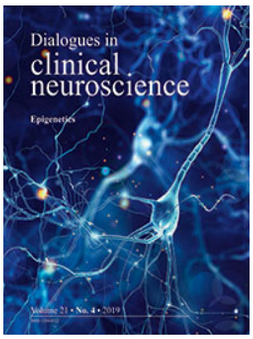

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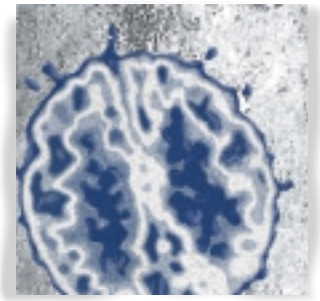
Neurotoxicity of NMDA antagonists: a glutamatergic theory of schizophrenia based on selective impairment of local inhibitory feedback circuits

Heinz Grunze, MD; Andreas Bender, MD; Stefan Wendhof, MD; Martin Schäfer, MD; Dan Rujescu, MD



Neuroimaging studies have implicated the limbic and language regions of the temporal lobe, especially the medial temporal lobe and the superior temporal gyrus, as sites of significant cell loss in schizophrenia.[1] This is supported by neuroanatomical studies showing a significant decrease in neuronal volume in hippocampal structures.[2] The main excitatory input of these limbic-hippocampal structures derives from excitatory amino acids (EAA),[3] mostly glutamate. Several research groups have proposed a central role of glutamatergic receptors—the amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptor, the *N*-methyl-D-aspartate (NMDA) receptor, and the metabotrophic glutamate (m-Glu) receptor—in schizophrenia.[4,5] It is assumed that hyperglutamatergic states are responsible for neurodegenerative cell loss in the course of the disease. However, both EAA agonists, such as kainate, and, paradoxically, NMDA antagonists are able to induce cell death, as shown in the cingulate by Olney.[6] Rats appear

Modulation of recurrent inhibition is critical not only for the normal function of highly excitable regions of the brain, especially the limbic system, but may also be a primary determining factor for the viability of neurons in these regions. Standard extracellular and intracellular recordings from in vitro brain slices of rat hippocampi were employed to show that recurrent inhibition onto CA1 neurons can be modulated by N*-methyl-*D*-aspartate (NMDA) antagonists. Besides reducing the amplitude of inhibitory postsynaptic potentials (IPSPs) at resting membrane potential conditions, different NMDA antagonists, including the endogenous substance* N*-acetyl-*L*-aspartyl-*L*-glutamic acid (NAAG), are able to block long-term potentiation (LTP) of recurrent inhibition completely at concentrations that are not sufficient to block LTP of the excitatory drive onto pyramidal neurons. This LTP of recurrent inhibition may play a significant role in stimulus discrimination and learning, as simulated in a biophysical computer model of a basic neuronal circuit. Both the amplitude of the IPSP and LTP of the recurrent inhibitory circuit also undergo developmental changes showing their highest expression and vulnerability to chronic NMDA antagonist injections in juvenile rats. Finally, blocking NMDA receptor–dependent transmission in the recurrent inhibition loop may lead to an overall increased excitability of the neuronal network. This may resemble the positive schizophrenic symptoms observed in man, presumably caused by elevated levels of the endogenous NMDA antagonist NAAG.*

Keywords: *NMDA antagonist; schizophrenia; recurrent inhibition; interneuron; parvalbumin*

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Selected abbreviations and acronyms

AMPA	*amino-3-hydroxy-5-methyl-4-isoxazole propionic acid*
APV	*2-amino-5-phosphovaleric acid*
DAPI	*4´,6-diamidino-2-phenylindole*
DNQX	*6,7-dinitroquinoxaline-2,3-dione*
EAA	*excitatory amino acid*
EPSP	*excitatory postsynaptic potential*
GABA	*γ-aminobutyric acid*
IPSP	*inhibitory postsynaptic potential*
LTP	*long-term potentiation*
NAAG	N-*acetyl-L-aspartyl-L-glutamic acid*
NMDA	N-*methyl-D-aspartate*
PCP	*phencyclidine*
PTP	*posttetanic potentiation*

most susceptible to NMDA antagonist–induced cell apoptosis in their early adulthood, which bears similarity to the usual time of onset of schizophrenia. Similarly, Benes[7] demonstrated a significant loss of GABAergic (GABA: γ-aminobutyric acid) inhibitory interneurons in the hippocampus in postmortem brains of schizophrenic individuals.

Currently, phencyclidine (PCP)- and ketamine-induced psychosis provides the best pharmacological model for the phenomenology of acute schizophrenic psychosis. The potent psychoactive effects of these substances seem to result, at least in part, from their action as NMDA antagonists. In healthy volunteers, PCP or ketamine at subanesthetic doses induces disturbances of attention, perception, and thought disorders, like symbolic thinking, that are very similar to those found in schizophrenia.[8,9]

In the search for a cellular model corresponding to the effects of PCP in humans, we conducted a series of experiments characterizing the effects of NMDA antagonists in vitro on local feedback inhibition in the hippocampal CA1 area of Long-Evans rats.

Figure 1 shows the basic neuronal circuit ubiquitous to cortical structures including the hippocampal CA1 area where the following experiments were performed. The projection neuron receives different excitatory and inhibitory inputs. When the overall excitatory input is sufficient, it will fire an action potential, leading to the release of EAA from its synapse. However, this action potential not only brings about the synaptic release of EAA onto another projection neuron, but also excites a local inhibitory interneuron through a recurrent axon collateral. The synapse onto this interneuron also uses glutamate as the neurotransmitter. This interneuron will then feed back onto the neuron from which it received the input and onto another 10 to 20 projection neurons (depending on the region of the brain), using GABA as its transmitter, thus limiting excitation. This recurrent inhibition is a most powerful means of limiting network excitability in cortical areas, thus preventing excitotoxic damage to the network. It is also a powerful means of synchronizing activity of projection neurons, as one inhibitory interneuron feeds back onto many principal cells.

In order to further characterize this local inhibitory loop and possible disturbances from NMDA antagonism, we performed intracellular recordings from the projection neuron and stimulated this circuitry with a bipolar electrode from the axon collateral *(Figure 2)*. To limit feed-forward excitation and inhibition from fibers from other layers, we transected the stratum lacunosum-moleculare, stratum radiatum, stratum pyramidalis, and stratum oriens, leaving only a bundle of alvear fibers intact. In a subset of experiments, we also used rats of which the commissural fibers had been transected 4 days prior to slice harvesting to exclude activation of feed-forward inhibition by axons from the contralateral dorsal hippocampus.

