

Role of nonsynaptic nicotinic acetylcholine and NMDA receptors in physiological and pathological conditions

„A NEMSZINAPTIKUS NIKOTINIKUS ACETILKOLIN ÉS NMDA RECEPTOROK SZEREPE ÉLETTANI KÖRÜLMÉNYEK KÖZÖTT ÉS PATHOLÓGIÁS ÁLLAPOTOKBAN”

OTKA NK 72959

FINAL REPORT

Introduction

Synaptic transmission is a rapid form of communication that involves the rapid (μs) diffusion of a high concentration (mM) of transmitters over a short distance (nm) between pre- and postsynaptic sites. An alternative form of chemical communication, non-synaptic transmission, also exists within the central nervous system (CNS) (Vizi 1984; Vizi 2000), providing a much slower (ms – s) means of communication that operates over longer diffusion distances (μm), uses relatively low transmitter concentrations (nM- μM) and exerts tonic influences. Synaptic functions are thought to be responsible for tasks that require high speed and precision, such as visual pattern recognition and motor pattern execution. Conversely, non-synaptic functions are thought to be responsible for modulating and tuning these processes by providing the emotional, attentional and motivational context. Many neurological and psychiatric disorders involve disturbances of these fine modulations and, therefore, have been most effectively combated by targeting the nonsynaptic modulatory system. Indeed, almost all pharmacological manipulations of the CNS are restricted to this latter realm, which predominantly involves nonsynaptic functions (Vizi, Fekete et al. 2010). Competitive antagonists preferentially affect nonsynaptic receptors because it is easier for the antagonist to displace agonists at low extrasynaptic concentrations than at high synaptic concentrations. Similarly, agents that inhibit transport systems predominantly affect the nonsynaptic systems because a minor increase in ambient concentrations has a large effect on high affinity extrasynaptic receptors, while the effect is negligible within the synaptic cleft where low affinity receptors are activated by millimolar transmitter concentrations. Thus, it is important to study the functions of these receptors and transporters, their sub-cellular distributions, and any functional differences (Vizi, Fekete et al. 2010) that depend on physiological and pathological conditions to develop effective drugs, particularly those that target extrasynaptic receptor subtypes.

The primary goal of the present study was to elucidate the function and pharmacology of nonsynaptic nicotinic acetylcholine receptors (nAChRs) (Chiovini, Turi et al. 2010; McCormack, Melis et al. 2010; Vizi, Fekete et al. 2010; Mike, Pesti et al. 2011) and NMDA receptors (Vizi,

Fekete et al. 2010; Katona, Kaszas et al. 2011; Lendvai, Halmos et al. 2011; Szasz, Fodor et al. 2011). The involvement of voltage-gated channels in neuropathological processes and possible pharmacological approaches for neuroprotection were also investigated.

Nonsynaptic and synaptic NMDA receptors

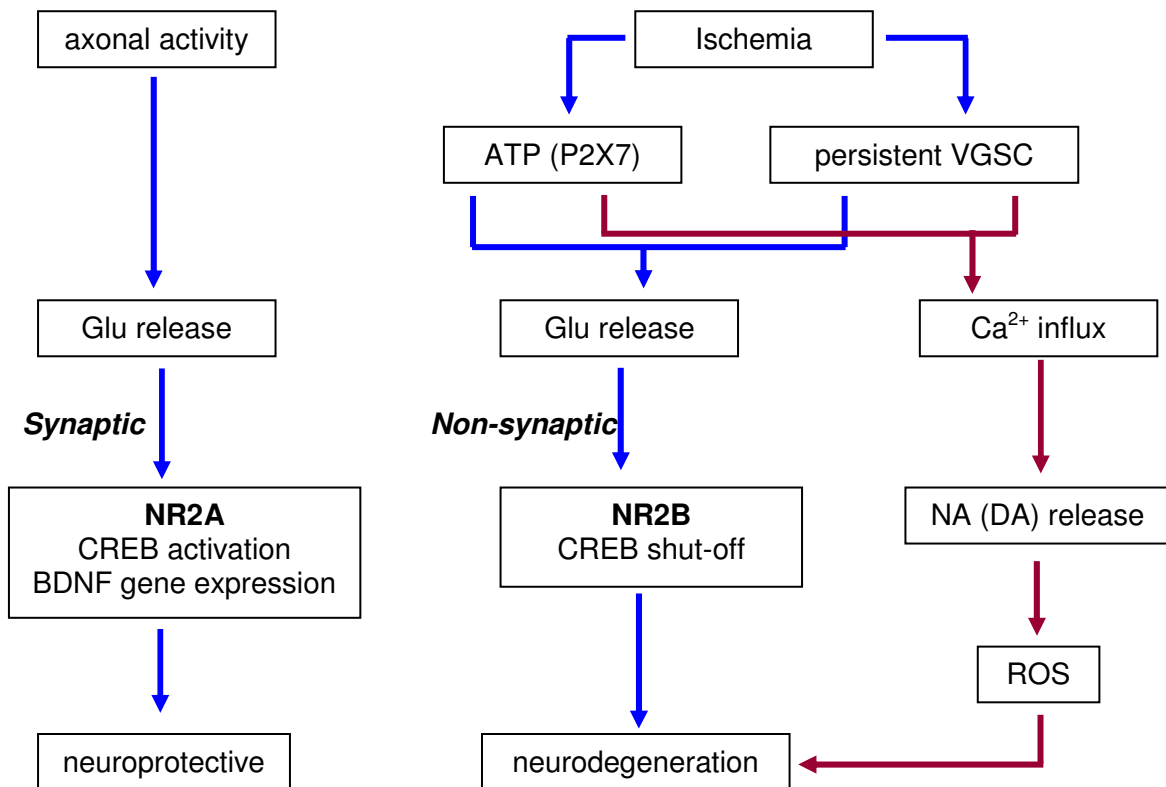


Fig. 1 Glutamate may have neuroprotective or neurodegenerative effects depending on physiological or pathophysiological conditions. The role of NR2A and NR2B receptors. Note that NR2A receptors located in the synapse, transmit information from the glutamatergic terminals to be neuroprotective. During ischemia glutamate released from nerve terminals $[Ca^{2+}]_o$ -independently exerts effects on extrasynaptic NR2B receptors, producing neurodegeneration.

