Crosstalk between NMDAR Antagonists and Potassium Channels in Murine and Human Lymphocytes

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1. Summary

N-methyl-D-aspartate receptors (NMDARs) are voltage- and ligand-gated ion channels of the brain. They are key glutamate receptors regulating neuronal synaptic transmission, learning and memory formation. They are also implicated in various neuronal disorders like excitotoxicity, Parkinson's disease, mood disorders or schizophrenia. NMDARs thus are attractive targets to treat neuronal diseases, and NMDAR antagonists like ifenprodil, MK801 or memantine are used to evaluate NMDAR function *in vitro*. Among them, only memantine is clinically used to treat Alzheimer's type of dementia as it is well tolerated. Memantine is a low-affinity, uncompetitive, voltage-dependent drug with a fast receptor kinetic. Its voltage-dependency is explained by its specific blocking site near to the Mg^{2+}-binding site or selectivity filter of NMDARs. As NMDARs have been described to be expressed on lymphocytes, the aim of this study was to determine how NMDAR inhibitors affect T- and B-cell function with regard to possible NMDAR activity, membrane potential, and Ca^{2+}-mobilization. The uncompetitive antagonists memantine and MK801 and the non-competitive inhibitor ifenprodil reduced T-cell receptor (TCR)- and B-cell receptor (BCR)-induced Ca^{2+}-flux in a concentration dependent manner indicating that these antagonists affect T- and B-cell proximal signalling and activation. Interestingly, TLR4/LPS-induced B-cell activation was also impaired by the antagonists as they depolarized the membrane potential. However, in patch clamp studies NMDAR currents on lymphocytes could not be detected. Instead, it was found that the used antagonists cross-inhibit $K_v1.3$ and $K_{Ca}3.1$ K^+-channels on primary murine and human lymphocytes and Jurkat and Raji lymphoma cell lines. $K_v1.3$ and $K_{Ca}3.1$ channels are the major K^+-channels expressed on lymphocytes that maintain the membrane potential as important parameter for Ca^{2+}-fluxes and, thereby, influence many Ca^{2+}-dependent cell responses. Notably, peripheral blood T cells of Alzheimer patients treated with memantine for 1 week showed a strong reduction of $K_v1.3$ channel activity, but after 12 weeks of treatment $K_v1.3$ channel activity was nearly similar to the values before treatment. In conclusion, using electrophysiological and immunological techniques, this study shows substantial effects of NMDAR antagonists on lymphocytes, most likely through cross-inhibition of $K_v1.3$ and $K_{Ca}3.1$ channels. The pharmacological cross-reactivity of memantine may be harmful for lymphocytes and, therefore, an immunological surveillance during memantine application is suggested.

2. Zusammenfassung

N-methyl-D-Aspartat-Rezeptoren (NMDARen) sind spannungs- und ligandengesteuerte Ionenkanäle des Gehirns. Sie spielen eine wichtige Rolle bei der synaptischen Übertragung und der Regulation von Lern- und Gedächtnisprozessen. Zu den NMDAR-assoziierten Störungen zählen Exzitotoxizität, Morbus Parkinson, affektive Störungen und Schizophrenie. Bei der Behandlung neuronaler Erkrankungen sind NMDARen daher wichtige pharmakologische Angriffspunkte. NMDAR-Antagonisten, wie Ifenprodil, MK801 und Memantin, werden bei *in vitro* Untersuchungen zur NMDAR-Funktionalität verwendet. Aufgrund der guten Verträglichkeit wird aber lediglich Memantin zur Behandlung von Alzheimer-Demenzen eingesetzt. Memantin ist ein niedrig-affiner, nicht kompetitiver und spannungsabhängiger Inhibitor mit schneller Rezeptorkinetik. Er blockiert die Mg^{2+}-Bindungsstelle, also den Selektivitätsfilter von NMDARen und behindert derart den Ca^{2+}-Einstrom in die Zelle. Die Expression von NMDARen wurde auch für Lymphozyten beschrieben. In der vorliegenden Studie wurde der Einfluss von NMDAR-Inhibitoren auf die NMDAR-Aktivität, das Membranpotential und die Ca^{2+}-Mobilisierung in Lymphozyten ermittelt. Die Antagonisten Memantin, MK801 sowie Ifenprodil reduzierten den T-Zellrezeptor- und B-Zellrezeptor-induzierten Ca^{2+}-Influx in konzentrationsabhängiger Weise. Dies weist darauf hin, dass die Antagonisten die proximalen Signalwege der Antigenrezeptoren und die Aktivierung von B- und T-Zellen beeinflussen. Auch die TLR4/LPS-induzierte B-Zellaktivierung wurde durch die Inhibitoren gehemmt, wobei auch hier eine Depolarisation des Membranpotentials durch die Inhibitoren nachgewiesen wurde. In den Lymphozyten wurden jedoch keine NMDAR-Ströme mittels Patch-Clamp vorgefunden. Stattdessen wurde aufgezeigt, dass die verwendeten Antagonisten $K_v1.3$ und $K_{Ca}3.1$ K^+-Kanäle in primären Lymphozyten sowie Jurkat- und Raji-Lymphom-Zellen kreuzinhibieren. Diese Kanäle sind die wichtigsten K^+-Kanäle der Lymphozyten zur Regulation des Membranpotentials und damit Ca^{2+}-abhängiger Zellantworten. T-Zellen von Alzheimer Patienten zeigten nach einwöchiger Behandlung mit Memantin eine Reduktion der $K_v1.3$-Kanalaktivität, die sich nach 12-wöchiger Behandlung wieder auf das Ausgangsniveau normalisierte. Mittels elektrophysiologischer und immunologischer Techniken wurden somit substantielle Effekte von NMDAR-Inhibitoren auf Lymphozyten nachgewiesen, die wahrscheinlich auf Kreuzreaktionen mit $K_v1.3$ und $K_{Ca}3.1$ K^+-Kanälen beruhen. Eine immunologische Überwachung bei Memantinbehandlung ist daher empfehlenswert.

3. Introduction

3.1 The Immune System

The cellular defence network of higher vertebrates against foreign antigens is a sophisticated system involving the interplay between several types of leukocytes, principally macrophages, dendritic cells (DCs), granulocytes, natural killer (NK) cells, T and B lymphocytes. The specificity, flexibility and efficiency of this security system are achieved by tightly regulated interactions between these immune cells.

The immune system is divided into two parts: the innate and adaptive arms provide the two lines of defence against invading pathogens. The cells of the innate immune system provide a first line of defence against many common microorganisms. However, they cannot always eliminate infectious agents, and some pathogens cannot be recognized due to the relatively poor diversity of specific recognition elements of innate cells. The lymphocytes of the adaptive immune system have evolved to provide a more versatile means of antigen-specific defence, which also provides increased protection against subsequent re-infection with the same pathogen. The cells of the innate immune system, however, play a crucial part in the initiation and subsequent direction of the adaptive immune response, and participate in the removal of pathogens that have been targeted by the adaptive immune response. Moreover, because there is a delay of ~1 week before the initial adaptive immune response becomes effective, the innate immune response has a critical role in controlling infections during this period (Rouzaire-Dubois B et al. 2002).

3.1.1 The Innate Immune System

The innate immune system consists of cells and proteins, like the complement cascade, that are always present and ready to be mobilized to fight foreign antigens at the site of infection. The main components of the innate immune system can be classified into anatomic, physiologic, phagocytic and inflammatory mediators for occluding the entry of foreign organisms. The anatomical barriers consist of skin and mucous membrane to inhibit the entry of foreign antigens into the body. Skin is the major mechanical barrier retarding the entry of microbes. The physiological obstacles are higher temperature, low pH, and chemical mediators like lysozyme, interferons (IFs)**,** complement, collectins, or Toll-like receptors (TLRs). TLRs can recognize microbial molecules like lipopolysaccharide (LPS) of gram-negative bacteria and send signals into the cells to secrete immune-stimulatory cytokines. Besides these two obstacles, there are cellular barriers formed by phagocytic and inflammatory branches. Blood monocytes, neutrophils, and tissue macrophages can phagocytose, digest and kill foreign microorganisms. The inflammatory process consists of a series

of events started by tissue damage and infection and is caused by the chemical mediators released by the phagocytic cells engulfing the pathogen, for instance bacteria. This whole process of tissue damage due to the phagocytosis process is characterized by four physical signs. These 'four cardinal signs of inflammation' are *rubor* (redness), *tumour* (swelling), *calor* (heat), and *dalor* (pain) as already described by the Roman physician Celsus in 1600 BC. Physiologically, this is caused by the vasodilation of the blood vessels, the increase in capillary permeability and influx of phagocytes from the capillaries into the tissues. Phagocytic cells accumulate at the site of inflammation, phagocytose bacteria, and release lytic enzymes, which can damage nearby healthy cells. Additionally, chemical mediators released due to tissue damage include serum proteins called acute-phase proteins. These, histamine, kinins, fibrin, and C-reactive protein become concentrated hugely in tissue-damaging infections and take part in the inflammation machinery (Kuby J 2003; Rouzaire-Dubois B 2002; Murphy K 2012).

3.1.2 The Adaptive Immune System

The innate immune response makes a crucial contribution to the activation of adaptive immunity. The inflammatory response increases the flow of lymph containing antigen and antigen-bearing cells like DCs into lymphoid tissue, while complement fragments on microbial surfaces and induced changes in activated phagocytes provide signals that synergize in activating lymphocytes whose antigen-receptors bind to specific microbial antigens. Macrophages that have phagocytosed bacteria and become activated can activate T lymphocytes. However, the cells that specialize in presenting antigen to T lymphocytes and are most effective in initiating adaptive immunity are the DCs.

The induction of an adaptive immune response begins when a pathogen is ingested by an immature DC in the infected tissue. These specialized phagocytic cells are resident in most tissues and are relatively long-lived, turning over at a slow rate. They derive from the same bone marrow precursor as macrophages, and migrate from the bone marrow to their peripheral locations, where their role is to survey the local environment for pathogens. After antigen-uptake, tissue-resident DCs migrate through the lymph to the regional lymph nodes where they interact with naive T lymphocytes. On activation, the immature DC matures into a highly effective antigen-presenting cell (APC) and undergoes changes that enable it to activate antigen-specific T cells in the lymph node. APCs process and present antigens bound to major histocompatibility complex (MHC) molecules which are recognized by the antigen-specific T-cell receptor (TCR) expressed on the T cells (Samelson et al. 1985). MHC class I and class II molecules are expressed on all nucleated cells and professional APCs, respectively. MHC-I molecules consist of an α-chain and β_2-microglobulin and are

recognized by CD8 co-receptors and TCRs of cytotoxic T-cells (T_c). MHC-II molecules consist of an α- and β-chain and are recognized by CD4 co-receptors and TCRs present on T helper cells (T_H).

The adaptive immune response is mediated through humoral and cell-mediated responses. Humoral immunity is mediated by B cells, which produce antibodies after activation and differentiation into plasma cells. Antibodies are secreted into the circulation and mucosal fluids, neutralize extracellular microbes, microbial toxins and virus present in the blood and lumen of mucosal organs, such as the gastrointestinal and respiratory tracts. Defence against intracellular microbes and virus relies on cell-mediated immunity by T lymphocytes. T_H cells secrete cytokines which support phagocytosis to destroy ingested microbes and B cell differentiation. T_c cells destroy host cells harbouring intracellular virus and tumor cells. T cells recognize only processed protein antigens, whereas B cells and antibodies are able to recognize different types of antigens, including proteins, carbohydrates, nucleic acids, and lipids.

The defence system of innate immunity is effective in combatting many pathogens. However, it is constrained by relying on relatively few fixed germline-encoded receptors to recognize microorganisms which evolve more rapidly than the host cells they infect. In contrast, the antigen recognition mechanism used by lymphocytes in adaptive immune response has evolved to overcome the constraints faced by the innate immune system to specifically recognize an almost infinite diversity of antigens. The diversity and specificity of the antigen receptors of T and B cells is determined by a unique genetic mechanism, named gene recombination that operates during lymphocyte development in the bone marrow (where B cells are formed) and thymus (where T cells are generated) and generates a huge diversity of antigen-specific TCR and BCR molecules. In 1950, Macfarlane Burnet already postulated in the ‘clonal selection theory’ the pre-existence of many different potential antibody-producing cells in the body, each having the ability to make antibody of a different specificity (Rouzaire-Dubois 2002). Thus, although an individual lymphocyte carries antigen receptors of only one specificity, the antigen receptor specificity of each lymphocyte is different. On binding specific antigen, T and B cells are activated to divide and produce many identical progeny, known as clonal selection. Expanded B cells differentiate to produce antibodies with specificity identical to that of the BCR that triggered activation and clonal expansion (Kuby J 2003; Rouzaire-Dubois B 2002; Murphy K 2012).

A hallmark of the adaptive immune response is the generation of memory cells which get activated when the same pathogen is encountered another time and leads to a faster immune response. The immunological memory is the basis for vaccination or transplantation

studies. All human memory T cells express the surface marker $CD45RO^+$, whereas $CD45RA^+$ and CCR7 (CD197) mark the naive and central memory cell population, respectively (Mackay 1999).

3.2 Signalling Pathways in Lymphocytes

3.2.1 T cell Signalling

TCR ligation induces a number of signalling events that ultimately determine the T cell fate through regulating cytokine production, cell survival, proliferation, and differentiation. T-cell activation is initiated by ligation of the membrane-associated TCR and 'cluster designation' molecules CD4 or CD8 by MHC-II or MHC-I molecules presenting specific peptide on the surface of an APC. TCR activation is initiated by the phosphorylation of immunoreceptor tyrosine-based motifs (ITAMs) within the TCRς chains of the TCR/CD3 complex by lymphocyte protein tyrosine kinase (Lck). The CD45 receptor tyrosine phosphatase dephosphorylates and activates Lck and other Src family tyrosine kinases. Zeta-chain associated protein kinase (Zap-70) is recruited to the phosphorylated TCRς chains where it becomes activated by Lck and then phosphorylates downstream adaptor or scaffold proteins like LAT (Linker for Activated T cells). Phosphorylation of SH2-domain–containing leukocyte protein of 76 kD (SLP-76) by Zap-70 promotes recruitment of Vav (a guanine nucleotide exchange factor) and inducible T cell kinase (Itk). Phosphorylation of phospholipase Cγ1 (PLCγ1) by Itk results in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce the second messengers diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG activates protein kinase Cθ (PKCθ) and the mitogen-activated protein kinase (MAPK)/Erk pathway, both promoting activation of the transcription factor nuclear factor-κB (NF-κB). IP_3 triggers the release of Ca^{2+} from the endoplasmic reticulum (ER), which promotes entry of extracellular Ca^{2+} into the cell through calcium release-activated Ca^{2+} channels (CRAC) (Weiss 2009, 2010).