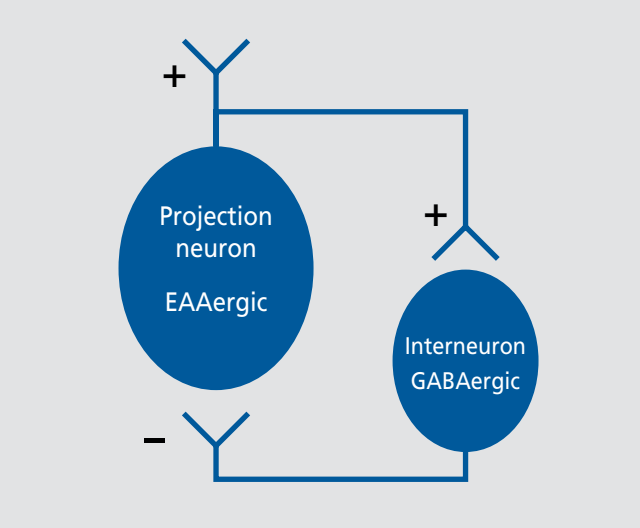


Figure 1. The basic neuronal unit in the cortex consists of a pyramidal cell (projection neuron) and a local inhibitory interneuron which together form an recurrent inhibition loop. EEA, excitatory amino acid; GABA, γ-aminobutyric acid.

These experiments were designed to address the following:

- The impact of acute NMDA antagonist application on the generation of IPSPs.
- The modulation of long-term potentiation (LTP) of IPSPs in vitro by NMDA antagonists.
- The impact of age and gender on recurrent inhibition.
- The impact of chronic in vivo injections of low-dose MK-801 during puberty on the expression of IPSPs and LTP in the recurrent inhibition loop.

Methods

Whole-cell patch clamp recordings from the CA1 region of rat hippocampal slices were performed using electrode placement as mentioned. Slices were prepared from rats of both sexes, aged 25 to 40 days, and maintained using standard procedures. Rats were decapitated under halothane anesthesia, and the brains rapidly removed. Using a vibratome (Model 820, Spencer Inc), 300- to 400-µm thick transverse slices were cut from the hippocampus. The slices were then placed in oxygenated artificial cerebrospinal fluid (ACSF) at room temperature. Whole-cell recordings were obtained with the technique described in reference 10. Briefly, borosilicate glass electrodes (resistance 4-6 MΩ) were filled with 100 mM potassium citrate, 20 mM KCl, 1 mM $CaCl_2$, 3 mM $MgCl_2$, 2 mM MgATP, 2 mM sodium guanosine 5´-triphosphate, 3 mM ethyleneglycotetraacetic acid, and 40 mM HEPES. Recordings were made with an Axoclamp 2A amplifier (Axon Instruments, Burlingame, CA) and Basic Fastlab software (Indec Systems, Sunnyvale, CA). ASCF contained (in mM): NaCl 124; KCl 3.75; KH_2PO_4 1.25; $MgCl_2$ 1.3; $CaCl_2$ 3.5; $NaHCO_3$ 26; glucose 10; it was bubbled with 95% O_2/5% CO_2 and maintained at 30±2°C throughout the recordings. Detailed information on the methods are described in another article,[11] where some of the results were originally published.

Excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials were evoked by stimulation of the alvear pathway *(Figure 2)*. In one set of experiments, the amplitudes of the EPSP and IPSP were measured under baseline conditions, after systemic application of different NMDA antagonists, and after washout. In a second set of experiments, a tetanus (20 stimuli of 100 Hz) was applied after measurement of the baseline IPSP using the same pathway. During the tetanus, the recorded neuron was hyperpolarized to -85 mV, with DC current injection, to prevent the induction of glutamatergic LTP onto the recorded neuron itself. The peak baseline value of the IPSP was compared with peak values obtained continuously until 21 minutes after tetanus. After characterizing the LTP of recurrent inhibitory circuits, we finally tested the sensitivity of this LTP to NMDA antagonists in comparison to excitatory, feed-forward LTPs in the same slice, using a second stimulus electrode in the stratum radiatum.

To examine the effect of a shift in the relationship between the strength of excitatory to inhibitory LTPs and its impact on learning and recall, we used a computer model of a local neuronal circuit resembling the typical cell population of the hippocampus. In this model, the functional role of synaptic modification of the excitatory input to inhibitory interneurons was explored in a network biophysical simulation of cortical autoassociative memory function, containing 240 pyramidal cells and 58 inhibitory interneurons activating chloride and potassium currents. Starting parameters for some currents were derived from previous simulation of the piriform cortex and of region CA3.[12,13] The simulation of pyramidal cells contained three compartments, with a range of synaptic and voltage-dependent currents. Both dendritic compartments contained excitatory synaptic sodium currents, while the proximal dendritic compartment contained inhibitory synap-

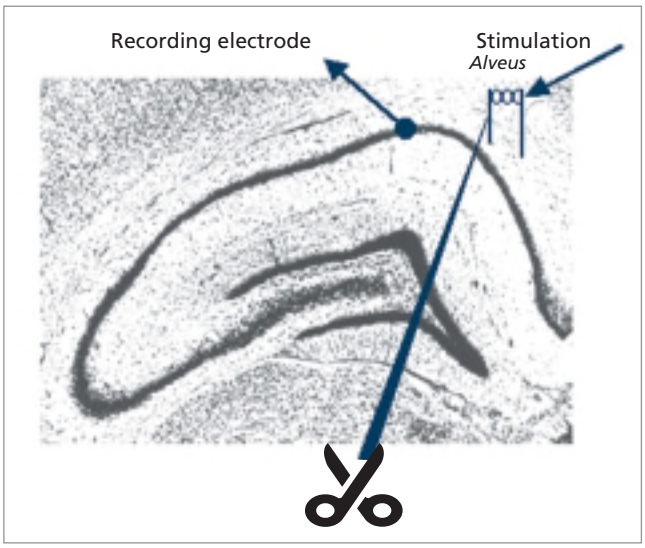


Figure 2. Placement of recording and stimulation electrodes for intracellular whole cell patch recordings. A transection, leaving only the alveus intact, divides the sides of stimulation and recording.

tic potassium currents and the soma contained inhibitory synaptic chloride currents. These currents include a fast activating voltage-dependent sodium current (I_{Na}), a delayed rectifier potassium current ($I_{K(DR)}$), voltage-dependent potassium currents ($I_{K(A)}$) and ($I_{K(M)}$), a high-threshold voltage-dependent calcium current (I_{Ca}), and a calcium-dependent potassium current ($I_{K(AHP)}$). Excitatory synapses between pyramidal cells, and from pyramidal cells to the class of inhibitory interneurons activating chloride currents, were modified according to a Hebbian learning rule. When a spike arrived along a presynaptic axon, the maximal conductance of the synaptic current was changed in proportion to the amount by which the average of the postsynaptic membrane potential exceeded a modification threshold. Further methodological details are explained in the legend of *Figure 6* below.