It has been demonstrated that calcium entry through synaptic NMDA receptors induces cAMP response element binding protein (CREB) activity and CREB-evoked brain derived neurotrophic factor (BDNF) gene expression (Leveille, El Gaamouch et al. 2008). In contrast, Ca^{2+} entry through non-synaptic NMDA receptors, which is activated by Glu that is added to the bath or released by hypoxic/ischaemic conditions, triggers the dominant CREB shut-off pathway and, therefore, blocks the induction of BDNF expression (Hardingham, Fukunaga et al. 2002) (Fig. 1). These findings indicate that synaptic NMDA receptors exhibit anti-apoptotic activity, whereas the stimulation of extrasynaptic NMDA receptors causes a breakdown of the mitochondrial membrane potential(s). The latter is an early marker for Glu-induced neuronal damage, ROS generation (Adam-Vizi 2005) and cell death. It has also been reported that hypoxic/ischaemic conditions achieved through the

stimulation of extrasynaptic Glu receptors may activate Ca^{2+} -activated chloride channels (ClCa1) (Wahl, Buchthal et al. 2009), which are part of the genomic death programme and are also involved in causing neuronal damage.

Thus, NMDA receptor antagonists are expected to provide some neuroprotective effects. However, a number of clinical trials have failed to demonstrate the expected effects of these drugs (Editorial 2006) to reduce brain injury or prevent neurodegeneration (Ikonomidou and Turski 2002; Kemp and McKernan 2002). Recent advances in NMDA receptor research have provided an explanation for this failure. It has been shown that NR2A subunit-

containing NMDA receptors are mainly located synaptically, whereas NR2B subunits are predominantly expressed in extrasynaptic locations and are primarily responsible for NMDA's excitotoxic effects (Leveille, El Gaamouch et al. 2008) (Fig. 2). Compounds that affect both subtypes have been studied clinically without any progress. Consequently, in the last few years, the specific blockade of non-synaptic NMDA receptors and the development of selective NR2B antagonists have begun to dominate the field of neuroprotective drug development. Pharmacological studies using NR2B receptor antagonists, therefore, promise interesting and clinically relevant results that could lead to the development of drugs for the treatment of dementia, acute brain injuries and neurodegenerative diseases.

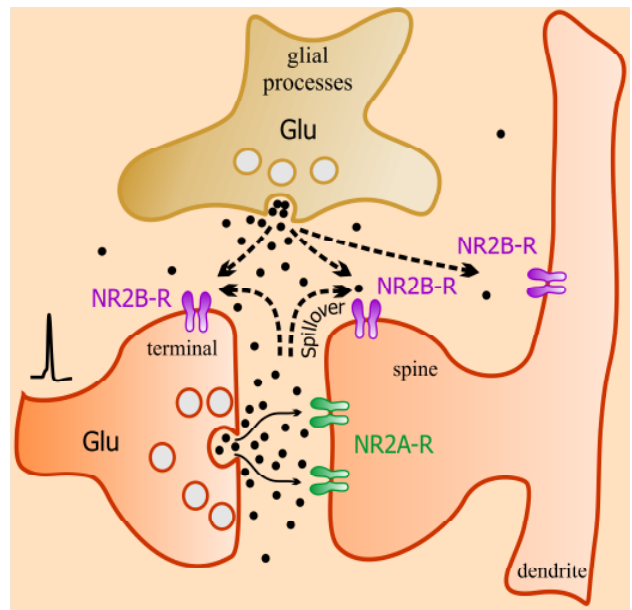


Figure 2. Nonsynaptic NMDA receptors: localisation and possible functions. (schematic drawing taken from Vizi et al. (Br. J. Pharmac. 160: 785-809, 2010).

Mayer A, Szasz BK, Kiss JP.

Inhibitory effect of antidepressants on the NMDA-evoked [^3H]noradrenaline release from rat hippocampal slices.

Neurochem Int, 2009, 55, 383-388

In this study we have shown that desipramine and fluoxetine were able to inhibit the NMDA-evoked [^3H]noradrenaline release with an IC_{50} 14.6 and 41.1 μM , respectively. These two antidepressants inhibited only the NMDA-evoked response, they were without effects on the release of [^3H]noradrenaline evoked by axonal activity. The non-competitive NMDA receptor antagonist MK801 also inhibited the release of [^3H]noradrenaline produced by NMDA receptor activation. These data indicate that the inhibitory effect of antidepressants on NMDA receptors might contribute to the therapeutic effect of these drugs.

Vizi, E. S., Szasz, B. K., Fodor, L., Mike, A., Lenkey, N., Kurko, D., Nagy, J., Kiss, J. P.
GluN2B-NMDA receptors as possible targets for the neuroprotective and antidepressant effects of fluoxetine.
Neurochem Int, 2012, accepted for publication

It is interesting that, although they belong to different superfamilies of ligand-gated ionotropic receptors, nAChRs and NMDA receptors share several pharmacological properties. For instance, the channel blocker-type nAChR antagonist mecamylamine is able to block NMDA receptors (Snell and Johnson 1989), while the channel blocker-type NMDA receptor antagonist MK-801 inhibits nAChRs (Ramoia, Alkondon et al. 1990). In addition, fluoxetine and desipramine have been shown to inhibit NMDA-evoked inward currents in a dose-dependent manner (Szasz, Mike et al. 2007), resulting in similar IC_{50} values for both drugs (3.13 μ M for desipramine and 10.51 μ M for fluoxetine (Fig. 3)). These concentrations fall in the therapeutically relevant range, suggesting that the clinical effects of neuroprotective drugs may involve the inhibition of NMDA receptors (Vizi 2000; Kiss 2008).