3.2.2 B cell Signalling

Similar to T cells, the B-cell receptor (BCR) complex consists of two modules: the antigen-binding and the signal transducing moiety. The antigen-binding moiety in essence is an immunoglobulin that is integrated into the lipid bilayer of the plasma membrane through a hydrophobic transmembrane domain. The signal-transducing moiety is a disulphide-linked heterodimer (CD79) consisting of CD79a and CD79b, also called Igα and Igβ, respectively. Binding of antigen to the BCR triggers phosphorylation of the ITAM tyrosine residues in Igα and Igβ by the tyrosine protein kinase Lyn which initiates a signalling cascade through activation of bruton tyrosine kinase (Btk) and PLCγ2 followed by IP_3 and DAG generation and

Ca^{2+}-influx. Subsequent signals involve activation of phosphatidylinositol 3-kinase (PI3-K) and Akt, Erk and NF-κB pathways, which contribute to B cell activation and proliferation (Wienands and Engels 2001).

3.2.3 TLR Signalling

Besides ligation of the BCR, B cells can be activated by TLRs. The Interleukin-1 receptor (IL-1R)/TLR superfamily of single transmembrane domain receptors comprises 24 members, including five adaptor proteins, which share a cytosolic domain named Toll/IL-1 receptor (TIR) domain. The TLR family includes 10 members, which play a key role in activating innate immune cells and B cells. TLRs recognize pathogen associated molecular patterns (PAMPs), which are highly conserved motifs of common pathogens. Each TLR has a distinct pattern-recognition specificity (Lee and Kim 2007). For example, TLR4 recognizes LPS of bacteria. However, a number of endogenous molecules (named as 'damage-associated molecular patterns' or 'danger signals') released by injured tissue can also activate the innate immune system via stimulation of certain TLRs (Bianchi 2007; Tsan and Gao 2004). IL-1R/TLR receptors are ubiquitously expressed by leucocytes (Janeway and Medzhitov 2002), epithelial cells (Yoshimoto and Nakanishi 2006) and endothelial cells (Gibson et al. 2006). Members of this family of receptors have also been shown to be expressed on CNS neurons, microglia and astrocytes (Vezzani et al. 2011). The critical role of the TLR4 pathway in treating severe sepsis and septic shock was delineated in many clinical trials (Wittebole et al. 2010).

Ligand binding of the TLRs leads to the recruitment of MyD88 and other cytosolic adaptor proteins to the TLR complex, activating IL-1R-associated kinase1/4 (IRAK1/4) and tumor necrosis factor receptor associated factor 6 (TRAF6), leading eventually to the expression of genes involved in inflammation, including the transcription factors NF-κB, activator protein-1 (AP-1) and interferon regulatory transcription factors (IRFs) (O'Neill and Bowie 2007). TLR3 and TLR4 can also signal using a MyD88-independent pathway which involves TIR-domain-containing adaptor-inducing interferon-ß (TRIF). The TRIF-dependent signalling cascade results in the activation of IRF-3, which then induces IFNα and IFNß. Induction of PI3-K can also occur in response to TLR stimulation, presumably using a MyD88-independent pathway (Davis et al. 2006; Diem et al. 2003).

These signalling pathways are orchestrated through the interplay of different ion channels (CRAC, $K_v1.3$, $K_{Ca}3.1$, TRPM4 and P2X). These ion channels regulate the normal membrane potential, which when altered affects the activation of downstream signalling cascades leading to cell proliferation, differentiation or cell death.

3.3 The Function of the Membrane Potential and Ion Channels in Immune Cells

3.3.1 Reasons for Ions to Cross through the Cell Membrane

Physiological processes depend on the continued flow of ions into and out of cells. Ions are more 'stable' in water than in the lipid layer inside the membrane. Thus, the hydrophobic membrane acts as a serious energy barrier for transporting ions. In a situation without biological pumps and ion channels, there can be large ion potential differences between the two sides of a biological membrane so that the predominant ions Na^+, K^+, Ca^{2+}, and Cl^- can never cross it. To resolve this issue, ion pumps, ion exchangers ('active' transport) and ion channels ('passive' transport) are used in cells. An ion channel needs a single gate and ion pump works with at least two gates. A gate or a selectivity filter is considered to be a part of a protein that hinders ion movement along the translocation pathway in the prohibitive confirmation but not in the permissive confirmation. The ion channels, like voltage-gated Na^+-, Ca^{2+}- or K^+-channels, are opened when a change of membrane potential displaces the voltage sensors connected to a cytoplasmic side of the 'activation gate'. They can be closed by reversal of those displacements ('deactivation') in response to an opposite change of membrane voltage. But even with their activation gates in the permissive position, the ion pathway through those channels can be closed by a separate gating process called 'inactivation'. Both these gates should be in a permissible position for the channel to conduct ions, and closure of either gate obstructs the ion flow (Gadsby 2009). In contrast, ion pumps are controlled by timely cohesion of two gates which are never open simultaneously (Lauger 1979). Instead, the chosen ions are allowed to enter the pathway from one side of the membrane while one of the gates is open, and then to leave at the other side of the membrane through another gate after the first one shuts down (Vidaver 1966; Gadsby 2009). Although these two transport systems work differently, ion selectivity is a prime criterion for both of them. The ion pumps generally transport ions against the electrochemical gradient with the use of energy like adenosine tri-phosphate (ATP) and a relatively slow speed. In contrast, ion channels are passive transporters of ions with a very high ion conduction rate to maintain the proper membrane potential. The membrane potential is defined as the electrical potential difference between the interior and exterior of the cell. It can be defined with Ohm's law (V = IR, where V = voltage, I = current and R = resistance). Voltage in this law is synonymous to the difference in the electrical potential, thereby, the ability to drive electric current across a resistance. The plasma membrane of a cell acts as an electrical resistor as it has low intrinsic permeability to ions. Insertion of ion-specific channels creates a membrane potential, which depends on the ion selectivity of the ion channels. Most eukaryotic cells possess selective channels for K^+ and Cl^- resulting in a membrane potential between -40 to -80 mV depending on the cell type (Gouaux and Mackinnon 2005; Dubyak 2004).

The resting potential of excitable cells like neurons and muscles varies from -70 to -80 mV, whereas for non-excitable cells like immune cells it is between -40 to -50 mV. The difference in the resting potential of these cell types relates to their function (Hille B 2001). The opening and closing of transmembrane ion channels alter the resting potential. It is called depolarization, if the interior voltage becomes less negative, and hyperpolarization, if the voltage becomes more negative. In excitable cells, a sufficient depolarization of the membrane can evoke an action potential for a short time period, which transiently repolarises the membrane. In immune cells, changes in the membrane potential are less transient and depend on the activation state of the cell. Maintaining a normal membrane potential is important for any immune cell. Thus, several ion channels (described in **section 3.3.3**) are expressed in the plasma membrane to allow the modulation of the membrane potential.

In non-excitable cells including immune cells, the membrane potential plays a prominent role in setting the electrical driving force for Ca^{2+}-influx. In cells where voltage-independent Ca^{2+}-channels like TRPM4 and K_{2P} are present, Ca^{2+}-influx only depends on the electrochemical gradient over the membrane and is stronger if the membrane potential is more negative (hyperpolarised). In contrast, Ca^{2+}-influx is mediated through voltage dependent Ca^{2+}-channels in excitable cells, like neurons. Functional coupling of Ca^{2+}-activated K^+-channels (K_{Ca}) with other predominant ion channels leads to a positive feedback cycle promoting a sustained Ca^{2+}-influx in case of immune cells (**Figure 1**) (Gao et al. 2010) or a negative feedback to terminate the flux by hyperpolarising the membrane potential and hence closing voltage-activated Ca^{2+}-channels in neurons (Fakler and Adelman 2008).

3.3.2 Regulation of Intracellular Ca^{2+}-level

Na^+ and K^+ are the most abundant cations in biological systems. Na^+ ions are most often present at high concentrations outside the cell, and K^+ is present at high concentrations inside the cell. Gradients for these ions across the cell membrane provide the energy source for action potentials generated by opening Na^+ and K^+-channels (Murata et al. 2005; Meier et al. 2005), and for moving solutes and other ions across the cell membrane via coupled transporters. Among several ions, the gradient for Ca^{2+} ions is the largest. It helps in controlling several physiological processes like secretion, excitation, contraction, and cellular proliferation (Berridge 1995; Berridge et al. 2000). The cytosol is surrounded by two massive Ca^{2+}-stores: the extracellular space, where the Ca^{2+} concentration is ~1.8 mM, and the sarco-endoplasmic reticulum (SER), where the Ca^{2+} concentration varies from 300 µM to 2 mM (Hannaert-Merah et al. 1995). In immune cells, the intracellular Ca^{2+} concentration is ~0.1 µM in the resting state, but it is increased 10-fold when the cells are activated (Feske et al. 2012; Hoth and Penner 1992).

Plasma membrane Ca^{2+}-channels are specifically important in cell-cycle progression and proliferation of immune cells. Accumulating evidence suggests that Ca^{2+}-influx is important at different steps of the cell cycle, the progression at late G1 into S phase as well as G2/M transition (Takuwa et al. 1991; Takuwa et al. 1992; Nordstrom et al. 1992). The nature of Ca^{2+}-channels involved in proliferation is well-defined and can be separated into voltage-activated (VACC), receptor-activated (RACC), store-operated (SOCC) and second messenger-operated (SMOCC) channels. RACC, SOCC and SMOCC are ubiquitous, whereas VACC is expressed in excitable cells only. VACC (e.g. L-, T-, N-, P-, Q-type Ca^{2+}-channels) open when the membrane is depolarized (Tsien et al. 1995). RACC (e.g. P2X purinergic receptors) open when a ligand binds to the channel (MacKenzie et al. 1999), whereas SOCC (e.g. transient receptor potential (TRP) (Clapham et al. 2001) and archetype CRAC (Hoth and Penner 1992) are activated when the level of Ca^{2+} within the lumen of the ER drops below a threshold level (Putney 1986; Putney and McKay 1999; Putney et al. 2001). Another type, SMOCC (e.g. arachidonic acid-regulated Ca^{2+} current) is activated by intracellular second messengers like arachidonic acid (Shuttleworth 1996). The role of CRAC, TRPM4 and P2X channels are important in case of immune cells in the continuous struggle to keep Ca^{2+} at an optimal level important for the maintenance of cellular functions in parallel with ion pumps like Na^+/K^+ pumps (Mijatovic et al. 2007; Lefranc and Kiss 2008).

Among the different ion channels mentioned in **Figure 1** involved in the regulation of Ca^{2+} homeostasis, CRAC channels are the most important ones. CRAC channels have been extensively characterized (Hoth and Penner 1992; Zweifach and Lewis 1993) and are distinguished by an extremely high ion selectivity for Ca^{2+} and a low conductance (Prakriya 2009). CRAC channels are activated through the binding of the ER Ca^{2+} sensors stromal interaction molecule 1 (STIM1) and STIM2 to the CRAC channel proteins ORAI1-3 (also known as CRACM1-3) (Hogan et al. 2010). ORAI1 is a widely expressed surface glycoprotein with four predicted transmembrane domains, intracellular amino- and carboxyl-termini and no sequence homology to other ion channels except for its homologues ORAI2 and ORAI3. All three ORAI proteins form Ca^{2+}-channels with broadly similar functional properties when ectopically expressed, although they differ in their inactivation characteristics, pharmacological properties and tissue expression (Lis et al. 2007; DeHaven et al. 2007). The activation of ORAI CRAC channels involves a complex series of coordinated steps, during which STIM proteins fulfil two crucial roles. Firstly, they sense the depletion of ER Ca^{2+}-stores, and secondly, they communicate store depletion to the CRAC channels (Liou et al. 2005; Roos et al. 2005; Cahalan 2009). In resting cells with repleted Ca^{2+}-stores, STIM proteins are diffusely distributed throughout the ER membrane. Following the depletion of Ca^{2+}-stores, STIM proteins are activated, oligomerize and redistribute into discrete

punctae located in junctional ER sites that are in close proximity to the plasma membrane (Cahalan 2009; Luik et al. 2008). Lymphocytes express two closely related STIM isoforms, STIM1 and STIM2, and both mediate store-operated Ca^{2+}-entry (SOCE) in B and T cells (Matsumoto et al. 2011; Oh-Hora et al. 2008). $CD4^+$ and $CD8^+$ T cells from ORAI1- and STIM1- deficient patients and mice show defective production of many cytokines, including IL-2, IL-17, IFN-γ and tumour necrosis factor (TNF) (Feske 2009; Feske et al. 2001). However, despite the profound defects in SOCE in B cells from ORAI1- and STIM1-deficient patients and mice, CRAC channels do not have a major role in antibody production (Feske et al. 2010).