In order to examine the effects of age and hormonal status on recurrent inhibition, in vitro experiments similar to those described above were conducted on a cohort of rats aged 6 to 9 months or older. The examined groups of Long-Evans rats consisted of "younger" females aged 200 days, ovariectomized females aged 200 days, "older" females aged 380 days, and males aged 250 days. One hemisphere of each brain was used for electrophysiological studies while the contralateral one was processed for immunohistochemistry.

Elevated levels of the endogenous partial NMDA antagonist *N*-acetyl-L-aspartyl-L-glutamic acid (NAAG) are one of the striking findings in postmortem brains of schizophrenic patients.[14] To mimic this condition and to characterize the effect of chronic low-dose NMDA antagonist exposure during puberty, we injected MK-801 (0.02 mg/kg body weight) intraperitoneally in 52-day-old rats for 2 weeks (MK-801: n=6; saline controls: n=6) before harvesting the hippocampi. During this period, rats showed no abnormalities of behavior or neurological signs of acute intoxication. Whole-cell patch clamp recordings were performed from CA1 principal neurons 3 days after the last injection. In addition, a relative cell count was performed on 15-µm cryostat slices triple-stained with antiparvalbumin, anticalretinin, and

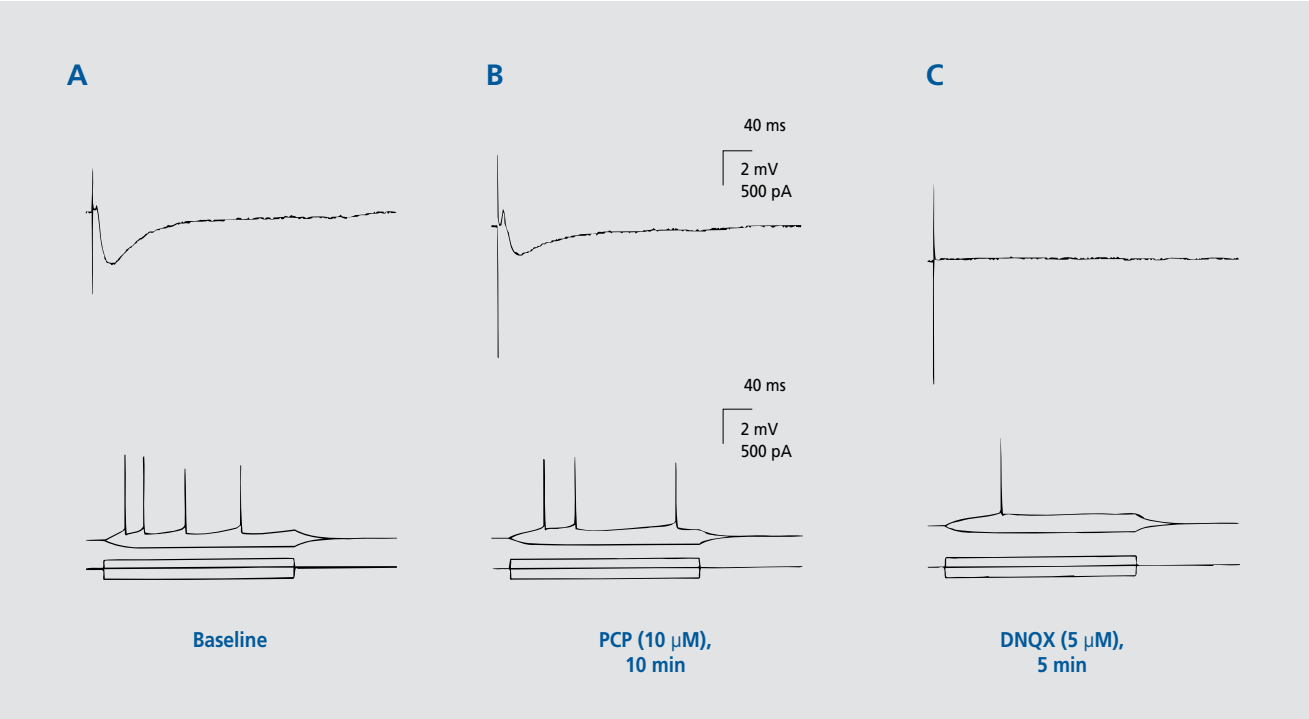


Figure 3. Phencyclidine (PCP) (10 µM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) (5 µM) decrease the inhibitory postsynaptic potential (IPSP). Upper traces: IPSP of an CA1 pyramidal cell in response to alvear stimulation, holding potential -60 mV. Lower traces: Corresponding responses to intracellular current injections of ±150 pA to check the stability of the input resistance. A: Baseline, B: after 10 min PCP (10 µM), C: after addition of DNQX (5 µM) for another 5 min.

DAPI, as markers for the interneuronal subpopulation and total cell number, respectively, on the hippocampus not used for electrophysiology.