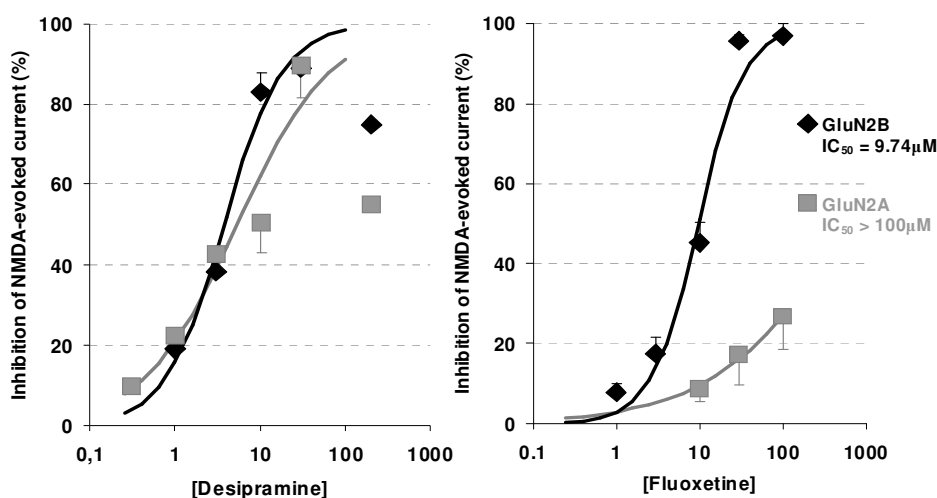


Fig. 3 Inhibitory effect of fluoxetine (FLX) and desipramine on the NMDA-evoked currents in HEK 293 cells stably expressing rat recombinant NMDA receptors with NR1a/NR2A or NR1a/NR2B subunit compositions. The cells were stimulated with NMDA (10 μ M). Each point represents the mean \pm S.E.M. of 4-6 independent experiments. The IC_{50} values were determined by non-linear regression (Prism 3.0). (Figure taken from Vizi et al., *Neurochem Int* 2012).

Lendvai, B., Halmos, G. B., Polony, G., Kapocsi, J., Horvath, T., Aller, M., Vizi, E. S., Zelles, T.

Chemical neuroprotection in the cochlea: the modulation of dopamine release from lateral olivocochlear efferents.

Neurochem Int, 2011, 59, 150-158

The role of extrasynaptic NR2B subunit-containing receptors in excitotoxicity-related hearing loss has also been discussed at length in our review of the neuroprotective role of dopamine in the cochlea. We propose that, under conditions of hearing loss resulting from ischaemia, age

progression, noise, and aminoglycoside exposure, elevated levels of extracellular glutamate evoke excitotoxic effects via nonsynaptic NMDA receptors.

Katona, G., Kaszas, A., Turi, G. F., Hajos, N., Tamas, G., Vizi, E. S., Rozsa, B.
Roller Coaster Scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons.
Proc Natl Acad Sci USA, 2011, 108, 2148-2153

Dendrites have long been thought to be electrically passive objects that can be described by the cable theory. It has only recently been discovered that dendrites exhibit active conductances and are even capable of producing local regenerative activities (dSpikes). It is thought that dSpikes may change the mode of integration from linear to supralinear within individual dendritic branches, a process that has been proposed to underlie memory formation. To date, such mechanisms have not been described for inhibitory interneurons. Using the 3 dimensional scanning method developed in our laboratory (which provides uniquely high spatial and temporal resolutions), we detected dSpikes in hippocampal interneurons for the first time. Subsequently, we explored the mechanisms behind interneuronal dSpikes, investigated the role of NMDA receptors in this phenomenon, and described a special integration method (which is unlike that previously reported in excitatory pyramidal cells).

Nonsynaptic nAChRs

Investigating this phenomenon in parvalbumin-positive GABAergic interneurons is important because clinical studies have shown that working memory impairments are accompanied by decreased power and synchrony of gamma oscillations in the brain (Gruber and Muller 2006). This effect has been observed in conditions such as psychosis, social withdrawal, and cognitive impairment, all of which are symptoms of schizophrenia (Lewis, Hashimoto et al. 2005). Similarly, chronic antagonism of NMDA receptors reduces gamma activity in the brain (Cunningham, Hunt et al. 2006; Kittelberger, Hur et al. 2011), indicating that NMDA receptors are involved in memory impairment, one of the earliest and most consistent manifestations of the disease.

Nicotine, a tertiary amine, has long been recognised as the primary psychoactive agent in tobacco. In addition to being an addictive drug, nicotine has many effects in the central nervous system (CNS) that are of therapeutic significance (Lloyd, Menzaghi et al. 1998). Nicotine and compounds that act on nicotinic receptors exhibit analgesic properties and improve several cognitive functions, including attention, learning and memory (Levine, Levine et al. 1998). Nicotine has been shown to enhance cognitive functions in both healthy subjects and schizophrenia (SP) patients (Jacobsen,

D'Souza et al. 2004; Barr, Culhane et al. 2008). Some studies (Guan, Zhang et al. 1999) have reported reduced expressions of $\alpha 7$ nAChRs in the frontal cortices of patients with schizophrenia. More importantly, accumulating scientific results support nAChRs as possible therapeutic targets for schizophrenia. Given the strong scientific evidence that diminished NMDA receptor activity (Gordon 2010) and subsequent cortical GABAergic dysfunction may underlie the pathophysiology of psychiatric disorders, including schizophrenia (Benes and Berretta 2001), it is certainly worth investigating the effect of nicotine on Ca^{2+} transients recorded on boutons of parvalbumin-positive GABAergic interneurons. Indeed, nicotine has been shown to facilitate the Ca^{2+} transients in boutons in response to soma stimulation(s), indicating that it enhances the release of GABA from axon terminals. This effect may help to restore gamma activity, thereby helping to alleviate memory dysfunction. In our earlier publication (Seršen, Toth et al. 1995) we firstly provided evidence that nicotine acts on preterminal axon able to release transmitter. We have recently shown that nicotine facilitates axonal Ca^{2+} transients measured by 2-foton laser scanning microscopy (unpublished data; Fig. 4).