3.3.3 Role of Ion Channels in Maintaining the Membrane Potential

The resting potential of a lymphocyte membrane is ~-50 mV (Lewis and Cahalan 1995). Membrane potential alterations mainly occur when lymphocytes get activated (Crabtree 1999). TCR engagement activates PLCγ1, which catalyses the hydrolysis of PIP_2 into IP_3 and DAG. IP_3 stimulates the release of Ca^{2+} from intracellular ER stores which triggers the opening of plasma membrane CRAC channels. It is the resulting influx of extracellular Ca^{2+} that is responsible for the sustained rise in cytoplasmic Ca^{2+} after TCR stimulation. Ca^{2+} binds to the cytoplasmic Ca^{2+}-dependent protein calmodulin which then activates the phosphatase calcineurin. This phosphatase dephosphorylates and activates the nuclear factor of transcription of activated T cells (NFAT), which enters the nucleus and helps to initiate interleukin-2 (IL-2) gene transcription (Serfling et al. 2007; Rao 2009). During the activation of immune cells opened CRAC-channels raise the intracellular Ca^{2+} level. To maintain the balance in membrane conductance, K_{Ca} channels get opened to hyperpolarize the membrane as this channel helps in Ca^{2+}-efflux. A negative feedback loop is established until Ca^{2+} reaches high enough levels inside the cell to inhibit CRAC-channels. With the Ca^{2+}-dependent activation of TRPM4 channels in T cells, there is also involvement of $K_v1.3$ channels to repolarize the membrane (as illustrated in **Figure 1**). Along with these conventional ion channels, the two-pore K^+-channels (K_{2P}) TASK-1 and TASK-3 are known to regulate immune cell effector functions by hyperpolarizing the membrane (Meuth et al. 2008). Although the best characterized channel for Ca^{2+}-influx in T cells is CRAC, several other channels may also mediate Ca^{2+}-influx in T cells, including members of the transient receptor potential (TRP) family, P2X receptors and voltage-gated Ca^{2+} (Ca_v) channels. Compared to CRAC channels, however, their contribution to TCR-induced Ca^{2+}-influx in immune cells is less well-defined (Feske 2013).

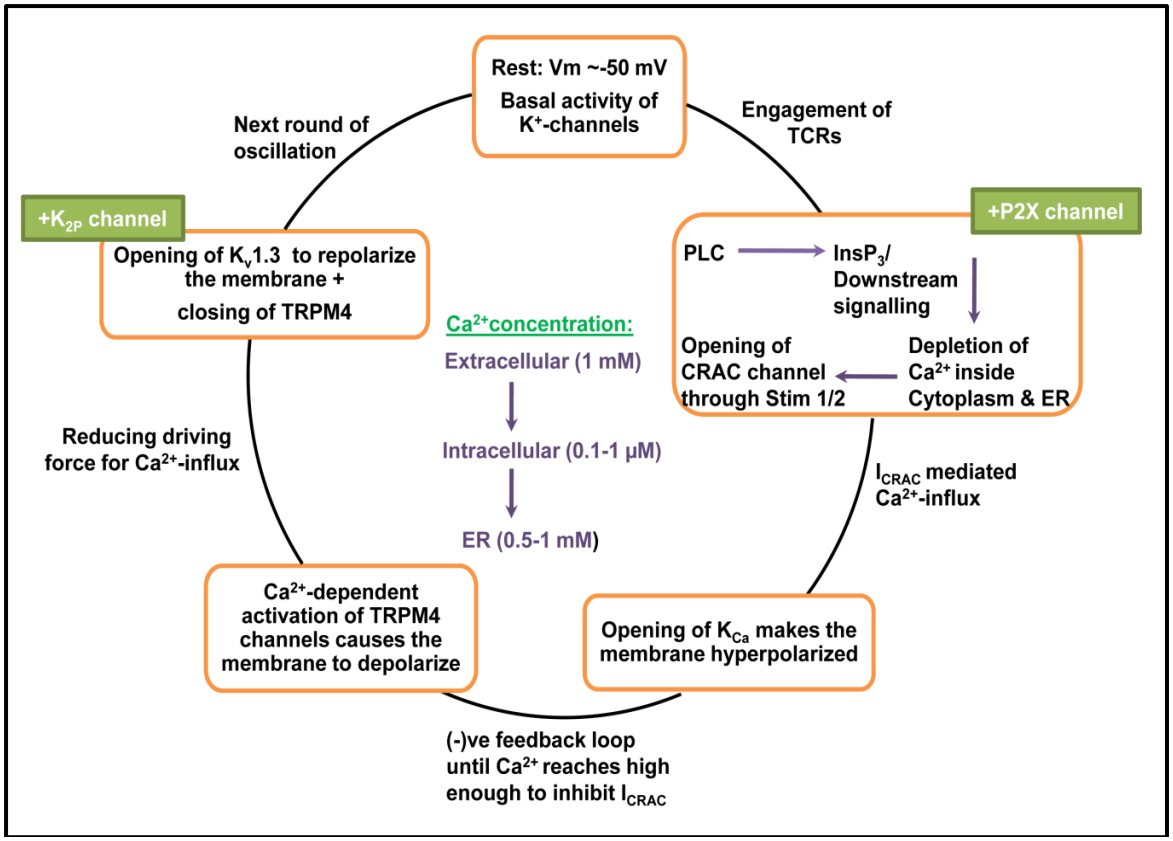


Figure 1. Oscillatory changes of the membrane potential in lymphocytes. Ca^{2+}-influx in lymphocytes depends on the gradient between the extracellular Ca^{2+} concentration (~1 mM) and the intracellular Ca^{2+} concentration (~0.1 µM) and on an electrochemical gradient established by the K^+-channels ($K_v1.3$, $K_{Ca}3.1$ and partially by K_{2P} channels) and the Na^+-permeable channel TRPM4 (Cahalan and Chandy 2009; Chandy et al. 2004). CRAC channels are activated following the engagement of the TCR or BCR and is mediated through the activation of PLCγ, the production of IP_3 and the release of Ca^{2+} from ER Ca^{2+}-stores. The ensuing activation of STIM1 and STIM2 results in the opening of ORAI1 CRAC channels and SOCE. Sustained Ca^{2+}-influx through CRAC channels leads to the activation Ca^{2+}-dependent enzymes and transcription factors, including calcineurin and NFAT (Lewis 2001; Hogan et al. 2010; Feske 2007). Additionally, P2X receptors (e.g. P2X4 and P2X7), which are non-selective Ca^{2+}-channels, are activated by extracellular ATP mediating Ca^{2+}-influx (modified from *Launay, P., 2004)* (Feske et al. 2012; Launay et al. 2004).

3.3.3.1 K^+-channels

K^+-channels are encoded by a superfamily of 78 genes (Harmar et al. 2009) and are involved in diverse physiological and pathological processes (Wulff et al. 2009). Structurally, K^+-channels are classified into three major groups: six transmembrane (**Figure 2A**), two transmembrane (Figure 2B) and four transmembrane (**Figure 2C**) K^+-channels. Voltage-gated K^+-channels (the first type) can further be subdivided into four families: K_v (shaker-like), Ether-a-go-go (EAG), KCNQ and K_{Ca} (Ca^{2+}-activated K^+-channels) (Yellen 2002; Shieh et al. 2000). K_{Ca} channels can be classified into three types: BK_{Ca} ($K_{Ca}1.1$), IK_{Ca}

($K_{Ca}3.1$) and SK_{Ca} ($K_{Ca}2.1$, $K_{Ca}2.2$, $K_{Ca}2.3$) (Wei et al. 2005). Among K^+-channels, immune cells mainly express voltage-activated ($K_v1.3$), calcium-activated ($K_{Ca}3.1$) and two-pore K_{2P} channels (TASK-1, TASK-3). K^+-channels protect against membrane depolarization by mediating the efflux of K^+ to depolarize the plasma membrane (Cahalan and Chandy 2009). $K_v1.3$ is a homotetramer of four α-subunits, each composed of six transmembrane segments (S1-S6), and is activated by membrane depolarization (Cahalan et al. 1985). Depolarization of the membrane is sensed by four arginine residues that are localized in the S4 segment and results in a conformational change that causes channel opening (Bezanilla 2008). $K_{Ca}3.1$ is a Ca^{2+}-activated K^+-channel, but it has similar membrane topology and pore architecture as $K_v1.3$. However, rather than containing a voltage sensor, the C- terminus of $K_{Ca}3.1$ is constitutively bound to calmodulin, and channel opening occurs after Ca^{2+} binds to calmodulin (Xia et al. 1998). $K_{Ca}3.1$ channels powerfully hyperpolarize the membrane following elevations in the intracellular Ca^{2+} concentration and thus help to sustain the driving force for Ca^{2+}-entry. In addition to the requirement of Ca^{2+}, $K_{Ca}3.1$ channel activity depends on a class II PI3-K, which increases the concentration of phosphatidylinositol 3-phosphate (PtdIns(3)P) in the plasma membrane (Feske et al. 2012). TASK-1 and TASK-3 also contribute to hyperpolarizing activities (Meuth et al. 2008).

The relative contribution of $K_v1.3$ and $K_{Ca}3.1$ in lymphocyte Ca^{2+}-influx are determined primarily by their expression level, which depends on the lymphocyte subset and its state of activation. Under resting conditions, $CCR7^+CD45RA^+$ naïve human T cells predominantly express $K_v1.3$ channels and depend on $K_v1.3$ for activation (Leonard et al. 1992). Following activation, naïve human T cells up-regulate $K_{Ca}3.1$ expression (Ghanshani et al. 2000), and inhibition of $K_{Ca}3.1$ in pre-activated T cells blocks TCR-stimulated Ca^{2+}-influx and proliferation (Fanger et al. 2000; Fanger et al. 2001). Furthermore, mouse T_H1 and T_H2 cells predominantly express $K_{Ca}3.1$ and depend on $K_{Ca}3.1$ for TCR-mediated Ca^{2+}-influx and cytokine production, whereas T_H17 cells mainly express $K_v1.3$ and require $K_v1.3$ for their activation and production of IL-17 (Di et al. 2010). Differential use of K^+-channels is also observed in effector memory T cells (T_{EM}) and central memory T cells (T_{CM}) (Cahalan and Chandy 2009; Srivastava et al. 2006; Beeton et al. 2001). When activated at sites of inflammation, T_{EM} cells, which have the phenotype $CCR7^-CD62L^{low}CD45RA^-$, produce various cytokines including IFNγ, IL-4 and IL-5, and exclusively up-regulate $K_v1.3$ expression. In contrast, T_{CM} cells, which are $CCR7^+CD62L^{hi}CD45RA^-$, up-regulate the expression of $K_{Ca}3.1$ following their activation in lymph nodes and mucosal lymphoid organs. As a result, $K_v1.3$ blockers are effective inhibitors of T_{EM} cells, whereas $K_{Ca}3.1$ blockers are effective at inhibiting T_{CM} cells.

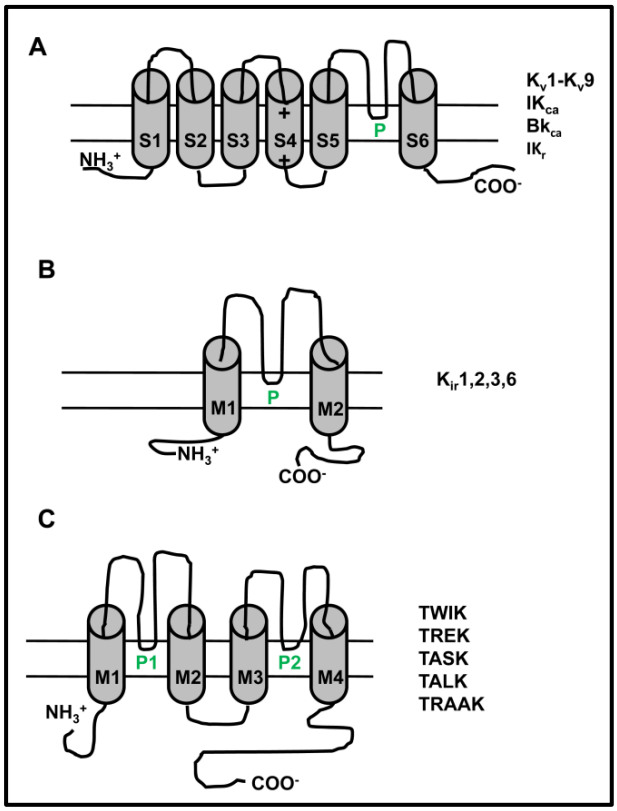


Figure 2. Scheme of structural classification of K^+-channel subunits. Structurally, K^+-channels are classified into three types. (A) Six-transmembrane one-pore. These channels are composed of four subunits, each of them with six transmembrane segments (S1-S6) with a voltage-sensor domain (S4) and a conducting pore between S5 and S6. (B) Two-transmembrane one-pore. The inward-rectifying channels belong to this group with four subunits each consisting of two transmembrane segments (M1 and M2) with a conducting pore in between. (C) Four-transmembrane two-pore. The ion channels belonging to this group consist of four-transmembrane (M1-M4) and two-pore domains (P1 and P2) (Modified from *Shieh, C.C. et al., 2000)* (Shieh et al. 2000). K_v: voltage-gated; IK: delayed-rectifier; K_{ir}: inward-rectifier; TWIK: Two-pore weak inward rectifier; TREK: TWIK-related; TASK: TWIK-related acid-sensitive; TALK: TWIK-related alkaline pH activated; TRAAK: TWIK-related arachidonic acid-stimulated K^+-channel.

The finding that $K_v1.3$ and $K_{Ca}3.1$ function to activate distinct lymphocyte subsets provides an opportunity to more selectively target lymphocyte subsets for therapeutic purposes. The relevance of these findings to humans was demonstrated by the observation of high levels of $K_v1.3$ expression by myelin-reactive T cells isolated from patients with multiple sclerosis (Wulff et al. 2003). Similar studies have shown an increase of $K_v1.3$ channels in disease-associated T_{EM} cells in patients with type I diabetes, rheumatoid arthritis (RA) and psoriasis, and the treatment of these diseases with $K_v1.3$ blockers lead to the amelioration of the disease (Beeton et al. 2006; Fasth et al. 2004; Friedrich et al. 2000; Gilhar et al. 2011). By contrast, inhibition of $K_{Ca}3.1$ protected mice from developing colitis in two mouse models of inflammatory bowel disorder (Di et al. 2010), suggesting that $K_{Ca}3.1$ may be a novel therapeutic target to treat patients with Crohn's disease or ulcerative colitis.

$K_v1.3$ is one of the first voltage-gated K^+-channels reported to be modulated during apoptosis (Szabo et al. 1996) and is shown to contribute to the increased K^+-efflux underlying the late phase of lymphocyte apoptosis. There is also proof of stimulation of these channels by death receptor CD95/Fas during apoptosis of Jurkat T lymphocytes (Storey et al. 2003). $K_v1.3$ expression also shows a correlation with tumour progression as

exemplified by the up-regulation of $K_v1.3$ expression in diffuse human large B-cell lymphoma and glioma (Preussat et al. 2003). Mechanistically, pro-apoptotic mediator cytochrome C can activate K_v-channels while anti-apoptotic protein Bcl-2 inhibits them (Remillard and Yuan 2004). $K_v1.3$ depletion decreases the expression of Caspase-3, Caspase-9 and Bad, molecules that exacerbate apoptotic cell death (Wasserman and Koeberle 2009). This mechanism indicates that inhibition of $K_v1.3$ channels confers resistance to apoptosis while their over-expression favours this process.