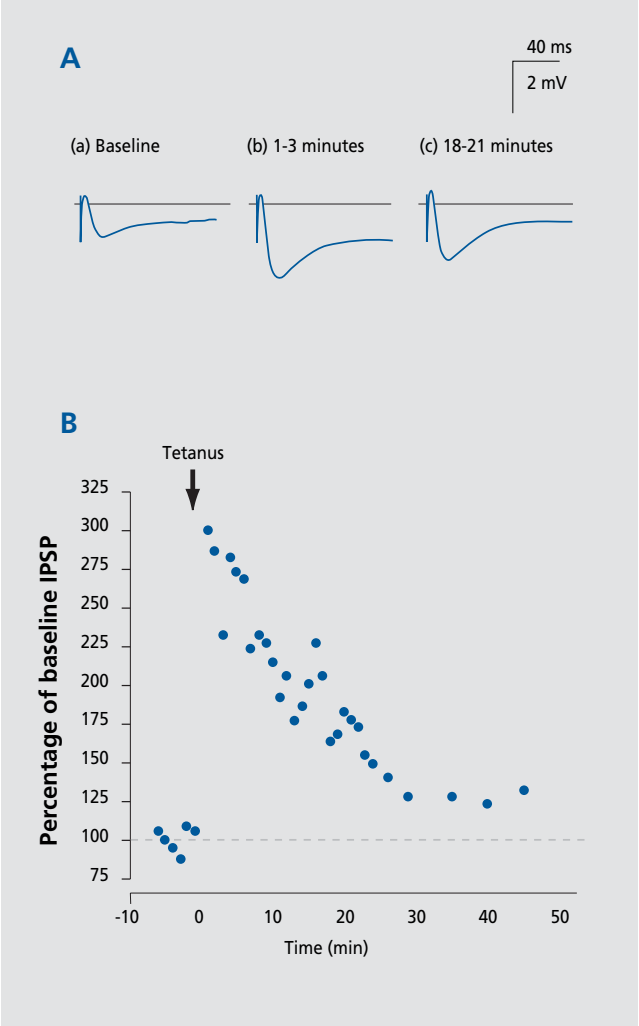


Figure 4. Intracellular whole-cell patch clamp recordings from CA1 pyramidal neurons. A: Postsynaptic potentials (a) before, (b) 1 to 3 minutes after tetanic stimulation, and (c) 18 to 21 minutes after tetanic stimulation of the alveus. All traces are averages of 8 recordings during 3 minutes with a 20-s interval. B: Graphic representation of the peak amplitude of the inhibitory postsynaptic potential (IPSP) in another CA1 pyramidal cell, before and after tetanic stimulation of the alveus. The amplitude of the IPSP is given as the percentage increase in the baseline IPSP amplitude before tetanus (first six points).

Results

The impact of acute NMDA antagonist application on the generation of IPSPs

Whole-cell patch clamp recordings were obtained from pyramidal CA1 cells, manually held at -60 mV, and the IPSP amplitude in response to low frequency (0.05 Hz, 0.5 -1 mA, 400 ms) was measured. Under drug-free baseline conditions, the mean amplitude of the IPSP was 6.7±0.5 mV (mean±SE). All NMDA antagonists tested, both competitive and noncompetitive, decreased the IPSP amplitude in a dose-dependent manner. The following results are given as a percentage decrease in the baseline IPSP amplitude (±SE) for the largest series conducted with the competitive NMDA antagonist 2-amino-5-phosphovaleric acid (APV) and the noncompetitive NMDA antagonist PCP:

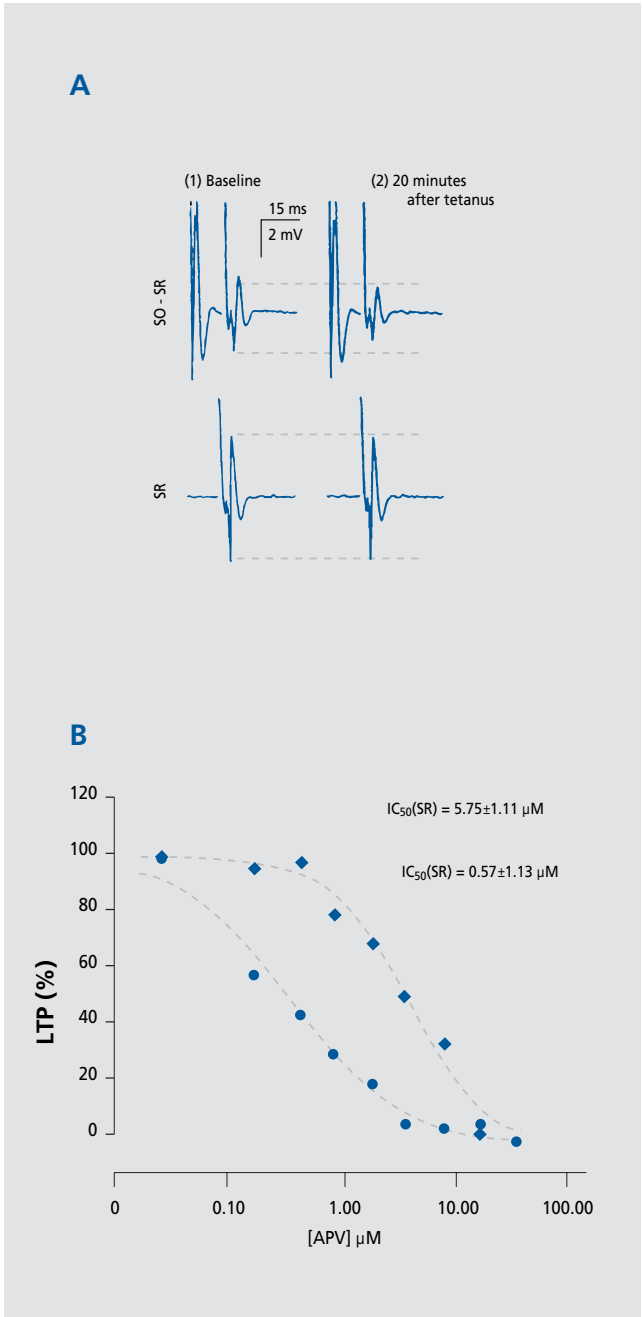
- APV—0.4 µM: -12±13%; 1 µM: -8±16%; 1.5 µM: -12±9%; 3 µM: -15±3%; 5 µM: -15±4%; 10 µM: -36±13%; 25 µM: -31±9%; 50 µM: -47±9%. Thus, APV significantly reduced the inhibition in the circuitry even at the smallest concentrations, whereas an effect on EPSPs was only seen at concentrations above 10 mM.
- PCP—10 µM: -24±21%; 25 µM: -9±16%; 50 µM: -46±6%; 100 µM: -48±19%. For 50 µM PCP, the reduction in the IPSP amplitude in the presence of naloxone (10 µM) was, at -40% of the same magnitude, verifying the response as NMDA- and not σ-receptor–related. In 4/8 neurons tested, 6,7-dinitroquinoxaline-2,3-dione (DNQX) (5 µM) abolished the IPSP completely, and in the other 4 neurons partially (-80±22% compared with the baseline value, *Figure 3*).

These results indicate that NMDA receptors on inhibitory interneurons may play a role not only in LTP as they do on excito-excitatory synapses, but may also have an impact on network excitability under resting membrane potential conditions. Thus, at low concentrations, they may increase network excitability and only at higher doses cause overall inhibition.