Nicotine facilitates axonal Ca^{2+} transients by trains

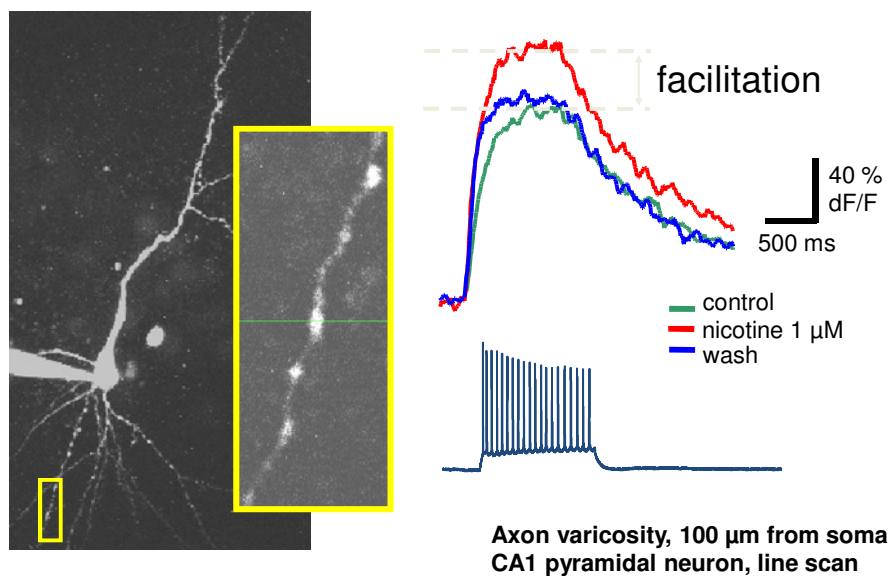


Fig. 4 The first evidence that nicotine facilitates Ca^{2+} transient evoked by soma stimulation (unpublished) in axonal bouton of Pv+ GABAergic interneurons. Two-photon laser scanning microscopy (for Method see Rózsa et al. 2011). The subtype of nAChR remained to be classified. Note the low concentration of nicotine acting on varicosities (in the chain-smokers brain nicotine can reach 0.2-2 μM) indicating extrasynaptic action.

Chiovini, B., Turi, G. F., Katona, G., Kaszas, A., Erdelyi, F., Szabo, G., Monyer, H., Csakanyi, A., Vizi, E. S., Rozsa, B.

Enhanced dendritic action potential backpropagation in parvalbumin-positive basket cells during sharp wave activity.

Neurochem Res, 2010, 35, 2086-2095

Furthermore, we have studied the modulatory role of nonsynaptic nicotinic receptors on backpropagating action potentials in hippocampal parvalbumine positive interneurons. Backpropagating action potentials are fundamental to the Hebbian concept of plasticity because synaptic potentiation or depression depends on the relative timing of action potentials and synaptic inputs. Thus, we investigated the effect of *in vitro* sharp-wave oscillations on backpropagating action potentials. This activity pattern occurs in the hippocampus during the awake resting state, consummation (feeding) and slow wave sleep and is, therefore, in contrast to theta oscillation, which occurs during exploration. Memory acquisition is hypothesised to take place during the theta phase, while memory consolidation is presumed to occur during sharp wave oscillation. We observed that, similar to sharp-wave oscillations, activation of nonsynaptic nicotinic receptors produced action potential backpropagation-enhancing effects (Fig. 5).

Fig. 5 Action potential backpropagation is enhanced during nAChR activation. **a Top**, Dendritic Ca^{2+} transients elicited by rapid ACh injections (1 mM for 200 ms) in the presence of atropine (1 μM), bicuculline (20 μM), AP5 (40 μM) and CNQX (10 μM). **Bottom**, Corresponding somatic membrane potential with a brief train of APs. **b** Subthreshold voltage and Ca^{2+} responses at more hyperpolarized membrane potential. **c Top**, Backpropagating action potential induced Ca^{2+} transients in control conditions (BAP5, *thick black traces*) and in the presence of rapid nAChR activation (BAP5 + ACh, *thick gray traces*). In case of small nAChR activation the difference of BAP5 + nACh and BAP5 (*Diff. thin black traces*) was larger as compared to the nAChR-induced transient, suggesting supralinear summation of the response; however, summation was sublinear (*Bottom*) when nAChR activation was large. **d** The difference of BAP5 + nACh and BAP5 transients as a function of nAChR activation. Each point represents different cells

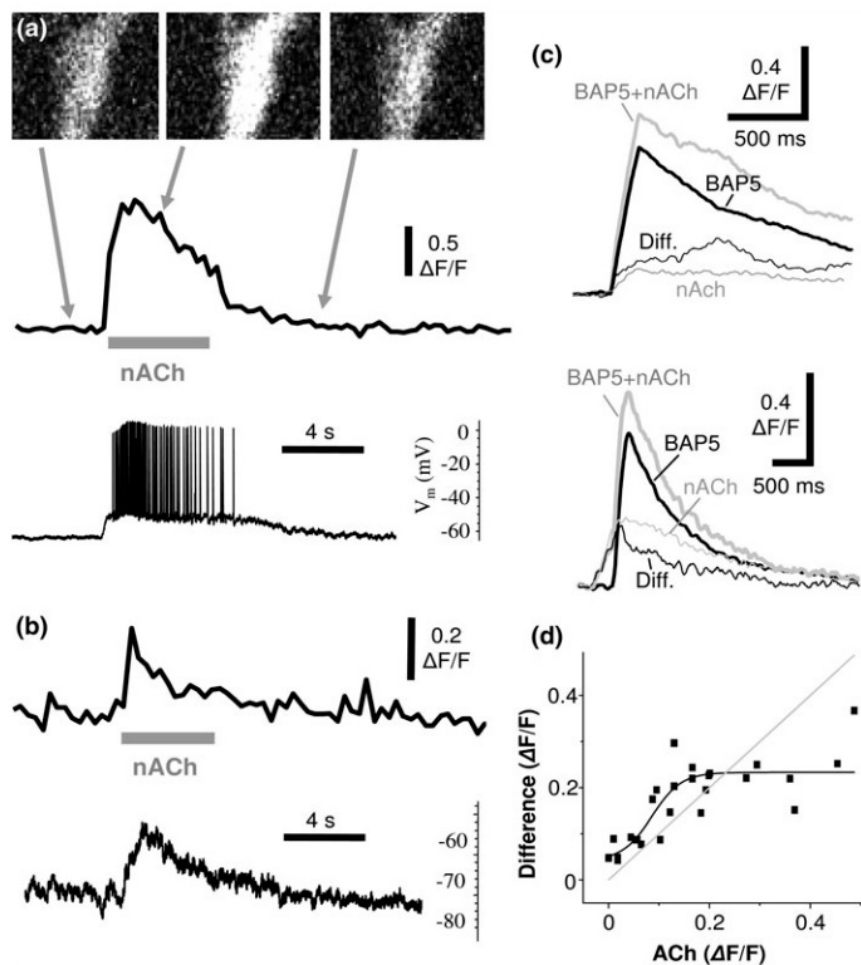


Fig. 5 Effect of nicotine on extrasynaptic nAChRs located on the dendrites of GABA-ergic interneurons. Figure from Chiovini et al., 2010.