3.3.3.2 Transient Receptor Potential (TRP) Channel

In humans, TRP channels form a large superfamily of 28 cation channels, which can be divided into 7 subfamilies (Venkatachalam and Montell 2007). T cells predominantly express channels belonging to TRPC and TRPM subfamilies, including TRPC1, TRPC3, TRPC5, TRPM2, TRPM4, and TRPM7 (Wenning et al. 2011). Most TRP channels are non-selective and permeable to several cations, including Ca^{2+} and Na^{+} (Ramsey et al. 2006; Owsianik et al. 2006). The function of TRPM4 channels is well documented in T cells and other immune cells unlike most other TRP channels. TRPM4 channels mainly conduct Na^{+} and K^{+} and, in contrast to other TRP channels, are only weakly permeable to Ca^{2+} (Vennekens and Nilius 2007). The activation of TRPM4 channels, which occurs in response to an increase in intracellular Ca^{2+} concentration, results in Na^{+}-influx, membrane depolarization and a reduction in the electrical driving force for Ca^{2+}-influx. TRPM4 channels thus provide a negative feedback mechanism for the regulation of SOCE and were proposed to prevent cellular Ca^{2+}-overload. Given that TRPM4 and K_v channels elicit opposing effects on the membrane potential, it remains to be elucidated precisely how TRPM4 works together with $K_v1.3$ and $K_{Ca}3.1$ to regulate changes in the membrane potential and intracellular Ca^{2+} concentration (Feske et al. 2012).

3.3.3.3 Purinergic P2X Receptor Channel

P2X receptors are a family of non-selective ion channels that are activated by extracellular ATP and regulate the influx of Na^{+}, Ca^{2+} and other cations (Junger 2011). At least three different P2X receptors have been implicated in Ca^{2+}-influx in human T cells: P2X1, P2X4 (Woehrle et al. 2010) and P2X7 (Yip et al. 2009). Their opening, especially that of P2X7, causes Ca^{2+}-influx and the activation of downstream signalling molecules such as calcineurin, resulting in the proliferation of B and T cells (Baricordi et al. 1996; Padeh et al. 1991) and IL-2 production (Adinolfi et al. 2005; Woehrle et al. 2010). Potential sources for the ATP required for P2X receptor activation include the T cells themselves, which are reported to release ATP in an autocrine manner through pannexin 1 hemichannels that co-

localize with P2X7 at the immunological synapse (Woehrle et al. 2010; Schenk et al. 2008). It has been suggested that autocrine ATP signalling in T cells via P2X receptors serves to amplify weak TCR signals, gene expression and T cell effector functions (Junger 2011).

The bi-directional connection between the neuronal and immune system is established by the presence and functional relevance of molecules and ion channels present in both systems as explained in the next chapter.

3.4 Connection between the Neuronal and Immunological Systems

Although there has been evidence for years for a crosstalk between the immune and nervous systems following injury, the 'dogma' in the field of neuro-immunology has been that the healthy central nervous system (CNS) is 'immune-privileged' because it lacks classical immune molecules (Joly et al. 1991; Murphy and Sturm 1923). However, the role of MHC-I molecules in neuronal synapse development and synaptic plasticity is quite evident in brain cells of the visual system (Huh et al. 2000), and TCR/CD3ζ molecules contribute to the spatial learning and memory function (Xu et al. 2010). Furthermore, the role of MHC-II molecules in the degradation of neurons in Alzheimer's disease (AD) is established (McGeer et al. 1989). Several inflammatory molecules like IL-1, TLR4 and TLR3 are expressed during brain inflammation (Rogers et al. 1988) and antagonists to these inflammatory mediators were shown to protect the brain. In addition, auto-antibodies generated against transmitter-receptors or voltage-gated ion channels in the brain influence the function of neuronal ligand- and voltage-gated ion channels, leading to synaptic dysfunction, and are found in Rasmussen's encephalitis, Lambert-Eaton Myasthenic Syndrome (LEMS) or NMDAR-associated encephalitis, as shown in **Table 3**. In the same way as neurons are regulated by molecules predominantly expressed in immune cells, brain molecules can regulate immune function, like regulation of immune cells by glutamate as described in **section 3.7**.

3.5 Glutamate Receptors

3.5.1 Ionotropic and Metabotropic Glutamate Receptors

Glutamate (Glu) can interact with multiple receptor types, which are divided into two main groups: ionotropic glutamate receptors (iGluRs), which form homo- or heteromeric ion channels from several subunits with four transmembrane domains and mediate fast excitatory glutamate responses, and metabotropic glutamate receptors (mGluRs), which have seven transmembrane domains and belong to the superfamily of G protein-coupled receptors (Nakanishi 1992). On the basis of sequence homology and agonist preference, iGluRs are classified into N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxasole-4-

propionate (AMPA) and kainate (KA) receptors, which are associated with permeability to particular cations (Wisden and Seeburg 1993). The first type is highly permeable to Ca^{2+}, whereas AMPA and KA receptors are mostly permeable to Na^{+} ions (Nakanishi 1992). mGluRs are classified into three subgroups (I, II, III) and there are eight family members identified so far (Pin and Duvoisin 1995). Group I contains mGlu1R and mGlu5R subtypes, which are mainly coupled to PLC, and quisqualic acid is their most potent agonist. Group II consists of mGlu2R and mGlu3R, which negatively couple to adenylate cyclase and for which L-2-(carboxycyclopropyl)-glycine is a potent agonist. Group III contains mGlu4R, mGlu6R, mGlu7R, and mGlu8R, which have the same property as type-II, but they have a different agonist, namely L-2-amino-4-phosphonobutyric acid (Pin and Duvoisin 1995; Pin and Acher 2002). This classification is schematically represented in **Figure 3**.

3.5.2 Ionotroic Glutamate Receptors in the Brain

NMDARs and AMPARs are the main ionotropic GluRs involved in glutamatergic neurotransmission in the CNS. Their functions in synaptic transmission and plasticity are well established including long term potentiation/depression and excitotoxicity. NMDARs are hetero-tetramers consisting of the obligatory GluN1 subunit and two accessory subunits named GluN2A-D, GluN3 or GluN4. Activation of NMDARs requires the binding of glutamate or aspartate, the co-agonists glycine or D-serine and membrane depolarization. The GluN2 family of NMDAR subunits contains a binding site for glutamate, the endogenous agonist, whereas the GluN1 subunit binds glycine (Johnson and Ascher 1987) as illustrated in **Figure 4**. There is another component, D-serine, which binds to the glycine site of classical NMDARs. Although levels of glycine are 10-fold higher than D-serine, several reports indicate that endogenous D-Serine and not glycine is the dominant exogenous co-agonist for NMDAR-mediated neurotoxicity (Shleper et al. 2005; Mothet et al. 2000; Wolosker et al. 1999). When glutamate and glycine/D-serine bind and the cell is depolarized to remove the Mg^{2+}-block of NMDARs, the channel opens with consequent influx of Ca^{2+} and Na^{+} into the cell, the amount of which can be altered by higher levels of agonists and by substances binding to one of the modulatory sites on the receptor (**Figure 4**). The opening kinetic of NMDARs depends on the subunit composition and has profound consequences for downstream signalling pathways. Thereby, NMDARs can sense different activation patterns and trigger specific intracellular signalling pathways via the induction of intracellular Ca^{2+} changes within small domains below the neuronal membrane (Paoletti et al. 2013).

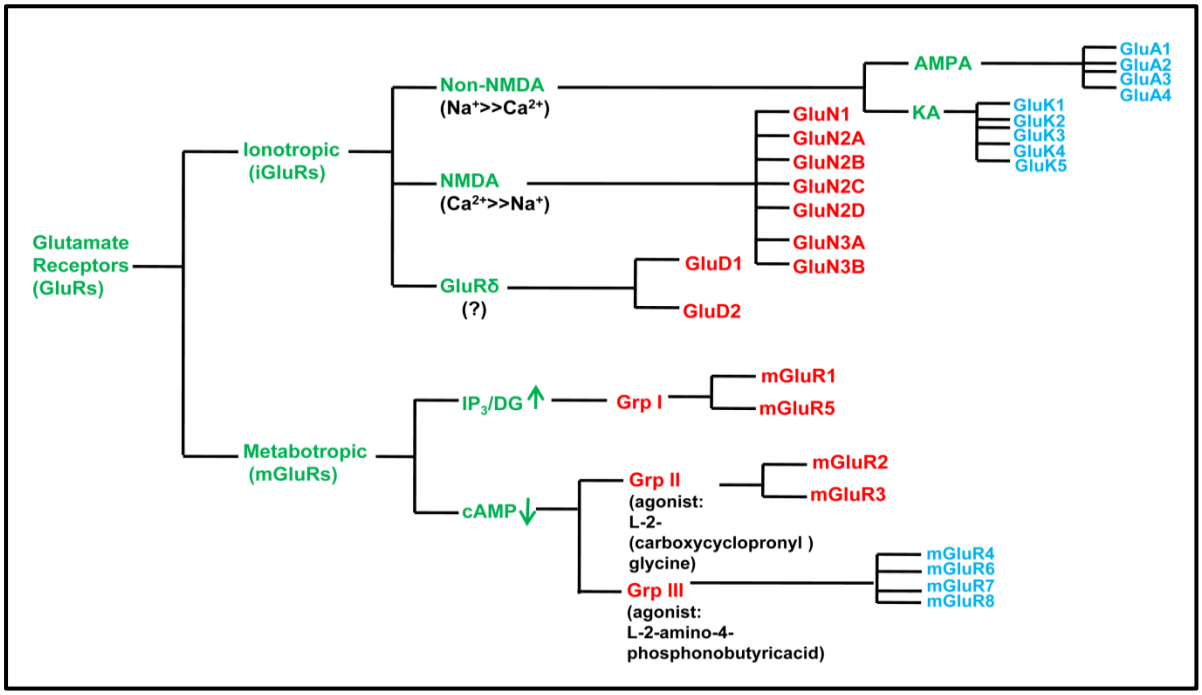
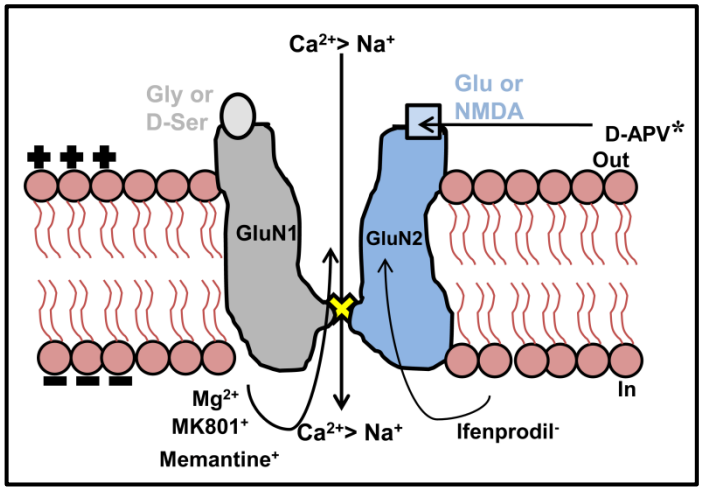


Figure 3. Classification of glutamate receptors (GluRs). GluRs are divided into two major classes, according to their differential intracellular signal transduction mechanisms as well as sequence homologies. iGluRs are sub-classified on the basis of sequence homologies and agonist preference into NMDA, AMPA and KA receptors, which are all associated with ion channels permeable to particular cations. There are three distinct subtypes of mGluRs, classified by their sensitivity to exogenous agonists and intracellular second messengers employed (modified from *Hinoi, E. et al.*) (Hinoi et al. 2004; Pacheco et al. 2007; Collingridge et al. 2009).

As NMDARs are important for several neuronal functions, they are implicated in various neuronal disorders like traumatic brain injury and ischemic stroke (Shohami and Biegon 2013), Huntington's disease (Daggett and Yang 2013), AD (Malinow 2012), schizophrenia (Paoletti et al. 2013), mood disorders (Machado-Vieira et al. 2010), and encephalitis (Finke et al. 2013). The physiological functions of NMDARs are mediated by the signalling events occurring downstream of their activation. Ca^{2+}-activated kinases like Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) and members of the protein kinase C (PKC) family mediate Early-Long-Term-Potentiation (E-LTP). The src kinase Fyn enhances NMDAR currents by phosphorylation of GluN2 subunits. More recent studies show NMDAR-induced activation of the MAPK, Erk1/2 and PI3-K/Akt pathways. NMDAR signals culminate in the activation of a cohort of transcription factors that orchestrate specific gene expression programs guiding neuronal homeostasis, cell death or plasticity. The localization and composition of the NMDARs in the neuronal membrane is fundamental for the initiation of these intracellular signalling events (Groc et al. 2006; Cognet et al. 2006; Kahlfuss et al. 2014).

Figure 4. NMDAR model illustrating important binding sites for agonists and antagonists. The agonists glycine (Gly)/D-serine (D-Ser) and glutamate/N-methyl-D-aspartate (Glu/ NMDA) are shown bound to their respective binding sites. The binding sites for Mg^{2+} (Mori et al. 1992; Mayer et al. 1984), MK801 (Huettner and Bean 1988) and memantine (Chen and Lipton 1997; Chen et al. 1992) are within the ion channel pore region and ifenprodil binds to the GluN2B subunit (Kew et al. 1996). The different kinds of antagonists are marked as: *competitive, +uncompetitive, -non-competitive (modified from *Lipton, S.A. et al.)* (Lipton 2006).



Excessive stimulation of NMDARs leads to excessive intracellular Ca^{2+}-influx, generation of free radicals such as nitric oxide and reactive oxygen species, collapse of the mitochondrial membrane potential, loss of ATP, and eventually neuronal apoptosis or necrosis depending on the intensity of the initial insult and the extent of energy recovery. This process is termed excitotoxicity and appears to be an integral component in a final common pathway to neuronal injury in neurodegenerative disorders including HIV-associated dementia (Yeh et al. 2000).