The modulation of long-term potentiation of IPSPs in vitro by NMDA antagonists

In 12 out of 15 neurons tested, posttetanic potentiation (PTP) of the IPSP was observed followed by significant LTP (mean 52±16%) of more than 20 minutes ($P<0.005$, Mann-Whitney U test, *Figure 4*). Neither PTP nor LTP of

the IPSP required $GABA_B$ receptor activation, as both were insensitive to the $GABA_B$ receptor antagonist saclofen (250 µmol/L). When a tetanus was applied during APV (50 µmol/L) superfusion, 4 out of 7 neurons showed PTP (mean 13%) but none showed LTP of the IPSP.



In order to obtain more stable and lasting recordings and to compare the LTP of orthodromically evoked EPSPs with recurrent inhibition LTP, we conducted a series of extracellular experiments. Population spikes (PS) of CA1 pyramidal neurons were evoked using a bipolar stimulating electrode placed in the stratum radiatum. This orthodromically (o) evoked PS could be reduced by applying an antidromic (a) stimulus via the alvear pathway at an appropriate time interval prior to the orthodromic stimulus. The reduction in the orthodromic PS results from activation of axon collaterals of CA1 pyramidal cells that project onto recurrent inhibitory interneurons. The size of the orthodromic PS was compared with its size after antidromic-orthodromic stimulation (means of 10 recordings at 20-s intervals), and the ratio of PS[a-o]/PS[o] was determined. A tetanus (4 trains of 10 stimuli at 100 Hz) was then applied via the alvear electrode. The PS[a-o]/PS[o] ratio, determined before tetanus, was compared with three time intervals (2, 10, and 20 minutes) after tetanus.

In 92% (24/26) of the recordings, a clear reduction in the PS[a-o]/PS[o] ratio was observed 20 minutes after tetanus compared with baseline values (mean reduction: 18.7±11.7%; $P<0.005$, Wilcoxon matched pair signed rank test). These data suggest long-lasting amplification of the recurrent inhibitory drive. *Figure 5* depicts a typical

Figure 5. Extracellular recordings of the CA1 stratum pyramidalis. A: Population spikes in response to orthodromic (o) stratum radiatum (SR) stimulation (lower trace) and combined antidromic (a) stimulation of alvear fibers in the stratum oriens (SO)/orthodromic (SR) stimulation (upper trace). The antidromic stimulus precedes the orthodromic by 12 ms (1) before tetanic alvear stimulation, (2) 20 minutes after tetanus. Note the clear decrease in the population spike with a-o stimulation. Traces are averages of 10 stimulations with a 20-s interval. B: Long-term potentiation (LTP) of local circuit inhibition shows differential sensitivity to *N*-methyl-D-aspartate (NMDA) receptor antagonists compared with excitatory LTP. There is a dose-response relationship for the percentage of maximal LTP versus 2-amino-5-phosphovaleric acid (APV) concentration (range 0.3-50 µM). The two data points at 0.05 µM (indicated by the overlapping symbols, · and ·) reflect the absence of APV and are used to facilitate curve fitting on the logarithmic scale. Each point represents the average of at least 4 experiments. LTP of recurrent inhibition (·) shows a 10-fold greater sensitivity to blockade by APV than excitatory LTP (·).

Figure 5B reproduced from reference 11: Grunze HC, Rainnie DG, Hasselmo ME, et al. NMDA-dependent modification of CA1 local circuit inhibition. *J Neurosci.* 1996;16:2034-2043. Copyright © 1996, Society for Neuroscience.

recording showing traces for baseline and 20 min after tetanus for orthodromic and antidromic-orthodromic stimulation.

In 7 out of 8 recordings, no change in the PS[a-o]/PS[o] ratio was observed following tetanic stimulation in the presence of APV (50 μM, $P<0.025$). PCP, applied during tetanus (n=6, $P<0.025$), mimicked the effect of APV. These data suggest that NMDA receptor activation is required for this long-lasting enhancement of inhibition. NAAG, 50 μM, applied during tetanus, also attenuated the reduction in the PS[a-o]/PS[o] ratio by 28% compared with the control (n=1), and abolished it at a concentration of 100 μM (n=2).

The ability of APV and NAAG to suppress LTP of the recurrent inhibitory drive was compared with its influence on LTP of the excitatory drive onto pyramidal cells with this antidromic-orthodromic stimulus paradigm. The dose-response curve obtained *(Figure 5)* shows a significant 10-fold increased susceptibility of recurrent inhibition LTP to NMDA antagonists compared with the more resistant LTP of excitatory input.

What may be the physiological use of recurrent inhibition LTP? Besides counteracting hyperexcitability through excitatory LTP, it may contribute to filtering stimuli under physiological conditions.

In a realistic biophysical model, we demonstrated that modification of excitatory input to inhibitory interneurons prevented interference between different stored patterns. As shown in *Figure 6*, we tested the ability of the network to store two patterns of 40 neurons, each with an overlap of 8 neurons. Strengthening of the excitatory synapses between pyramidal cells mediated the auto-associative memory storage of the patterns in the network, allowing completion of missing elements of a degraded pattern. However, the overlap between patterns could cause interference during learning and recall, where activity elicited by one input pattern would spread into neurons which were only part of the other pattern. This interference could be prevented by simultaneously enhancing the strength of excitatory transmission from pyramidal cells to inhibitory interneurons. The enhanced activation of inhibitory currents prevented the excessive spread of activity within the network during learning and recall. This effect may prevent the breakdown of function due to the development of excessive associations between different stored activity patterns.