McCormack, T. J., Melis, C., Colon, J., Gay, E. A., Mike, A., Karoly, R., Lamb, P. W., Molteni, C., Yakel, J. L.

Rapid desensitization of the rat $\alpha 7$ nAChR is facilitated by the presence of a proline residue in the outer beta-sheet.

J Physiol (Lond), 2010, 588, 4415-4429

Alpha7 subunit-containing neuronal nicotinic receptors are the second most abundant nicotinic receptors in the CNS (after the alpha4-beta2 subunit-containing type). Their unique properties include extremely fast activation and desensitisation, extremely low open probability, and high Ca^{2+} permeability. The receptors are partly synaptic but are more typically extrasynaptic, and both activity/location types are distinguishable by their known functions (learning, memory, cognition, reward, sensory information processing, motor control, arousal, pain, neuroprotection, etc.). Agonists and positive allosteric modulators (PAMs) of $\alpha 7$ nAChRs are prospective pro-cognitive drugs for treatment of schizophrenia and different types of dementia, as demonstrated by several preclinical and clinical studies. The advantage of PAMs over agonists is that the physiological spatio-temporal pattern of neuronal activation is preserved with the former; only $\alpha 7$ nAChR-mediated signals are augmented. This is the basis of the effectiveness of GABA_A receptor PAMs (such as benzodiazepines). In the case of $\alpha 7$ nAChRs, however, most PAM compounds have only recently been developed, and much less is known about their mode(s) of action. Some very basic questions remain unanswered regarding (a) the operation of the $\alpha 7$ nAChR itself, and (b) the effect of PAMs on receptor function.

In this paper we address the operation and the kinetics of the receptor by studying the effect of an important mutation on agonist affinity and gating of the receptor.

Mike, A., Pesti, K., Szabo, A., Vizi, E. S.

Mode of action of the positive modulator PNU 120596 on alpha7 nicotinic receptors.
Neuropharmacology, submitted for publication

In recent work (Neuropharmacology, submitted), we investigated the mode of action of PNU 120596, one of the best known PAMs. For the first time, we determined the maximal open probability at the agonist-evoked current peak (3.85 ± 0.72 %), as well as the open probability in the presence of the modulator (66.8 ± 6.25 %). We have shown that PNU 120596 is unable to bind to the resting state of the receptor; instead, it binds to the desensitised state, thereby re-activating desensitised receptors. We observed, however, that a second desensitised state with slower onset and recovery kinetics can develop and that this slower desensitised state is insensitive to PNU 120596. Furthermore, our results indicate that binding of the modulator greatly increases agonist affinity by slowing down dissociation.

Other nonsynaptic mechanisms

Reactive oxygen species (ROS) production.

Milusheva E, Baranyi M, Kormos E, Hracskó Z, Sylvester Vizi E, Sperlágh B.

The effect of antiparkinsonian drugs on oxidative stress induced pathological [³H]dopamine efflux after in vitro rotenone exposure in rat striatal slices.
Neuropharmacology, 2010, 58, 816-825.

This non-synaptic DA release could serve as a source of further self-amplifying ROS production imposing an extra oxidative burden to dopaminergic neurons (Tretter, Sipos et al. 2004) and sensitizing them to a relatively mild mitochondrial deficit alternative ROS-producing pathways not selective to the dopaminergic neurons, such as direct mitochondrial or microglial superoxide production, may also contribute to the oxidative damage of dopaminergic and nondopaminergic terminals after rotenone treatment (i.e. on aging patients or on patients suffering from mild PD).

Parkinson's disease (PD) is the second most common neurodegenerative disease, which primarily affects the aging population. An in vitro model of mitochondrial dysfunction with subsequent oxidative stress was elaborated and utilized to study the effect of L-deprenyl and rasaglyline, currently used for the treatment of Parkinson's H₂O₂-induced ³H-dopamine release and formation of toxic (Ros and dopamine quinone) compounds in rat striatal slices. For the mechanism see Fig. 1. In this paper we suggested that L-deprenyl is a neuroprotective drug!

Role of extracellular adenosine.

Sperlagh, B., Vizi, E. S.

The role of extracellular adenosine in chemical neurotransmission in the hippocampus and Basal Ganglia: pharmacological and clinical aspects.
Curr Top Med Chem, 2011, 11, 1034-1046.

During hypoxia/ischemia not only neurotransmitters such as Glu, GABA, monoamines are released due to the reverse operation of transporters, but there is a release of ATP from different cells which is immediately decomposed into adenosine and thereby concentration of extracellular adenosine increases. The activation of presynaptically located A₁ receptors results in a decrease of transmitter release (Vizi and Knoll 1971) including Glu and exerts a neuroprotective action against ischemia (Stone, Ceruti et al. 2009).

Activation of adenosine receptors is another key protection mechanism against excitotoxicity. The dynamics of extracellular adenosine, the localisation of receptors, the function of individual subtypes and some potential targets for pharmacological intervention have been reviewed in this paper.

Role of sodium channels in excitotoxicity.

Activations of NMDA receptors and sodium channels are interconnected in pathological overexcitation. The contribution of persistent sodium channels is especially significant (Fig. 1). The persistent sodium current amplitude is less than 1% of the transient amplitude (French, Sah et al. 1990; Hammarstrom and Gage 1998; Stone, Ceruti et al. 2009), but the resulting total current is functionally very significant at subthreshold voltages (Stafstrom, Schwindt et al. 1982; Stafstrom, Schwindt et al. 1985; Bean 2007). This persistent current functions by amplifying sub-threshold depolarisation and generating an endogenous rhythm (Bean 2007; Tazerart, Vinay et al. 2008). Upregulation of the persistent component has been described for most major sodium channel isoforms under pathological conditions (e.g., epilepsy, traumatic injury of the nervous system, chronic neurodegenerative diseases such as multiple sclerosis or ALS, cardiac ischaemia, long QT syndrome, chronic inflammation, various pain syndromes, muscle spasms, etc.). The resulting overexcitability is amplified by the increased Ca^{2+} influx through NMDA receptors. Overactivation of NMDA receptors has been hypothesised to play a pivotal role in producing neuronal death by increasing intracellular Ca^{2+} activated nucleases, cytosolic proteases and kinases, destroying the composition of the cell. Due to Na^+ entry and the resulting increase in intracellular Na^+ concentration, the osmotic balance of the cell is also disturbed during persistent depolarisation. The entry of Na^+ is followed by the passive entry of Cl^- ions and water, which causes an increase in the cell volume (osmotic swelling) (Fig. 1) and the release of cell contents into the extracellular space. These findings indicate that both antagonism of NMDA receptors and inhibition of sodium channels are viable strategies against conditions of hyper-excitability (e.g., to prevent neuronal loss after stroke and head trauma).