As NMDARs are involved in many neuronal disorders, modulation of their activity is important in clinical perspective. Among the NMDAR modulators clinically used for the treatment of neuronal disorders, memantine hydrochloride was approved by the U.S. Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMEA) in 2003 for moderate to severe kinds of AD. Another NMDAR inhibitor ketamine, which originally was developed as an anesthetic, is able to improve depressive symptoms within hours in subjects with treatment-resistant depression (Berman et al. 2000). Experimentally, ketamine mainly acts through Erk and Akt, which activate the kinase mTOR (mammalian target of rapamycin) enabling the translation of synaptic proteins (Zunszain et al. 2013).

3.6 NMDAR Inhibitors

A competitive antagonist is defined as an inhibitor which competes with the agonist binding site present in the receptor. This kind of antagonist competes with glutamate for binding to the glutamate receptor site on the GluN2 subunit of the NMDAR. Examples for competitive

antagonists are D-APV (mostly used in neurobiological studies) and selfotel (anti-convulsant with side-effects), as illustrated in **Figure 4***. A non-competitive antagonist can work via two mechanisms: it can bind to the active site of the receptor or to an allosteric site of the receptor. An allosteric site is defined as a binding site distinct from the active or agonist binding site. The end result of these two non-competitive mechanisms is quite similar. They reduce the magnitude of maximum response attained by any amount of agonist, whereas competitive antagonists reduce the quantity of agonist required to achieve a maximal response. In case of NMDAR antagonists, these two kinds of non-competitive mechanisms are exemplified by ifenprodil and ketamine. Ifenprodil binds to the GluN2B subunit and aptiganel to the Mg^{2+}-binding site (active site) of NMDARs. Ketamine, which acts as an analgesic and is in clinical trial for depression and mood disorders, appears to bind to the allosteric site of the channel (Orser et al. 1997), as shown in **Figure 4-**. Finally, an uncompetitive antagonist is defined when receptor activation by an agonist is required before it can bind to a separate allosteric binding site of the receptor. This type of antagonist follows a kinetic rule where the 'same amount of antagonist blocks higher concentration of agonist better than the lower concentration' (Lipton 2004). The prominent examples in this group are memantine (used for the treatment of AD), MK801 (used in scientific research) and, amantidine (used for treating influenza and Parkinson's disease) as shown in **Figure 4+**.

Cross-reaction of an antagonist with other channels is quite common within the neuronal ion channel population. Memantine and MK801 are known to cross-react with α-7-nicotinic acetylcholine (α-7-nAchR) and serotonin receptors in heterologous expression systems and rat hippocampal neurons (Aracava et al. 2005; Amador and Dani 1991; Rammes et al. 2001; Iravani et al. 1999). Furthermore, memantine is known to block dopaminergic (D2) receptors in pituitary cells (Seeman et al. 2008), and ifenprodil blocks ionotropic serotonin receptors (5-HT3) (Barann et al. 1998), presynaptic P/Q type Ca^{2+}-channels (Delaney et al. 2012) and K^+ inward rectifier channels (Kobayashi et al. 2006).

3.6.1 Pharmacological Quantification: Hill slope, Dwell time, Type of Inactivation

In pharmacological research, there are several parameters used for showing the binding kinetic of a drug to its target. These terms are described in the following:

The Hill slope (n) provides a way to quantify whether one or more ligands bind to a receptor. It describes the co-operativity of ligand binding to its receptor in this way: If n> 1, then the receptor has affinity to bind to more than one molecule (positively co-operative binding).

If n< 1, then it does not bind to more than one molecule (negatively co-operative binding), and if n = 1, then the affinity does not depend on whether there is any chance of binding any other molecule (non-cooperative binding).

The Dwell time (τ) gives information on the kinetic processes. It defines the amount of time a channel remains in the closed position and is used to describe the amount of time an ion spends in an ion channel pore at a particular binding site. This information is important when choosing a drug to be pharmacologically important. For example, memantine is preferred among other pharmacological NMDAR inhibitors like MK801 or D-APV because of its small τ. The Dwell time describes the kinetics of binding of an inhibitor to its receptor electrophysiologically, whereas the behaviour of the ion channel can be described biochemically with rate constants K_{on} and K_{off}. The biomolecular scheme of macroscopic blocking and unblocking are hypothetically shown with this equation:

$$\text{channel+ MEM} \underset{K_{off}}{\overset{K_{on}}{\leftrightarrow}} \text{channel- MEM (blocked channel)} \quad \ldots\ldots\ldots(1)$$

K_{on} is dependent on the memantine concentration and K_{off} in Eqn. (1) is inhibitor-independent. The macroscopic on-rate constant (K_{on}) is related to the time for onset constant (τ_{on}) by a sum of blocking rate (K_{on}) and unblocking rate (K_{off}) constants. Here, K_{off} is the reciprocal of the measured unblocking time constant (τ_{off}). Thus, the equation is:

$$K_{on} = 1/\tau_{on} - 1/\tau_{off} \quad \ldots\ldots\ldots(2)$$
$$K_{off} = 1/\tau_{off} \quad \ldots\ldots\ldots(3)$$

K_{on} calculated from Eqn. (2) experimentally is dependent on an increasing memantine concentration, whereas K_{off} in Eqn. (3) remains relatively constant (Nelson DL 2004).

$$K_i = K_{off}/(K_{on}/[MEM]) \quad \ldots\ldots\ldots(4)$$

The dissociation constant (K_i) at equilibrium for memantine action can be calculated from Eqn. (4). It is found empirically that memantine is a low-affinity (apparent affinity of ~1 µM) open-channel blocker of the NMDAR and a major component of the affinity is determined by a K_{off} at clinically relevant concentrations in the low micromolar range (Chen and Lipton 1997).

Any voltage-gated ion channel exists in three different states: activation, deactivation and inactivation. In this context, the concept of gate should be described. The pore domain, as

explained in **Figure 2** with P marked in green colour of a voltage-gated ion channel as exemplified by a K^+-channel in **Figure 2**, acts as a permeation pathway, which is opened and closed by two distinct molecular gates: activation and inactivation gates. In most voltage-gated ion channels, the activation gate is closed at normal membrane potential. Membrane depolarization causes a conformational change in voltage-sensing domains, as shown in the S4 domain of **Figure 2A**, that is transmitted to the pore domain, as illustrated between S5 and S6 of **Figure 2A**, resulting in opening of the gate. Inactivation is a non-conducting state during maintained depolarization. Conventionally, there are two kinds of inactivation: N- and C-type. The different names come from the protein parts that are involved in the inactivation process. The fast inactivating N-terminal region is involved in N-terminal inactivation, in contrast to C-type, which includes the C-terminal part of the protein. Voltage-activated K^+-channels modulate through the slower C-type of inactivation. In case of N-inactivation, the N-terminal residues (amino acids 6-46) of the channel move into the internal vestibule, as described in the S4-S5 linker of **Figure 2**, to occlude the intracellular mouth of the ion-conducting pore (Isacoff et al. 1991; Hoshi et al. 1991). Once the pore is occluded, it is hard to close the pore similar to a 'foot-in-the-door' mechanism (Demo and Yellen 1991) keeping it in a deactivated state. In comparison, C-type inactivation involves a slower rearrangement of the outer mouth and specific residues in the pore region (Liu et al. 1996).

Inhibitors can change the property of an ion channel by binding to the sites which are involved in the inactivation phenomenon. For example, L-type Ca^{2+}-channel blockers like nifepidine and verapamil, used for cardiovascular disorders, stabilize the inactivated closed state. This effect delays the transition to the resting phase and, thereby, inhibits the depolarization-induced Ca^{2+}-influx. With this property, these drugs are clinically used for the treatment of hypertension and cardiac arrhythmias by decreasing blood pressure and cardiac contractility (Abernethy and Schwartz 1999; Striessnig et al. 1998).

3.6.2 Reasons for Selecting Memantine to Block NMDAR Activity

Memantine HCl (systematic name: 3, 5-dimethyladamantan-1-amine) was first developed by Eli Lily & Co. in 1963 as an anti-diabetic agent for lowering the blood glucose level. Merz & Co. then proved its effectiveness in the treatment of AD due to its antagonism of NMDARs (Parsons et al. 1999). This was succeeded by a series of clinical trials by French, UK and USA pharmaceutical companies. The results of these trials convinced the European Union in 2002 and the US FDA in late 2003 (Lipton 2006) to approve memantine for the treatment of AD. Among the different types of NMDAR inhibitors mentioned before, memantine is chosen as a drug of choice because of its low side-effects. To be clinically

acceptable, the anti-excitotoxic therapy must block the excessive activation of NMDARs while leaving NMDAR function relatively normal. Drugs that compete with Glu or Gly at the agonist-binding sites block normal function and therefore do not meet this requirement. Consequently, these drugs have failed in clinical trials because of severe side-effects such as drowsiness, hallucination and even coma (Lipton and Rosenberg 1994; Kemp and McKernan 2002; Koroshetz and Moskowitz 1996; Hickenbottom and Grotta 1998; Lutsep and Clark 1999; Rogawski 2000; Palmer 2001).

Although memantine and MK801 are uncompetitive inhibitors, memantine was chosen for clinical trials due to its small τ. The K_{off} rate of an inhibitor is a major determinant of clinical tolerability of open-channel blockers, because an excessively slow off-rate (associated with a long τ and higher K_{off}) causes the drug to accumulate in the channels, interfere with normal neurotransmission and to produce unacceptable adverse effects as in the case of MK801. These kind of drugs make patients hallucinate (e.g. phencyclidine, also known as Angel Dust) or so drowsy that they can serve as anaesthetics (as ketamine). The relatively small K_{off} and short τ of memantine prevent the drug from accumulating in the ion channels and interfering with normal synaptic transmission. Thus, memantine can provide neuroprotection without displaying adverse side effects (Lipton 2006). The neuro-protective properties of memantine have been confirmed in a large number of *in vitro* studies and *in vivo* animal models (Lipton 2006). Neurons were protected by memantine in several areas of the brain like cerebrocortical, cerebellar and retinal regions (Vorwerk et al. 1996; Lipton 1993; Chen and Lipton 1997; Chen et al. 1992; Lipton 1992; Pellegrini and Lipton 1993; Sucher et al. 1997; Osborne 1999). A series of human clinical trials have been completed or are nearly completed testing the efficacy of memantine in AD, vascular dementia, HIV-associated dementia, diabetic neuropathic pain, depression, and glaucoma. Along with mild to moderate vascular dementia (Orgogozo et al. 2002), randomized clinical trials reported that memantine was beneficial in severely demented patients, probably representing both AD and vascular dementia (Winblad and Poritis 1999).

In terms of binding to NMDARs, memantine has two binding sites. The specific site of memantine action is presumed to be near to the Mg^{2+}-binding site at the selectivity filter region of the NMDAR channel (Sakurada et al. 1993). This specific binding site manifests a slow unblocking rate, moderate voltage dependence and high affinity (Danysz and Parsons 2003). The voltage-dependency is exemplified by the increase of IC_{50} (inhibiting NMDAR responses by 50%) with depolarization (Johnson and Kotermanski 2006; Parsons et al. 2007; Rogawski and Wenk 2003). The second unspecific binding site of memantine is re-

ported to have a much lower affinity, minimal voltage dependence and a non-competitive mechanism of blocking (Bresink et al. 1996; Antonov and Johnson 1996).

Pharmacokinetics: Memantine (trade name: Namenda) is absorbed completely from the GI tract, and peak plasma concentrations are achieved within 6-8 h after oral intake. By repeated administration a steady-state plasma level is reached within 21 days. Under therapeutic conditions, the serum levels of memantine with daily maintenance doses of 20 mg range from 0.5 to 1.0 µM. The plasma clearance half-life is 60-100 h. The elimination of memantine is mainly performed by the kidneys as schematically showed in **Figure 26** as unchanged substance or hydroxylated metabolite. Memantine crosses the blood-brain barrier (BBB), but cerebrospinal fluid (CSF) concentration is ~20-50% lower due to its binding to albumin in the blood serum (Kornhuber and Quack 1995).

3.7 Glutamatergic Regulation of Immune Cells

3.7.1 Presence of Glutamate Receptors in Non-neuronal Organs

Dopamine, Glu, serotonin and other neurotransmitters constitute a group of physiochemically stable molecules, which may act on target cells relatively far from where they were originally released (volume transmission). In contrast, acetylcholine belongs to the group of labile compounds which, when released, achieve effective concentrations to act near to the target cells due to their rapid degradation by cholinesterases that are abundant in tissue and plasma (Danysz and Parsons 2003). Thus, substantial amounts of the former type of neurotransmitters can be detected in extracellular fluids including plasma. The Glu concentration in plasma is relatively high (50-100 µmol/L) compared with the CNS (0.5-2 µmol/L in extracellular fluids) (Morrell et al. 2008)and is tightly regulated by peripheral Glu transporters (Hinoi et al. 2004). For example, platelets express excitatory amino acid transporters (EAATs) to clear Glu from the extracellular environment (Morrell et al. 2008). Emerging evidence suggests that Glu can play a dual role in mechanisms underlying cellular homeostasis: as an excitatory neurotransmitter in the central neurocrine system and as an extracellular autocrine and paracrine signal mediator in peripheral tissues. This leads to the assumption that Glu receptors are present on different non-neuronal cells. Accumulating evidence indicates the expression of Glu receptors in the heart, spleen, testis, kidney, pancreas, and on osteoblasts, osteoclasts, and platelets (Morrell et al. 2008). Functional relation studies reveal stimulation of insulin release from pancreatic β cells by AMPA (Bertrand et al. 1993) and regulation of platelet production from megakaryocytes by NMDA (Hitchcock et al. 2003). Several of the Glu receptors were cloned and sequenced and are identical to those found in the CNS (Hinoi et al. 2004). Over the last years evidence has emerged that immune cells including macrophages, neutrophils, T cells, and DCs release glutamate and

can be regulated by glutamate found in the blood stream, peripheral organs or CNS (Pacheco et al. 2007).