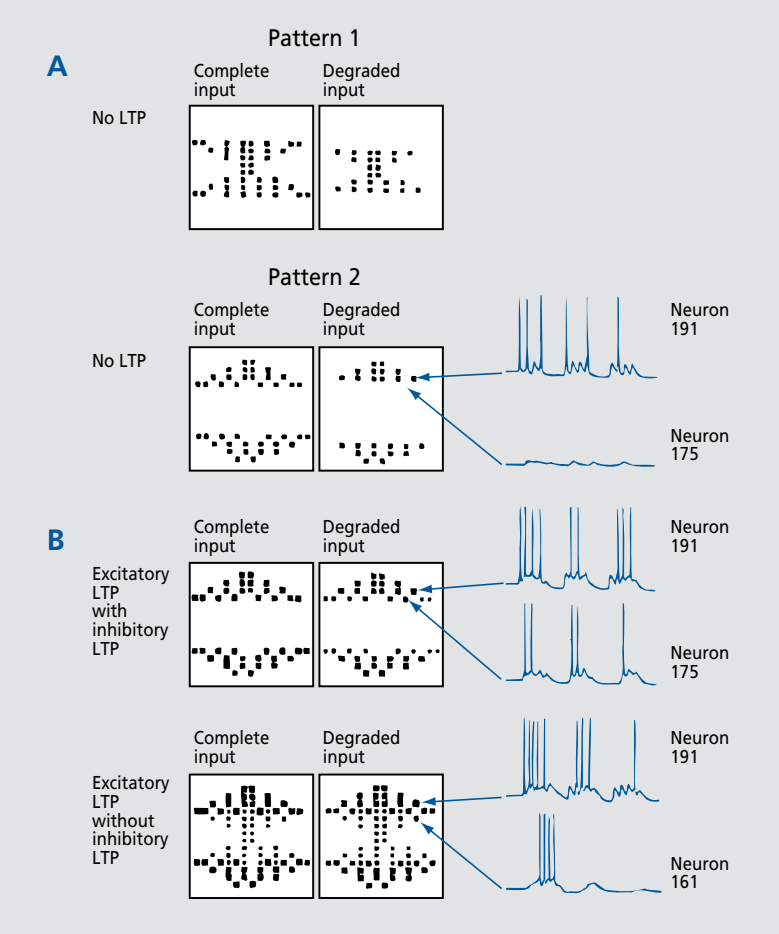
This biophysical model has remarkable similarities to thought disorder in schizophrenic patients. Deficits in learning new verbal associations in the Verbal Paired Associate Learning Test, which also correlate with a reduced volume of the left posterior superior gyrus, have been demonstrated in schizophrenic patients.[15] Impairment of recall may be reflected by the greater "search activity" for congruent sentence endings seen in the N400 studies conducted by McCarley et al.[16]

The capability of local interneurons to synchronize cortical network activity has already been mentioned. This triggers population bursts that may shape local functional connectivity in the cortex,[17] and also strengthen the overall excitatory limbic input onto mesolimbic GABAergic neurons. These neurons integrate cortical glutamatergic and the dopaminergic input from the ventral tegmentum. High synchronicity of the cortical glutamatergic input is more likely to overcome the dopaminergic inhibition, leading downstream from the mesolimbic projection neuron finally to an improvement in the thalamic filter function.[18] Some neuroleptics may exert a dual action in balancing the relationship between dopaminergic and glutamatergic input, eg, haloperidol, which also has glutamatergic agonist properties by enhancing NMDA receptor sensitivity.[19-21]

Another physiological correlate of the glutamatergic interplay between excitatory projection neurons and GABAergic interneurons is γ-range neural synchronization.[22] In an auditory evoked potential paradigm, schizophrenic patients are distinguished from controls by a reduced EEG power at 40 Hz, but not at lower frequencies of stimulation. They exhibit a delayed onset of entrainment, poorer synchronization, and any longer persistence of entrainment after the end of the 40-Hz stimulation, consistent with impaired generation of γ-synchronization.[16]

The impact of age and gender on recurrent inhibition

To further examine the compatibility of our model with schizophrenia, we conducted similar in vitro experiments on the impact of age and gender on recurrent inhibition. Aged rats of both sexes showed decreased amplitudes of the IPSPs (1.1±0.9 mV in rats of 9 to 12 months, compared with 6.7±0.5 mV in prepubescent rats). Only one third of all slices tested exhibited any measurable IPSP at all. The EPSP, however, appeared more prominent compared with juvenile rats. This suggests that recurrent inhibition has a more prominent role in shaping networks and limiting excitation in youth; in old rats, however,



extrinsic modulation of hippocampal networks may be more decisive. This idea is supported by the findings of decreased NMDA receptor density in the hippocampus of older rats.[23,24] However, the functionality of the single NMDA receptor complex increases with age[25] and, thus, also their sensitivity to excitotoxic damage.[26]

Slices from old female rats exhibited a tendency toward higher IPSP amplitudes compared with males (2.2±0.4% versus 0.9±0.5%). This is in line with a previously demonstrated higher functionality of the NMDA receptor in female rats.[27] To examine whether female sex steroids exert a neuroprotective effect and/or male sex steroids impair recurrent inhibition, we also examined rats which had been castrated prior to puberty. In fact, the IPSP in these castrated rats was significantly increased compared with age-matched male controls (2.4±1.3 mV, $P<0.25$, Mann-Whitney U test). Furthermore, the m-Glu agonist *trans*-1-amino-1,3-cyclopentadicarboxylic acid (ACPD) increased the IPSP amplitude in aged animals whereas it had no effect in young rats. Taken together, these data suggest that inhibitory local circuits undergo age-dependent changes, possibly with an important modu-

Figure 6. Activity in a network biophysical simulation showing the significance of long-term potentiation (LTP) of recurrent inhibition for recall of stored input patterns. This figure illustrates activity during recall. Activity during learning is not shown. A. Spiking activity induced in a network of 240 pyramidal cells without any strengthening of excitatory intrinsic synapses. Each enclosed panel contains 240 neurons plotted in a 15 × 16 matrix. The size of the black squares represents the number of action potentials generated by each pyramidal cell during a 500-ms period of recall. (To the right of pattern 2 is shown the membrane potential of neurons 191 and 175. The black square for 191 represents the six spikes generated by that neuron, which receives direct afferent input. The absence of a black square for 175 represents the absence of spiking activity due to the absence of afferent input to this neuron.) For each pattern, the left panel shows the response to the complete input version of the pattern (with 40 neurons active) and the right square shows the response to a degraded input version of the pattern (with 24 neurons activated by afferent input). B. The spiking activity in the network after learning strengthens the excitatory connections between active neurons (excitatory LTP). The network has been trained on both pattern 1 and pattern 2, but recall is only shown for the complete and degraded version of pattern 2. Activity is shown during a 500-ms recall period with and without the strengthening of synapses between pyramidal cells and inhibitory interneurons (Inhibitory LTP). Top: With both excitatory LTP and inhibitory LTP, the network responds to the complete pattern with no excess spread of activity, and responds to the degraded input with activity spreading only into neurons which were a component of the original learned pattern. For example, on the right, the membrane potential traces show neuron 191 responding to afferent input, while neuron 175 responds due to synaptic activity spreading from other neurons. Bottom: With excitatory LTP in the absence of inhibitory LTP, the network responds to both the complete and degraded versions of pattern 2 with activity which spreads to components of the other stored pattern, pattern 1. This is due to the spread of activity from neurons which were components of both patterns. This additional spread can be prevented by stronger recurrent inhibition. On the right, membrane potential traces show the response of neuron 191 and the excess spread of activity into neuron 161. In this case, differentiation of the two patterns is prevented because input of either pattern recalls elements of both patterns.