Because of the significant involvement of voltage-gated channels in pathophysiological processes of neurodegeneration, we studied the role of sodium channels as well as their pharmacology. Sodium channels are not only important targets of anti-epileptic drugs but are also promising candidate targets for the treatment of acute and chronic neurodegenerative processes and different pain syndromes.

Persistent sodium current is not caused by a specific channel isoform but most probably reflects a specific phosphorylation state of the channels. A major challenge in current pharmacological research regarding sodium channels is the development of persistent-selective compounds. For this reason, it is especially important to understand the different modes of action of different types of sodium channel inhibitors.

Fekete, A., Franklin, L., Ikemoto, T., Rozsa, B., Lendvai, B., Sylvester Vizi, E., Zelles, T.
Mechanism of the persistent sodium current activator veratridine-evoked Ca elevation: implication for epilepsy.
J Neurochem, 2009, 111, 745-756.

In this study we studied the mechanisms by which activation of a persistent sodium current is linked to elevation of intracellular Ca^{2+} levels. The sources of calcium increase, and their temporal aspects were investigated. Ca^{2+} influx through voltage-gated Ca^{2+} channels and mitochondrial Ca^{2+} sequestration dominated the first, rapid phase, which was followed by a robust second phase, which involved reverse operation of the Na^+ - Ca^{2+} exchanger and mitochondrial Ca^{2+} release.

Lenkey, N., Karoly, R., Lukacs, P., Vizi, E. S., Sunesen, M., Fodor, L., Mike, A.
Classification of drugs based on properties of sodium channel inhibition: a comparative automated patch-clamp study.
PLoS One, 2010, 5, e15568.

In an attempt to identify sodium channel inhibitors with different state-selectivity, we performed a comparative study of 33 SCI drugs using an automated patch clamp instrument. We have shown that SCIs are diverse in their modes of action; and identified at least four distinct groups with different chemical properties and modes of action. This study was the first step to identify chemical properties which determine effectiveness in specific therapeutic actions. We found that aromaticity and logD are especially important determinants of inactivated state affinity, and showed that – contrary to textbook knowledge – positive charge is not a requirement (it was only found important for resting state affinity).

Lenkey, N., Karoly, R., Epresi, N., Vizi, E., Mike, A.
Binding of sodium channel inhibitors to hyperpolarized and depolarized conformations of the channel.
Neuropharmacology 60, 191-200.

One of the major hindrances in the development of novel sodium-channel inhibiting drugs is the lack of detailed comparative analyses of drug actions. Individual laboratories often provide detailed analyses for individual drugs; however, these are not compared with studies in other laboratories on other compounds. Large-scale screens for SCIs, on the other hand, provide insufficient useful information regarding the modes of drug actions.

For these reasons, we wanted to devise a way to compare data from individual laboratories. Toward this end, we developed a method to calculate resting- and inactivated-state affinities from different sets of experimental protocols. Using this method, we compared the affinities of individual drugs. Interestingly, we found that SCIs that were therapeutically successful were among the least potent drugs, indicating that potency alone is not a good predictor of therapeutic value. It has been suggested that the extent of state-dependence (i.e., inactivated state affinity / resting state affinity) is

more important in judging the therapeutic potential of a novel compound. Therefore, we performed a meta-analysis to ascertain the most important chemical properties that determine a compound's affinity for resting and inactivated states. We showed that aromaticity and logD values are critical in determining inactivated-state affinity. (Lenkey, Karoly et al. 2011).

Karoly, R., Lenkey, N., Juhasz, A. O., Vizi, E. S., Mike, A.

Fast- or slow-inactivated state preference of Na⁺ channel inhibitors: a simulation and experimental study.

PLoS Comput Biol 6, 2010, e1000818.

One of the major mechanisms of inhibition by SCIs is related to their high affinities for the inactivated state, resulting in the stabilisation of particular inactivated states via drug-bound channels. A unique action is expected for each SCI, depending on which of the inactivated states it prefers. In recent years, several compounds have been shown to prefer the slow inactivated state. However, as we have discussed in an experimental and theoretical paper, there is interaction between binding and gating kinetics, making it easy to mistake slow binding kinetics for slow inactivated-state preference in studies of SCIs. We have detailed the special situations in which specific-state preferences can be determined with confidence.

Mike, A., Lukacs, P.

The enigmatic drug binding site for sodium channel inhibitors.

Curr Mol Pharmacol 2010, 3, 129-144.

We deduced that if SCIs are functionally heterogeneous, they must be heterogeneous in their interactions with specific residues within the SCI binding region. We collected all available mutagenesis data, and compared data for individual drugs plotted on a homology model of sodium channels. We found that while the role of Phe1764 (Nav1.2 numbering) is unquestionably crucial for the majority of drugs, its mutation did not cause a significant change of the affinity of eight out of 28 drugs. This suggests that an alternative binding site must exist. The other residue, Tyr1771 which is regarded as the other crucial element of the binding site, did not prove to be special at all; mutation of at least five other residues caused similar changes in affinity. Furthermore, based on mutagenesis data we proposed that a highly conservative ring of asparagine residues located at the outer surface of the pore domain is involved in voltage sensor-gate coupling.

Role of different neurotransmitters released into the extracellular space.

Vizi, E. S., Fekete, A., Karoly, R., Mike, A

Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment.

Br J Pharmacol, 2010, 160, 785-809.