3.7.2 Glutamate Receptors in Immune Cells

Immune cells were shown to express NMDARs, AMPARs (GluA3-subunit) (Ganor et al. 2003) and metabotropic Glu receptors (group I, II and III mGluRs) (Pacheco et al. 2007), which modulate their functions. Glu receptors were found to be expressed on murine thymocyte subsets (double negative (DN) immature, double positive (DP) and mature thymocytes), peripheral T cells and human peripheral blood lymphocytes (PBLs). Glu transporters are reported to be expressed on macrophages and DCs (Pacheco et al. 2007). Although less explored, mGluRs are present on B cells (Rush et al. 2004) and DCs (Rezzani et al. 2003). Functionally, effects of NMDARs and AMPARs on the migration and apoptosis of immune cells were described. NMDARs on rodent lymphocytes seem to mediate an increase of intracellular Ca^{2+} and reactive oxygen species (Boldyrev et al. 2004), and AMPARs may play a role in the integrin-mediated adhesion to laminin and fibronectin (Ganor et al. 2003). Furthermore, inhibitory effects of CNS Glu on myelin basic protein (MBP)- and myelin oligodendrocyte glycoprotein (MOG)-specific lymphocyte activation were described in case of multiple sclerosis (MS) patients (Sarchielli et al. 2007). In case of another autoimmune disorder, Rasmussen's Encephalitis, antibodies to AMPAR GluR3 subunits are found in paediatric patients (Levite and Hermelin 1999) and auto-antibodies to NMDARs may be involved in several neuronal disorders (Kleopa 2011). In 2011, when our studies on NMDAR function in T and B cells had already commenced, *Affaticati et al.* showed that NMDAR GluN1 subunits accumulate in the synaptic contact region formed between OT-II TCR transgenic (tg) thymocytes and DCs presenting cognate ovalbumin (OVA)-peptide. Inhibition of NMDARs by the antagonists MK801 or memantine altered the duration of TCR-induced Ca^{2+}-flux and, thereby, influenced the apoptosis of DP thymocytes (Affaticati et al. 2011). This study further supported the idea that NMDARs are central regulators of T cell function.

However, it has to be noted that a major difference between the functional studies on neuronal and immune cells is the reported sensitivity to NMDAR-specific pharmacology. In immunological studies, NMDAR antagonists were often used in 10-fold higher concentrations than in neurons raising the question to what extent NMDARs are functional and important in lymphocytes.

3.8 Aim of the Study:

In view that NMDARs were reported to be expressed in lymphocytes and to affect human T cell function, the major aims of my thesis were

a) to proof the expression and functionality of NMDARs in lymphocytes
b) to determine the effects of several types of NMDAR inhibitors on the membrane potential and Ca^{2+}-flux of murine and human lymphocytes.

For this analysis, electrophysiological and immunological methods were applied.

4. Material and Methods

4.1 Material

4.1.1 Mouse Lines

Experiments were conducted with wild-type (wt) C57BL/6 mice, OT-II TCR transgenic (tg) (Barnden et al. 1998) and OT-I TCR tg (Hogquist et al. 1994) mice on C57BL/6 background. Mice were at the age of 6-10 weeks. All animal work was performed in compliance with the German Guidelines for the Use of Experimental Animals. Animals were housed in the Animal Facility of the Medical Faculty, Magdeburg.

4.1.2 Cell Culture-related

4.1.2.1 Media

Chemicals	Amount
FBS (heat-inactivated)	5% or 10%
1x SC	25 ml
Streptomycin	100 U/ml
β/2-Mercaptoethanol	50 mM

in 500 ml RPMI-1640

20X SC contains

Chemicals	Amount
FBS (heat-inactivated)	500 ml
Na-Pyruvate	12.2 mM
Non-essential amino acids	10 mm
Penicillin/ Streptomycin	$1x10^4$ U/ml
L-Glutamine	5%

FBS was inactivated at 56°C, aliquoted and stored at -20°C. Ingredients used for supplementation of the medium were sterile-filtered prior to use. 1X SC is used for optimal culture conditions.

4.1.2.2 Cell Isolation Kits

Kit name (cell type)	Constituents (biotinylated Abs)
mouse $CD4^+/CD8^+$ T cells	NK 1.1 (PK136), CD8α (53-6.7), CD4 (GK1.5), I-A/I-E (2G9), CD45R/B220 (RA3-6B2), Ter-119
human T cells	CD14, CD16, CD19, CD36, CD56, CD123, Glycophorin A
mouse B cells	CD43 (Ly48), CD4 (L3T4), Ter-119

All kits were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany) and contain streptavidin-coupled microbeads along with the biotinylated Abs for isolation of the cell subsets.

4.1.2.3 Cell Stimulation

Antibodies and mitogens	**Company**
CD3 (2C11)	BD Biosciences (Heidelberg, Germany)
CD28 (CD28.2)	BD Biosciences
SuperAvidin coated microsphere beads	Bangs Laboratories (IN, USA)
LPS	Sigma-Aldrich (Steinheim, Germany)
α-IgM $(Fab')_2$ fragment	Jackson Immunoresearch (Hamburg, Germany)

4.1.2.4 Reagents

Cell culture reagents	**Company**
RPMI-1640	Biochrom AG (Berlin, Germany)
DMEM	Gibco AG (Darmstadt, Germany)
AIMV	Gibco AG
EDTA	Sigma-Aldrich
Trypsin	Gibco AG

FBS (Fetal Bovine Serum)	Pan Biotech (Aidenbach, Germany)
Hank's Buffer	Biochrom AG
PFA	Sigma-Aldrich
Triton X-100	Sigma-Aldrich
BSA	Sigma-Aldrich
Streptomycin	Biochrom AG
Gentamycin	Roth GmbH (Karlsruhe, Germany)
OVA- peptide	AnaSpec (Fremont, USA)
2-mercaptoethanol	Gibco AG
DMSO (Dimethylsulfoxide)	Roth GmbH
Na-pyruvate	Biochrom AG
NEA (Non-essential amino acids)	Gibco AG
L-Glutamine	Gibco AG
Trypan Blue	Roth GmbH
PBS	Biochrom AG
Poly-D-Lysine	Sigma-Aldrich
Poly-L-Lysine	Sigma-Aldrich
Mowiol® 4-88	Sigma-Aldrich

4.1.3 Microscopy-related

4.1.3.1 Immunofluorescence Abs

Antibodies	**Epitope**	**Company**
GluN1	extracellular rabbit α-mouse	Alomone labs (Jerusalem, Israel) and Synaptic systems (Göttingen, Germany)
GluN2A	extracellular rabbit α-mouse	Alomone labs
GluN2B	extracellular rabbit α-mouse	Alomone labs
GFP	monoclonal mouse-α-GFP	Roche GmbH (Penzberg, Germany)

IgG2b	rabbit IgG2b control	Dianova (Hamburg, Germany)
IgG2a	rabbit IgG2a control	Dianova
FITC-coupled	secondary Ab α-mouse	Invitrogen
Cy5-coupled	secondary Ab α-rabbit	Jackson Immuno Research

4.1.3.2 Solutions for Electrophysiological Analysis

4.1.3.2.1 Extracellular Solution

Channel	Chemical	Concentration (mM)
$K_v1.3$	NaCl	160
	KCl	4.5
	HEPES	5.0
	$MgCl_2$	1.0
	$CaCl_2$	2.0
$K_{Ca}3.1$	Na-aspartate	160
	KCl	4.5
	$CaCl_2$	2.0
	$MgCl_2$	1.0
	HEPES	10.0

4.1.3.2.2 Intracellular Solution

Channel	Chemical	Concentration (mM)
$K_v1.3$	KF	162
	EGTA	11
	HEPES	10
	$CaCl_2$	1
	$MgCl_2$	2
$K_{Ca}3.1$	K-aspartate	145
	$CaCl_2$	8.5
	$MgCl_2$	2
	EGTA	10
	HEPES	10

Extracellular and intracellular solutions were adjusted to pH7.4 and pH7.2, respectively. The osmolarity of both solutions was maintained at 300-340 mOsm.

4.1.3.3 Inhibitors

Inhibitors (blocked channel)	Company
Margatoxin ($K_v1.3$)	Tocris Biosciences (Bristol, UK)
Charybdotoxin ($K_{Ca}3.1$)	Tocris Biosciences
TRAM-34 ($K_{Ca}3.1$)	Tocris Biosciences
Ifenprodil (GluN2B of NMDARs)	Tocris Biosciences
MK801 (open channel of NMDARs)	Tocris Biosciences and Alomone labs
Memantine (open channel of NMDARs)	Tocris Biosciences
Ketamine (open channel and allosteric site of NMDARs)	Tocris Biosciences
D-APV (competitive for Glu-site of NMDARs)	Tocris Biosciences

All inhibitors were reconstituted in ddH_2O and stored at -20°C for 3 months.

4.1.3.4 Ca^{2+}-imaging Dyes/Chemical

Dye	Company	Activity	Use
Fura-2 AM	Molecular Probes, Invitrogen (Darmstadt, Germany)	double-excitation (at 340***** and 380^+ nm) and single-emission (510 nm)	fluorescence microscopy
Indo-1 AM	Molecular Probes	single-excitation (350 nm) and double- emission (400^+ and 475 nm*****)	flow cytometry
Ionomycin	Molecular Probes and Calbiochem (Merck, Darmstadt, Germany)	acts as ionophore to raise the intracellular Ca^{2+} concentration	flow cytometry and fluorescence microscopy

*****: Ca^{2+}-free; **+**: Ca^{2+}-bound

Both Ca^{2+}-imaging dyes are ratiometric, which reduces the effects of uneven dye loading, leakage of dye, photobleaching, and problems associated with measuring Ca^{2+} in cells of unequal diameter.

4.1.3.5 Chemicals

Electrophysiological Chemicals	Company
NaCl	Roth GmbH
KCl	Roth GmbH
HEPES	Roth GmbH
$MgCl_2$	Roth GmbH
$CaCl_2$	Roth GmbH
Na-aspartate	Sigma-Aldrich
KF	Sigma-Aldrich
EGTA	Roth GmbH
K-aspartate	Sigma-Aldrich

4.1.4 DNA Constructs

Construct	Vector backbone	Insert	Source
GluN1-wt	pRcCMV	GluN1	gift from Dr. Paoletti (Bordeaux)
GluN2A-SEP (5.8 kb)	pCI (4 Kb)	GluN2A (1.1 Kb)+SEP (0.7 Kb)	Addgene
GluN2B-SEP (6.3 kb)	pCI (4 Kb)	GluN2B (1.6 Kb)+SEP (0.7 Kb)	Addgene
GluR2-SEP (9.6 kb)	pcDNA (not known)	GluR2 (~4 Kb)+SEP (0.7 Kb)	gift from Dr. Passafaro (Bordeaux)

SEP = Super-ecliptic protein

4.1.5 Instruments

Instruments	Company
pH Meter	Mettler-Toledo (Gießen, Germany)
OsmoMeter	Osmomat 320 Genotec (Berlin, Germany)

Vortex	Vortex Genie2 (New York, USA)
Light microscope	Olympus CK2 (Tokyo, Japan)
Inverted microscope (for Ca^{2+}-imaging)	Olympus
Inverted Microscope (for electrophysiology)	Zeiss (Oberkochen, Germany)
Epifluorescence Microscope	Zeiss
Water-bath chamber	Lauda AQUALINE AL5 (Lauda-Königshofen, Germany)
Centrifuge	Eppendorf AG 5415R, 5810R (Hamburg, Germany)
EPC 10 Amplifier	Warner Instruments (Hamden, USA)
Electrophysiological chamber and manipulator	Warner Instruments
LSR II Flow Cytometer	BD Biosciences
Neubauer Chamber	Roth GmbH (Karlsruhe, Germany)

4.1.6 Software

Software	**Company**
PatchMaster v.2.11	HEKA Electronic (Lambrecht (Pfalz), Germany)
FitMaster v2x53	HEKA Electronic
Metamorph	Molecular Devices (CA, Germany)
IgorPro5.04B	WaveMetrics Inc. (Portland, USA)
FlowJo v3.6.1	TreeStar (Ashland, USA)
Graphpad Prism 5.0	Prism (CA, USA)
ImageJ	National Institute of Health (USA)

4.2 Methods

4.2.1 Cell Culture-related

4.2.1.1 Cell Isolation

4.2.1.1.1 T cell Isolation

4.2.1.1.1.1 Mouse T cell Isolation

Mice were killed with CO_2. Spleen and lymph nodes were extracted and kept in RPMI-1640 medium supplemented with 10% FCS at RT. For preparation of single cell suspensions, spleens or lymph nodes were passed through a plastic cell strainer with a diameter of 100 µm in Petri dishes containing the medium. An aliquot of cells was diluted in 0.05% Trypan blue in PBS and counted in a Neubauer chamber to determine the cell number. Splenic cells were treated with Gey's solution to lyse erythrocytes. This solution was pre-warmed at 37°C in the water bath before use.

$CD4^+$ or $CD8^+$ T cells were isolated from pooled lymph nodes of wt, OT-II or OT-I TCR tg mice by negative selection using a cocktail of biotinylated Abs: NK1.1 (PK136), CD8α (53-6.7), CD4 (GK1.5), I-A/I-E (2G9), CD45R/B220 (RA3-6B2), Ter-119 (all from BD Bioscience) and streptavidin magnetic beads (Miltenyi Biotec) according to the manufacturer's protocol. Purity of $CD4^+$ or $CD8^+$ T cells after MACS isolation was routinely above 90%.

$CD4^+$ T cells were activated with CD3+CD28 antibodies (3+5 µg/ml) in supplemented RPMI-1640 medium for 48 h for patch clamp recording.

4.2.1.1.1.2 T cell Isolation from Human Peripheral Blood

Fresh $CD3^+$ T cells were isolated from the collected blood samples of healthy donors and dementia patients using the Pan and $CD4^+$ T cell isolation kit from Miltenyi Biotec and the Ficoll-Hypaque gradient procedure according to manufacturer's protocol. The dementia patients were prescribed to have a daily 10 mg memantine dose in the first week followed by 20 mg for the next 11 weeks. The isolation kit contained biotinalyted CD14, CD16, CD19, CD36, CD556, CD123, and Glycophorin A and anti-biotin microbeads for the conjugation to anti-biotin antibodies. The cells were maintained in AIMV medium. Purity of iolated T cells was 90%. All experiments with human cells were conducted in collaboration with Prof. Dr. Ursula Bommhardt (Institute for Molecular and Clinical Immunology) and Dr. Stefan Busse (Department of Psychiatry, Magdeburg) and were approved by the local Ethics Committee.