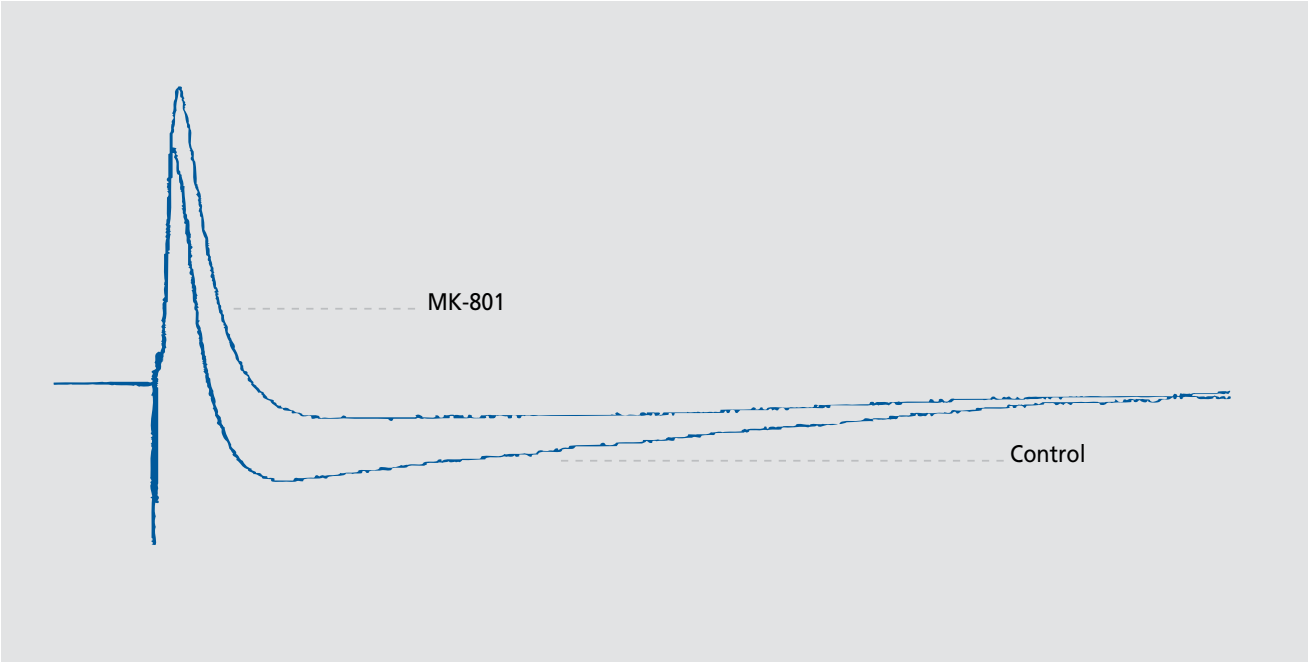


Figure 7. Comparison of typical inhibitory postsynaptic potentials (IPSPs) recorded from a rat chronically injected with low-dose MK-801 and a saline control rat. Chronic MK-801 injection in vivo causes a significant reduction in the IPSP amplitude of the in vitro recordings.

latory role of sex steroids, and that activation of m-Glu receptors can support IPSP generation in aged animals, whereas, in young animals, a maximum of IPSP amplitudes is already achieved by AMPA and NMDA receptor activation.

IPSP modulation and possible cell loss may result from chronic exposure to high levels of the endogenous NMDA antagonist NAAG, which may play a pathogenetic role in schizophrenia.[4] As puberty and early adolescence appear to be a vulnerable time for schizophrenia, we examined the effect of chronic, not acute toxic, low-dose application of MK-801 on electrophysiological and histological changes in two interneuronal subpopulations in the rat hippocampus. There was no difference in the mean membrane resting potential, action potential threshold and overshoot, $GABA_A$ reversal potential, or response to locally applied GABA between treated rats and saline controls. However, local inhibition evoked by alvear stimulation was significantly reduced in the MK-801 group (IPSP amplitude -1.6±1.3 mV versus -3.7±1.2 mV in controls, $P<0.025$, Mann-Whitney test) *(Figure 7)*. This finding is consistent with a reduced ratio of parvalbumin-positive (PV[+])/calretinin-positive (CR[+]) interneurons in the treated group *(Figure 8)*. The loss of PV[+] interneurons also seems to be related to chronic MK-801 application, as rats that received only one high dose of MK-801 (1 mg/kg BW) had no shift of the PV[+]/ CR[+] interneuron ratio.

Discussion

The cellular model of glutamatergic dysfunction presented here is based on the smallest cortical network loop, the local inhibitory feedback circuit. We demonstrated in vitro that both recurrent inhibition and its LTP can be selectively inhibited by low doses of NMDA antagonists. The nature of this differential sensitivity of NMDA receptors is still unclear, but may be related to the different subunit assembly of NMDA receptors on interneurons compared with pyramidal cells,[28] which also has consequences on the expression of NMDA-gated currents.[29] Selective impairment of local inhibition may lead to increased excitability of the cortical network, causing intrinsic excitotoxic damage with greatest vulnerability of PV[+] interneurons, and to functional impairment, which may also include higher cognitive