Finally, we wrote a general review on nonsynaptic neurotransmission for British Journal of Pharmacology, which included discussion of the role of nicotinic, NMDA, adenosine as well as all other major receptor types. In addition we considered specific aspects of non-synaptic transmission, such as synaptic–extrasynaptic receptor trafficking, neuron–glia communication and retrograde signaling. We reviewed structural and functional aspects of non-synaptic transmission, including (i) anatomical arrangement of non-synaptic release sites, receptors and transporters, (ii) intravesicular, intra- and extracellular concentrations of neurotransmitters, as well as the spatiotemporal pattern of transmitter diffusion. We emphasized the role of non-synaptic receptors and transporters as primary targets available for pharmacological intervention.

Summary:

There is a long list of neuroprotective compounds that have failed to be clinically useful in the treatment of ischaemic stroke (Editorial 2006). This is likely due, at least in part, to our inadequate knowledge regarding the core mechanisms of ischaemic diseases.

Neurodegeneration after a stroke is one of the major causes of present-day morbidity and mortality. Cell death produced by excitotoxicity resulting from acute ischaemic brain injuries and chronic neurodegeneration is associated with excessive and/or prolonged activation of Glu receptors, resulting in the persistent depolarisation of neurons, mitochondrial dysfunction leading to energy failure, increased mitochondrial Ca^{2+} uptake, increased production of reactive oxygen species (ROS), increased concentrations of cytosolic Ca^{2+} and the activation of self-destructing enzymatic and nuclear mechanisms. Depolarisation is initiated by the activation of AMPA receptors and voltage-dependent Na^+ channels, leading to Na^+ entry followed by Ca^{2+} influx and further depolarisation. If the cell becomes depolarised, NMDA receptors escape from the Mg^{2+} block and can be activated by synaptic Glu, resulting in further Ca^{2+} entry into the cell. In addition, the intracellular Ca^{2+} concentrations can be increased through the activation of voltage-dependent Ca^{2+} channels, the opening of NMDA receptor channels and the impairment of membrane $\text{Na}^+/\text{Ca}^{2+}$ exchangers.

- Most “novel” drugs that target the CNS are designed to act on neurotransmitter receptors or transporters that are localised within synapses. To develop the most effective drugs, it is

important to remember that there are extrasynaptic receptors and transporters that may outnumber those located within synapses and that, when malfunctioning, may be responsible for several symptoms of CNS disorders. There is strong morphological and functional evidence that nonsynaptic receptors and transporters are present in the brain and are of axonal or somatodendritic localisations. They can be present in silent forms, and they have high affinities, enabling them to respond to chemical messages arriving from distant axon terminals and at low ambient concentrations. (Vizi, Fekete et al. 2010).

- Extrasynaptic receptors and transporters are easily accessible by drugs and therefore present ideal targets for drug therapy at low, non-toxic levels, and their abnormal (excessive, tonic or lack of) activation by transmitters that are released into the extracellular space may be involved in the pathology of CNS diseases. Synaptic NMDA receptors show anti-apoptotic activity, whereas stimulation of extrasynaptic NMDA receptors causes a breakdown of the mitochondrial membrane potential. The latter is an early marker for Glu-induced neuronal damage, ROS generation (Adam-Vizi 2005) and cell death.
- Hypoxic/ischaemic conditions induced by the stimulation of extrasynaptic Glu receptors have also been found to activate Ca^{2+} -activated chloride channels (ClCa1) (Wahl, Buchthal et al. 2009), which are part of the genomic death programme and are also involved in causing neuronal damage. It is clear from these data that non-selective NMDA antagonists that exert effects on both NR2B and NR2A receptors cannot be efficient neuroprotective drugs because they inhibit both the neurotoxic and neuroprotective pathways of NMDA transmission. The specific blockade of nonsynaptic NMDA receptors and the search for selective NR2B antagonists have, therefore, become primary avenues for drug development.

We suggest that future drug development research consider the following:

- Compounds that are able to selectively inhibit non-synaptic NR2B Glu receptors and that are able to block Na^+ -channels (such as Fluoxetine);
- Compounds that are able to prevent the transporter-mediated release of DA (noradrenaline) in response to ischaemia (oxidative stress, H_2O_2), thereby inhibiting the production of toxic DA metabolites (i.e., DOPEGAL and DOPAL) and H_2O_2 production (oxidative stress). (Note that released DA produces H_2O_2 and H_2O_2 releases DA, thereby forming a destructive feedback loop) are candidates for successful treatment of neurodegenerative diseases.
- compounds able to enhance the effects of nicotinic agonists. They would be potential drugs in the treatment of memory problems and in smoking cessation.
-