4.2.1.1.2 B cell Isolation

Splenic or lymph node B cells were isolated with the B-cell isolation kit (Miltenyi Biotec) composed of a cocktail of biotinylated Abs: CD43 (Ly48), CD4 (L3T4) Ter119 and anti-biotin microbeads. The procedure was followed according to the manufacturer's protocol and purity of B cells was 90-95%.

B cells were cultured in complete RPMI-1640 medium supplemented with 10% FCS, 50 µM β-mercaptoethanol, 1% penicillin/streptomycin and were activated with goat anti-mouse α-IgM $(Fab')_2$ or LPS (10 µg/ml each) for 48-72 h and subsequently used for the experiments.

4.2.1.1.3 DC Isolation

4.2.1.1.3.1 Generation of Bone-marrow Derived Dendritic Cells (BMDCs)

Bone marrow (BM) was collected from femur and tibia of wt mice and cells were suspended in RPMI-1640 medium reconstituted with 1% non-essential amino acids, 5% FCS, 1% L-glutamine, 0.1% gentamycine, 0.1% 2-mercaptoethanol, IL-4 (48 ng/ml) and GM-CSF (10 ng/ml) from a hybridoma supernatant (a gift of Prof. Dr. Matthias Gunzer). A total of $3x10^6$ BM cells/5 ml BMDC medium were cultured for 7 days. At day 3, 2 ml medium was replaced by fresh BMDC medium, at day 6, total medium was replaced and cells were stimulated with LPS in a concentration of 20 ng/ml for 24 h. DCs used at day 9 or 10 were re-stimulated with LPS 24 h before experimental onset. Maturation of BMDCs was verified at day 7 by staining cells with Abs against MHC-II (NIMR-4), CD11c (HL3), CD80 (2D10) and CD86 (all from BD Bioscience).

4.2.1.1.3.2 Activation of DCs

Mature BMDCs ($MHC\text{-}II^+$ $CD11c^+$ $CD80^+$ $CD86^+$) were pulsed with OVA-peptide (aa 323-339, 10 µg/ml or aa 257-264, 5 µg/ml (SIINFEKL), AnaSpec) for 2 h and cultured with OT-II $CD4^+$ or OT-I $CD8^+$ T cells, respectively. $CD4^+$ T cells ($0.5x10^5$- $1x10^5$) were stimulated with CD3 Abs (3 or 10 µg/ml) or CD3 pus CD28 Abs (3 and 5 µg/ml, respectively).

For single-cell Ca^{2+}-flux measurement, a total of $1x10^6$ OT-II $CD4^+$ or OT-I $CD8^+$ T cells were left untreated or were activated with pOVA-loaded BMDCs in a DC-T cell ratio of 1:10. NMDAR-inhibitors were added during recording. Matured BMDCs were pulsed with the OVA-peptide for overnight.

4.2.1.2 Maintenance of Cell Lines

EL-4 (mouse T cell), JE6.1 (human T cell) and Raji (human B cell) lymphoma cells were grown in RPMI-1640 medium containing 10% FCS supplemented with nutrients at 37°C and 5% CO_2. Cells were diluted 1:10 after 3 days to maintain an optimal cell density ($1x10^5$–$1x10^6$cells/ ml). For freezing, the cells were diluted in DMSO at a ratio of 1:10.

Adherent HEK-293T cells were grown in Dulbecco's MEM (DMEM) containing 10% FCS, 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C and 5% CO_2. Cells were diluted 1:10 in 3 day cycles to avoid confluence of the cell layer. For splitting, adherent cells were washed once with PBS and subsequently treated with 5 ml Trypsin/EDTA solution (0.25% trypsin/0.02% EDTA in PBS without Ca^{2+}, Mg^{2+}) for 1 min at RT for cell detachment. Cells were suspended and diluted 1:10 in fresh medium for culture.

4.2.1.3 Determination of Cell Number

Determination of the cell number was done with the Neubauer chamber, which is subdivided into four quadrants (each having a surface area of 1 mm^2). Each quadrant is subdivided into 16 smaller quadrants. When cells are added to the chamber, each big quadrant receives a volume of 0.1 µl (0.1 mm^3). The cell suspension was diluted 1:50 in Trypan blue solution and counted with a light microscope of 20x magnification. Bright live cells in the quadrants were counted excluding the blue coloured ones representing dead cells. Calculation of cell count = counts of 4 bigger quadrants)/4 x dilution factor x 10,000 = cell count/ml.

4.2.1.4 Transfection Protocol

4.2.1.4.1 Fugene Method

600 µl of PBS was mixed with 2 µg of DNA and 50 µl Fugene (Roche, Berlin, Germany). This mixture was kept for 15 min at RT and then added to HEK-293T cells according to the manufacturer's protocol. Transfected cells were immuno-stained after 24-48 h. This method was used for transfecting the cell line to test NMDAR Ab specificity.

4.2.2 Microscopy-related

4.2.2.1 Immunofluoroscence Method

For live extracellular staining, HEK-293T cells were incubated with primary Abs (GluN1-wt, GluN2A-SEP and GluN2B-SEP) in DMEM medium for 30 min at 37°C. Then, the cells were fixed with 4% PFA and blocked with a solution containing 10% FCS in PBS, 1% Triton X-100. Then, the cells were washed three times with PBS. Afterwards, secondary Abs in

PBS/1% BSA were added to the cells and kept for 1 h in the dark. Finally, the cells were washed three times and mounted in mowiol.

For staining of fixed cells, the cells were initially fixed with 4% PFA and then blocked with 10% FCS/PBS/1% Triton X-100. Then, the cells were incubated with primary Abs in PBS for 1 h. This was followed by washing the cells three times with PBS/1% BSA. Finally, the cells were incubated with secondary Abs for 1 h in the dark followed by three times washing in PBS. At the end, the cells were mounted in mowiol. BSA (bovine serum albumin) inhibits the non-specific binding of Abs. Thus, its use in immunofluorescence stainings is preferred.

4.2.2.2 Electrophysiological Methods (Patch clamp)

All experiments were carried out in the whole-cell configuration of the patch-clamp technique using an EPC10 amplifier and PatchMaster v.2.11 (HEKA Electronic) at RT (20-24°C).

4.2.2.2.1 Voltage Clamp

Patch pipettes from borosilicate glass used for recordings had a resistance between 3-5 MΩ. $K_v1.3$ and $K_{Ca}3.1$ currents were recorded with external and internal solutions mentioned in **section 4.1.3.2**. Osmolarity was set to 300-340 mOsM by the Osmomat instrument. $K_v1.3$ currents were measured with depolarizing voltage steps up to +60 mV from a holding potential of -80 mV every 30 s in steps of 20 mV. $K_{Ca}3.1$ currents were elicited by a 200 ms voltage ramp from -120 to +40 mV from a holding potential of -80 mV every 15 s. Sampling rate was 50 kHz in case of $K_v1.3$ and 20 kHz in case of $K_{Ca}3.1$. The antagonists ifenprodil, MK801, memantine, ketamine or D-APV (Tocris) were added during the recording with a constant inhibitor concentration. As a positive control for the measured currents, margatoxin and TRAM-34 (Tocris) were used.

4.2.2.2.2 Current Clamp

For membrane potential experiments, activated mouse T or B cells and JE6.1 or Raji cells were recorded in the current clamp mode with 0 pA holding current immediately after establishment of the whole-cell configuration. Ifenprodil, memantine or D-APV were added during the recording with a constant inhibitor concentration to determine the membrane depolarization in the presence of the inhibitors.

4.2.2.3 Ca^{2+}-flux Measurement

4.2.2.3.1 Whole-cell Ca^{2+}-flux

For T cells, lymph node cells from wt mice were stained with 4 µM Indo-1 AM (Invitrogen, Molecular Probes) for 45 min at 37°C. After being washed with PBS, the cells were stained for CD8 and B220 or CD4 and B220 surface expression for 15 min, washed and re-suspended in Hank`s buffer (Biochrom AG) supplemented with 1 mM $CaCl_2$. CD3-biotin Abs (145.2C11, 10 µg/ml) plus streptavidin (25 µg/ml, Dianova) were added to induce Ca^{2+}-flux. The NMDAR antagonist ifenprodil (10 or 30 µM) was added for 5 min before CD3 Ab and streptavidin treatment. Additionally, external NMDA (100 µM) was added to induce Ca^{2+}-flux.

For B cells, splenocytes were stained with 4 µM Indo-1 AM for 45 min at 37°C. Cells were washed, stained for CD8 and CD4 surface expression and suspended in Hank's buffer supplemented with 1 mM $CaCl_2$. NMDAR antagonist ifenprodil (10 or 30 µM) or memantine (30 or 50 µM) was added for 5 min before B cells were activated with α-IgM (10 µg/ml) to induce Ca^{2+}-flux.

Towards the end of each measurement, ionomycin (2 µM, Calbiochem) was added as a positive control for cell reactivity. Ca^{2+}-flux was measured on a LSRII flow cytometer (BD Biosciences). Data files were transferred to FlowJo V3.6.1, mean Ca^{2+}-flux was determined for unlabelled $CD4^+$ or $CD8^+$ T cells and unlabelled B cells and data were further processed with IgorPro5.04B software. For each graph, ΔCa^{2+}-flux was defined as the difference between the maximum and minimum value of Ca^{2+} -intensity.

4.2.2.3.2 Single-cell Ca^{2+}-flux

Freshly-isolated $CD4^+$ or $CD8^+$ T cells were loaded with 2-4 µM Fura-2 AM for 45 min at 37°C. These Fura-loaded cells were measured with the MetaMorph Program (Molecular Devices) under a fluorescence microscope (Olympus) in 40x magnification along with the prepared DCs. Mean Ca^{2+}-flux was determined by plotting the 340 and 380 nm values in Excel and each trace was assessed in IgorPro 5.04B software. The T cells were stimulated with either OVA-presenting DCs or CD3/CD28 coated microbeads.

4.2.2.3.2.1 Bead Stimulation Protocol

Beads (mean diameter: 10.14 µm, 33 µl for $1x10^6$ cells) were incubated with CD3 and CD28 Abs (both 10 µg/ml) for 30 min at 37°C to activate the cells. Then, they are washed with PBS and added to the used cell culture medium.

4.2.3 Statistical Analysis

Data are given as mean values ± standard deviation (SD), if not stated otherwise. Student's *t* test and other statistical measurements were performed in GraphPad Prism 5.04B. Statistical significance was set as *p<0.05, **p<0.01 and ***p<0.001.

5. Results

5.1 NMDAR Expression in Murine Lymphocytes is Puzzling

In previous work GluR expression was detected in murine thymocytes and human peripheral lymphocytes by intracellular staining and flow cytometry, and localization of GluN1 subunits in the thymocyte-DC contact zone was shown by confocal microscopy (Affaticati et al. 2011; Ganor et al. 2003; Lombardi et al. 2001). Inspired by this work, experiments within our collaborative research project apparently confirmed the results for NMDAR subunit expression, namely expression of GluN1, GluN2A and GluN2B subunits in thymocytes and peripheral T cells (Kahlfuss et al., 2014). Upon co-culture of OT-II $CD4^+$ T cells with pOVA-presenting DCs, GluN1 and GluN2B subunits were detected in the immunological synapse, as shown by confocal microscopy. However, in Western blot analyses, GluN1 protein in thymocytes and $CD4^+$ T cells appeared at a lower molecular weight than GluN1 protein in brain lysate. To proof whether the detected protein is GluN1, we performed analyses on thymocytes obtained from newborn GluN1 knock out (ko) mice. PCR and RT-PCR analyses showed the deletion of GluN1 at the DNA and mRNA level in thymocytes, and GluN1 protein was absent in brain samples, but not in thymocytes. These observations strongly suggest that GluN1 protein is not detectable in thymocytes. Intracellular staining with two different GluN1 Abs and flow cytometry as well as immunohistochemistry also showed identical staining for wt and GluN1 ko thymocytes, although the used Abs for GluN1, GluN2A and GluN2B showed specificity for the subunits in transfected HEK-293T cells (**Figure 5**). Hence, on the protein level, there is no evidence for expression of the obligatory GluN1 subunit of NMDARs in murine thymocytes and T cells (Kahlfuss et al. 2014).

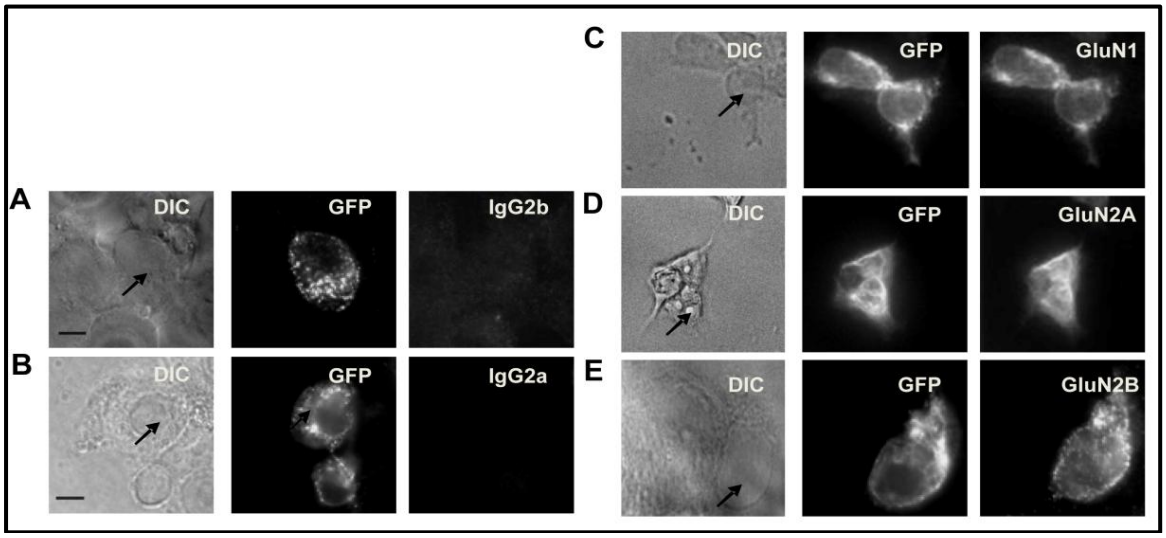


Figure 5. NMDAR subunit transfection for testing the specificity of Abs used to detect NMDAR subunit expression. Fluorescent images of HEK-293T cells transfected with the NMDAR subunits GluN1-wt, GluN2A-SEP and GluN2B-SEP were analysed by an epifluroscent microscope taken in

63x magnification and processed with ImageJ. Scale bar = 4 µm. Transfected cells were analysed for GFP(SEP)-expression to detect the individual subunits and were stained with (C) mouse-anti-GluN1 (Synaptic System), (D) rabbit-anti-GluN2A (Alomone labs), (E) rabbit-anti-GluN2B (Alomone labs), (A) mouse-IgG2b, (B) rabbit-IgG2a, and secondary PE-labelled Abs. (A) and (B) were used as controls for mouse-anti-GluN1 and rabbit-anti-GluN2A/GluN2B, respectively. The immunohistochemical stainings show strong overlap with GFP-expression indicating that the Abs are specific for the individual NMDAR subunits. Differential interference contrast (DIC) images show the position and shape of an individual cell. Arrows point towards a representative transfected HEK-293T cell.