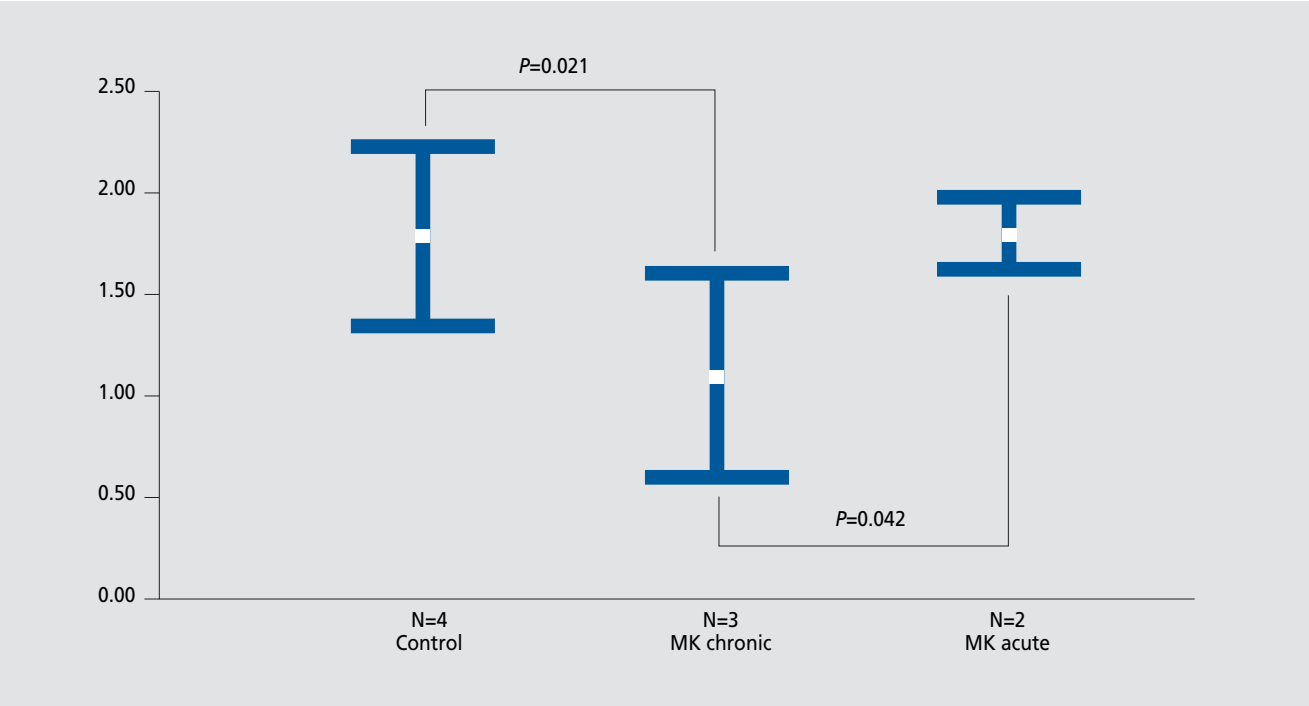


Figure 8. Ratio of parvalbumin-positive (PV[+])/calretinin-positive (CR[+]) interneurons in the rat hippocampus. The difference between controls and chronically MK-801–injected rats is highly significant as calculated with the post-hoc Scheffe test.

functions as learning and recall, as demonstrated in the computer model. Lack of synchronization of the cortical input onto mesolimbic cells may lead to a relative hyperfunction of the dopaminergic modulation of these cells, with consecutive impairment of the thalamic filter.

Consistent with schizophrenia, we also demonstrated that the vulnerability of NMDA-mediated recurrent inhibition may be most pronounced in adolescent animals and male animals, whereas female sex steroids may have a protective effect.

In conclusion, this model supplies a glutamatergic basis of schizophrenia. The next step is now to examine its modulation by other neurotransmitters implicated in schizophrenia, such as serotonin and dopamine.

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Neurotoxicidad de los antagonistas del NMDA: una teoría glutamatérgica de la esquizofrenia basada en el deterioro selectivo de los circuitos de feed-back inhibitorios locales

La modulación de la inhibición recurrente no es crítica sólo para el funcionamiento normal de las regiones altamente excitables del cerebro- especialmente el sistema límbico- sino que también puede ser un factor primario determinante de la viabilidad de las neuronas en estas regiones. Se utilizaron registros extra e intracelulares estándar de preparaciones cerebrales in vitro de hipocampo de rata para mostrar que la inhibición recurrente de las neuronas CA1 puede ser modulada por antagonistas del N-*metil*-D-*aspartato (NMDA). Además de reducir la amplitud de los potenciales inhibitorios postsinápticos (PIPS) en condiciones de reposo del potencial de membrana, diferentes antagonistas NMDA, incluyendo la sustancia endógena ácido* N-*acetil*-L-*aspartil*-L-*glutámico (NAAG), son capaces de bloquear completamente la potenciación a largo plazo (PLP) de la inhibición recurrente a concentraciones que no son suficientes para bloquear la PLP de la conducción excitatoria de las neuronas piramidales. Esta PLP de la inhibición recurrente puede jugar un papel significativo en la discriminación de estímulos y el aprendizaje, como es simulado en un modelo biofísico computarizado de un circuito neuronal básico. Tanto la amplitud del PIPS como la PLP del circuito inhibitorio recurrente sufren cambios con el desarrollo y muestran su mayor expresión y vulnerabilidad frente a la inyección crónica de antagonistas NMDA en ratas jóvenes. Finalmente, el bloqueo de la transmisión dependiente del receptor de NMDA en el circuito de inhibición recurrente puede conducir a un aumento total de la excitabilidad de la red neuronal. Esto puede asemejarse a los síntomas esquizofrénicos positivos observados en el hombre, presumiblemente causados por los niveles elevados del antagonista de NMDA, el NAAG.*

GRUNZE: Neurotoxicité des antagonistes de la NMDA : une théorie glutamatergique de la schizophrénie basée sur l'atteinte sélective des circuits locaux de rétrocontrôle de l'inhibition

La modulation de l'inhibition récurrente est non seulement importante pour le fonctionnement normal des régions cérébrales d'excitabilité élevée, en particulier le système limbique, mais pourrait également être considérée comme un facteur déterminant primaire pour la viabilité des neurones dans ces régions. Des enregistrements standard extracellulaires et intracellulaires obtenus à partir de coupes d'hippocampe de rats in vitro ont été utilisés pour montrer que l'inhibition récurrente dans les neurones CA1 peut être modulée par les antagonistes de la N-*méthyl*-D-*aspartate (NMDA). Parallèlement au fait qu'ils réduisent l'amplitude des potentiels post-synaptiques inhibiteurs (PPSI) dans des conditions de potentiel de membrane de repos, différents antagonistes de la NMDA, y compris la substance endogène, l'acide* N-*acétyl*-L-*aspartyl*-L-*glutamique acid (NAAG), sont capables de bloquer complètement la potentialisation à long terme (PLT) de l'inhibition récurrente à des concentrations qui ne sont pas suffisantes pour bloquer la PLT de la stimulation excitatrice vers les neurones pyramidaux. Cette PLT de l'inhibition récurrente pourrait jouer un rôle significatif dans la discrimination et l'apprentissage du stimulus, comme simulé dans un modèle biophysique informatisé d'un circuit neuronal fondamental. Aussi bien l'amplitude de la PPSI que la PLT du circuit inhibiteur récurrent sont également soumis aux modifications du développement montrant leurs expressions les plus élevées et leur vulnérabilité aux injections chroniques d'antagonistes de la NMDA chez les jeunes rats. Finalement, le blocage de la transmission dépendante du récepteur de la NMDA dans la boucle de l'inhibition récurrente pourrait conduire à une augmentation globale de l'excitabilité du réseau neuronal. Ceci pourrait ressembler aux symptômes schizophréniques positifs observés chez l'homme, dus a priori à des concentrations élevées de NAAG, l'antagoniste endogène du NMDA.*

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