticus models of epilepsy: rewiring the brain," *Progr. Neurobiol.*, **73**, No. 1, 1–60 (2004).
45. R. J. Mullen, C. R. Buck, and A. M. Smith, "NeuN, a neuronal specific nuclear protein in vertebrates," *Development*, **116**, No. 1, 201–211 (1992).
46. M. V. Nikolaev, L. G. Magazanik, and D. B. Tikhonov, "Influence of external magnesium ions on the NMDA receptor channel block by different types of organic cations," *Neuropharmacology*, **62**, No. 5–6, 2078–2095 (2012).
47. H. Ooigawa, H. Nawashiro, S. Fufui, et al., "The fate of Nissl-stained dark neurons following traumatic brain injury in rats: difference between neocortex and hippocampus regarding survival rate," *Acta Neuropathol.*, **112**, No. 4, 471–481 (2006).
48. J. H. Park, H. Cho, H. Kim, and K. Kim, "Repeated brief epileptic seizures by pentylenetetrazole cause neurodegeneration and promote neurogenesis in discrete brain regions of freely moving adult rats," *Neuroscience*, **140**, No. 2, 673–684 (2006).
49. C. G. Parsons, W. Danysz, and G. Quack, "Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist – a review of preclinical data," *Neuropharmacology*, **38**, No. 6, 735–767 (1999).
50. R. J. Racine, "Modification of seizure activity by electrical stimulation. II. Motor seizure," *EEG Clin. Neurophysiol.*, **32**, No. 3, 281–294 (1972).
51. M. A. Rogawski, A. Thurkauf, S. Yamaguchi, et al., "Anticonvulsant activities of 1-phenylcyclohexylamine and its conformationally restricted analog 1,1-pentamethylenetetrahydroisoquinoline," *J. Pharmacol. Exp. Ther.*, **249**, No. 3, 708–712 (1989).
52. R. S. Sloviter, "'Epileptic' brain damage in rats induced by sustained electrical stimulation of the perforant path. I. Acute electrophysiological and light microscopic studies," *Brain Res. Bull.*, **10**, No. 5, 675–697 (1983).
53. R. S. Sloviter and D. W. Dempster, "'Epileptic' brain damage is replicated qualitatively in the rat hippocampus by central injection of glutamate or aspartate but not by GABA or acetylcholine," *Brain Res. Bull.*, **15**, No. 1, 39–60 (1985).
54. T. P. Sutula, J. Hagen, and A. Pitkanen, "Do epileptic seizures damage the brain?" *Curr. Opin. Neurol.*, **16**, No. 2, 189–195 (2003).
55. F. F. Tian, C. Zeng, T. H. Guo, et al., "Mossy fiber sprouting, hippocampal damage and spontaneous recurrent seizures in pentylenetetrazole kindling rat model," *Acta Neurol. Belg.*, **109**, No. 4, 298–304 (2009).
56. S. F. Traynelis, L. T. Wollmuth, C. J. McBain, et al., "Glutamate receptor ion channels: structure, regulation, and function," *Pharmacol. Rev.*, **62**, No. 3, 405–496 (2010).
57. G. L. Wenk, C. G. Parsons, and W. Danysz, "Potential role of N methyl-D-aspartate receptors as executors of neurodegeneration resulting from diverse insults: focus on memantine," *Behav. Pharmacol.*, **17**, No. 5–6, 411–424 (2006).
58. M. Wong, D. F. Wozniak, and K. A. Yamada, "An animal model of generalized nonconvulsive status epilepticus: immediate characteristics and long-term effects," *Exp. Neurol.*, **183**, No. 1, 87–99 (2003).