- Adam-Vizi, V. (2005). "Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources." *Antioxid Redox Signal* **7**(9-10): 1140-1149.
- Barr, R. S., M. A. Culhane, et al. (2008). "The effects of transdermal nicotine on cognition in nonsmokers with schizophrenia and nonpsychiatric controls." *Neuropsychopharmacology* **33**(3): 480-490.
- Bean, B. P. (2007). "The action potential in mammalian central neurons." *Nat Rev Neurosci* **8**(6): 451-465.
- Benes, F. M. and S. Berretta (2001). "GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder." *Neuropsychopharmacology* **25**(1): 1-27.
- Chiovini, B., G. F. Turi, et al. (2010). "Enhanced dendritic action potential backpropagation in parvalbumin-positive basket cells during sharp wave activity." *Neurochem Res* **35**(12): 2086-2095.
- Cunningham, M. O., J. Hunt, et al. (2006). "Region-specific reduction in entorhinal gamma oscillations and parvalbumin-immunoreactive neurons in animal models of psychiatric illness." *J Neurosci* **26**(10): 2767-2776.
- Editorial (2006). "Neuroprotection: the end of an era?" *Lancet* **368**(9547): 1548.
- French, C. R., P. Sah, et al. (1990). "A voltage-dependent persistent sodium current in mammalian hippocampal neurons." *J Gen Physiol* **95**(6): 1139-1157.
- Gordon, J. A. (2010). "Testing the glutamate hypothesis of schizophrenia." *Nat Neurosci* **13**(1): 2-4.
- Gruber, T. and M. M. Muller (2006). "Oscillatory brain activity in the human EEG during indirect and direct memory tasks." *Brain Res* **1097**(1): 194-204.
- Guan, Z. Z., X. Zhang, et al. (1999). "Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain." *Neuroreport* **10**(8): 1779-1782.
- Hammarstrom, A. K. and P. W. Gage (1998). "Inhibition of oxidative metabolism increases persistent sodium current in rat CA1 hippocampal neurons." *J Physiol* **510** (Pt 3): 735-741.
- Hardingham, G. E., Y. Fukunaga, et al. (2002). "Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways." *Nat Neurosci* **5**(5): 405-414.
- Ikonomidou, C. and L. Turski (2002). "Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury?" *Lancet Neurol* **1**(6): 383-386.
- Jacobsen, L. K., D. C. D'Souza, et al. (2004). "Nicotine effects on brain function and functional connectivity in schizophrenia." *Biol Psychiatry* **55**(8): 850-858.
- Katona, G., A. Kaszas, et al. (2011). "Roller Coaster Scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons." *Proc Natl Acad Sci U S A* **108**(5): 2148-2153.
- Kemp, J. A. and R. M. McKernan (2002). "NMDA receptor pathways as drug targets." *Nat Neurosci* **5** Suppl: 1039-1042.
- Kiss, J. P. (2008). "Theory of active antidepressants: a nonsynaptic approach to the treatment of depression." *Neurochem Int* **52**(1-2): 34-39.
- Kittelberger, K., E. E. Hur, et al. (2011). "Comparison of the effects of acute and chronic administration of ketamine on hippocampal oscillations: relevance for the NMDA receptor hypofunction model of schizophrenia." *Brain Struct Funct*.
- Lendvai, B., G. B. Halmos, et al. (2011). "Chemical neuroprotection in the cochlea: the modulation of dopamine release from lateral olivocochlear efferents." *Neurochem Int* **59**(2): 150-158.
- Lenkey, N., R. Karoly, et al. (2011). "Binding of sodium channel inhibitors to hyperpolarized and depolarized conformations of the channel." *Neuropharmacology* **60**(1): 191-200.
- Leveille, F., F. El Gaamouch, et al. (2008). "Neuronal viability is controlled by a functional relation between synaptic and extrasynaptic NMDA receptors." *FASEB J* **22**(12): 4258-4271.
- Levine, T. B., A. B. Levine, et al. (1998). "The impact of beta-receptor blocker addition to high-dose angiotensin-converting enzyme inhibitor-nitrate therapy in heart failure." *Clin Cardiol* **21**(12): 899-904.
- Lewis, D. A., T. Hashimoto, et al. (2005). "Cortical inhibitory neurons and schizophrenia." *Nat Rev Neurosci* **6**(4): 312-324.
- Lloyd, G. K., F. Menzaghi, et al. (1998). "The potential of subtype-selective neuronal nicotinic acetylcholine receptor agonists as therapeutic agents." *Life Sci* **62**(17-18): 1601-1606.
- McCormack, T. J., C. Melis, et al. (2010). "Rapid desensitization of the rat 7 nAChR is facilitated by the presence of a proline residue in the outer beta-sheet." *J Physiol* **588**(Pt 22): 4415-4429.
- Mike, A., K. Pesti, et al. (2011). "Mode of action of PNU 120596 on alpha7 nicotinic receptors." *Neuropharmacology*.
- Ramo, A. S., M. Alkondon, et al. (1990). "The anticonvulsant MK-801 interacts with peripheral and central nicotinic acetylcholine receptor ion channels." *J Pharmacol Exp Ther* **254**(1): 71-82.
- Sershen, H., E. Toth, et al. (1995). "Nicotine effects on presynaptic receptor interactions." *Ann N Y Acad Sci* **757**: 238-244.
- Snell, L. D. and K. M. Johnson (1989). "Effects of nicotinic agonists and antagonists on N-methyl-D-aspartate-induced 3H-norepinephrine release and 3H-(1-[1-(2-thienyl)cyclohexyl]-piperidine) binding in rat hippocampus." *Synapse* **3**(2): 129-135.
- Stafstrom, C. E., P. C. Schwandt, et al. (1985). "Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex in vitro." *J Neurophysiol* **53**(1): 153-170.
- Stafstrom, C. E., P. C. Schwandt, et al. (1982). "Negative slope conductance due to a persistent subthreshold sodium current in cat neocortical neurons in vitro." *Brain Res* **236**(1): 221-226.

- Stone, T. W., S. Ceruti, et al. (2009). "Adenosine receptors and neurological disease: neuroprotection and neurodegeneration." Handb Exp Pharmacol(193): 535-587.
- Szasz, B. K., L. Fodor, et al. (2011). "GluN2B-NMDA receptors as possible targets for the neuroprotective and antidepressant effects of fluoxetine." Neurochem Int *accepted for publication*.
- Szasz, B. K., A. Mike, et al. (2007). "Direct inhibitory effect of fluoxetine on N-methyl-D-aspartate receptors in the central nervous system." Biol Psychiatry **62**(11): 1303-1309.
- Tazerart, S., L. Vinay, et al. (2008). "The persistent sodium current generates pacemaker activities in the central pattern generator for locomotion and regulates the locomotor rhythm." J Neurosci **28**(34): 8577-8589.
- Tretter, L., I. Sipos, et al. (2004). "Initiation of neuronal damage by complex I deficiency and oxidative stress in Parkinson's disease." Neurochem Res **29**(3): 569-577.
- Vizi, E. S. (1984). Non-synaptic Interactions Between Neurons: Modulation of Neurochemical Transmission. Pharmacological and Clinical Aspects.. New York, John Wiley and Sons, Chichester.
- Vizi, E. S. (2000). "Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system." Pharmacol Rev **52**(1): 63-89.
- Vizi, E. S., A. Fekete, et al. (2010). "Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment." Br J Pharmacol **160**(4): 785-809.
- Vizi, E. S. and J. Knoll (1971). "The effects of sympathetic nerve stimulation and guanethidine on parasympathetic neuroeffector transmission; the inhibition of acetylcholine release." J Pharm Pharmacol **23**(12): 918-925.
- Wahl, A. S., B. Buchthal, et al. (2009). "Hypoxic/ischemic conditions induce expression of the putative pro-death gene *C1ca1* via activation of extrasynaptic N-methyl-D-aspartate receptors." Neuroscience **158**(1): 344-352.
-