Although expression of NMDARs at the protein levels was elusive, we detected Ca^{2+}-flux in lymph node (LN) $CD4^+$ and $CD8^+$ T cells and LN and splenic B cells upon addition of the NMDAR agonist NMDA (100 µM). NMDA-induced Ca^{2+}-flux in T cells was reduced by 10 µM ifenprodil to ~80% and it was nearly abrogated with 30 µM ifenprodil (**Figure 6A**). In case of B cells, the reduction in Ca^{2+}-flux was even more prominent, as Ca^{2+}-flux was reduced to ~40% in LN and to ~20% in splenic B cells by 10 µM ifenprodil (**Figure 6B**).

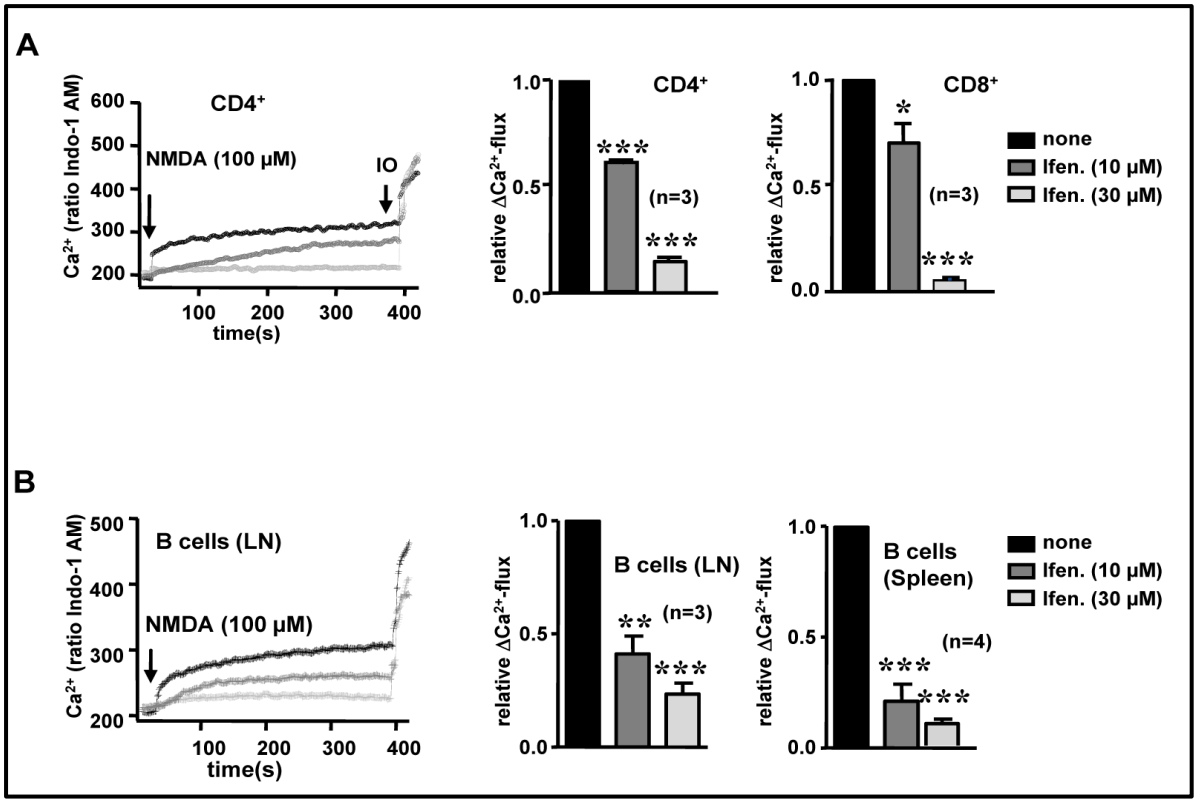


Figure 6. NMDAR antagonists attenuate NMDA-induced Ca^{2+}-flux. Indo-1 AM-loaded (A) LN $CD4^+$ and $CD8^+$ T cells and (B) LN and splenic B cells were activated with NMDAR agonist NMDA (100 µM) in the absence or presence of ifenprodil (Ifen.) and Ca^{2+}-flux was determined with flow cytometry. Ionomycin (IO) was added towards the end of each measurement to control cell reactivity. Left histograms in (A, B) show a representative experiment for LN $CD4^+$ T cells and B cells; right graphs show the relative ΔCa^{2+}-flux for $CD4^+$ and $CD8^+$ T cells and B cells calculated from 3-4 ex-

periments. ΔCa^{2+}-flux from cells activated without ifenprodil (none) was set as 1. Data represent mean values ± standard error of mean (SEM) from 3-4 independent experiments.

Although NMDA-induced Ca^{2+}-flux was detected in T and B cells, the presence of NMDARs should be further examined by performing Ca^{2+}-flux experiments in thymocytes from GluN1 ko mice. It should also be mentioned that external Glu (100 µM) did not induce any Ca^{2+}-flux. Thus, we hypothesize that NMDA either unspecifically binds to some other unknown ion channel(s) on lymphocytes or that NMDARs are expressed only transiently and in very small numbers and therefore are not detectable under the used experimental conditions.

Along with whole-cell Ca^{2+}-flux in mature T cells, single-cell Ca^{2+}-influx was performed, as *Affaticati et. al., 2011* showed Ca^{2+}-influx at the immune cell synapse formed between OT-II TCR tg $CD4^+/CD8^+$ thymocytes and OVA-presenting DCs. We used the DC system and CD3/CD28 Ab-coated microbeads to activate T cells. OT-I TCR tg $CD4^+$ and OT-II TCR tg $CD8^+$ T cells were loaded with 4 µM Fura-2 AM to monitor TCR-induced Ca^{2+}-flux changes using the MetaMorph Program and an inverted microscope. Matured DCs were loaded with cognate OVA-peptide for 24 h before use. A transient Ca^{2+}-flux was detected in OT-II $CD4^+$ and OT-I $CD8^+$ T cells after CD3/CD28 bead-stimulation as shown in **Figure 7A and C**. For DC/antigen-stimulated OT-II $CD4^+$ T cells, transient as well as sustained Ca^{2+}-fluxes were observed (**Figure 7B**). However, there was no reliable detection of a reduction of single-cell Ca^{2+}-flux in the T-DC or T-bead contact zone after addition of ifenprodil (data not shown). This was unexpected and puzzling and could be due to the more homogenous cell population using TCR tg T cells instead of heterogeneous whole cell populations used for the measurement of Ca^{2+}-flux by flow cytometry, or, the cells reacting to ifenprodil did not respond with any Ca^{2+}-flux at all or due to technical limitations.

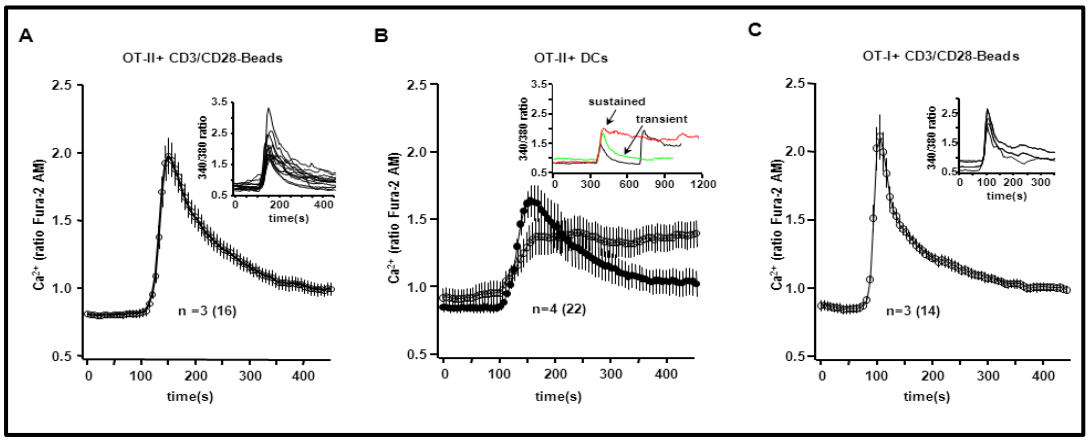


Figure 7. Single-cell Ca^{2+}-flux of TCR tg T cells in the presence of antigen-presenting DCs or CD3/CD28-coated beads. Single cell Ca^{2+}-flux was recorded for 500 s in OT-II $CD4^+$ T cells stimulated with (A) CD3/28-coated beads or (B) OVA-presenting DCs and in (C) OT-I $CD8^+$ cells after stimulation with CD3/28-coated beads. The data represent averaged Ca^{2+}-fluxes from 14-22 sin-

gle cells of n = 3-4 experiments out of 8-9 preparations from OT-I/II TCR tg mice. Insets show the representative flux for different stimulation conditions mentioned.

5.2 NMDAR Antagonists Attenuate TCR/BCR- induced Ca^{2+}-flux

5.2.1 TCR-induced Ca^{2+}-flux

In order to understand how NMDAR antagonists influence T-cell activation, their effect on proximal T-cell signalling was analyzed. I concentrated on the analysis of Ca^{2+}-flux, as this is pivotal for the activation of many Ca^{2+}-regulated proteins which guide T-cell activation.
LN $CD4^+$ and $CD8^+$ T cells, loaded with Indo-1 AM to monitor intracellular Ca^{2+}-changes by flow cytometry, responded to TCR ligation by cross-linked CD3 Abs with a rapid mobilization of Ca^{2+}. The Ca^{2+}-flux was significantly reduced by 10 µM and almost entirely blocked by 30 µM ifenprodil as shown in **Figure 8**.

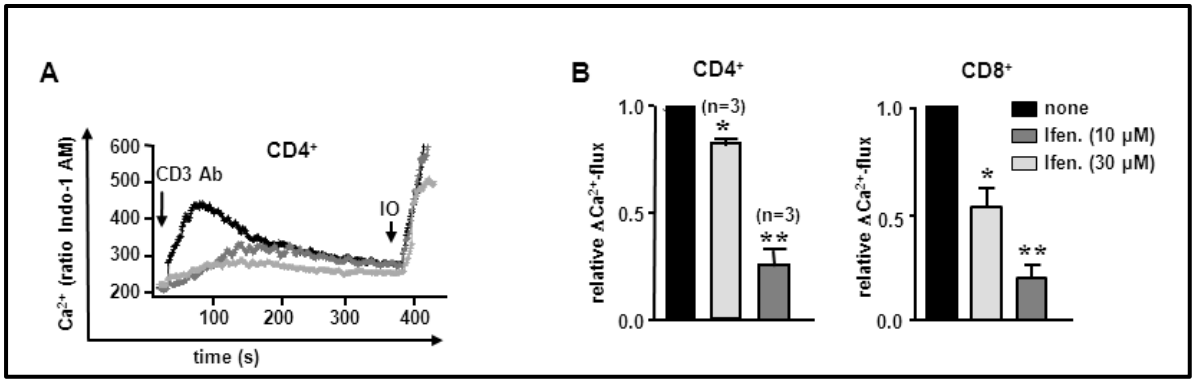


Figure 8. NMDAR antagonists attenuate TCR-induced Ca^{2+}-flux. (A) Indo-1 AM-loaded $CD4^+$ and $CD8^+$ T cells were activated with CD3 Abs (10 µg/ml) in absence or in presence of 10 and 30 µM ifenprodil (Ifen.) and Ca^{2+}-flux was determined by flow cytometry. IO was added towards the end of each measurement to control cell reactivity. The histogram in (A) shows a representative Ca^{2+}-flux measurement for $CD4^+$ T cells and the graphs in (B) shows the statistics for $CD4^+$ and $CD8^+$ T cells. Data in the the graphs provide the relative ΔCa^{2+}-flux as mean $\pm$ SEM calculated from 3 experiments each. The obtained ΔCa^{2+}-flux of cells activated in the absence of Ifen. (none) was set as 1.

5.2.2 BCR-induced Ca^{2+}-flux

As TCR-induced Ca^{2+}-flux was modulated by ifenprodil, it was intriguing to study B cells as several autoimmune disorders, as described in **Table 3**, involve B cells producing autoantibodies, for instance against NMDARs. Ligation of the BCR with IgM Abs (α-IgM) and LPS were used for polyclonal stimulation of B cells. Indo-1 AM-labelled splenic B cells showed a concentration-dependent inhibition of BCR-induced Ca^{2+}-flux when they were treated with ifenprodil or memantine. **Figure 9A** shows a reduction of Ca^{2+}-flux to ~80% of untreated cells in the presence of 10 µM and to ~30% by 30 µM ifenprodil. **Figure 9B** de-

picts the reduction of Ca^{2+}-flux to ~80% in the presence of 30 µM and to ~40% by 50 µM memantine. Thus, NMDAR antagonists also impair BCR-induced B-cell activation.

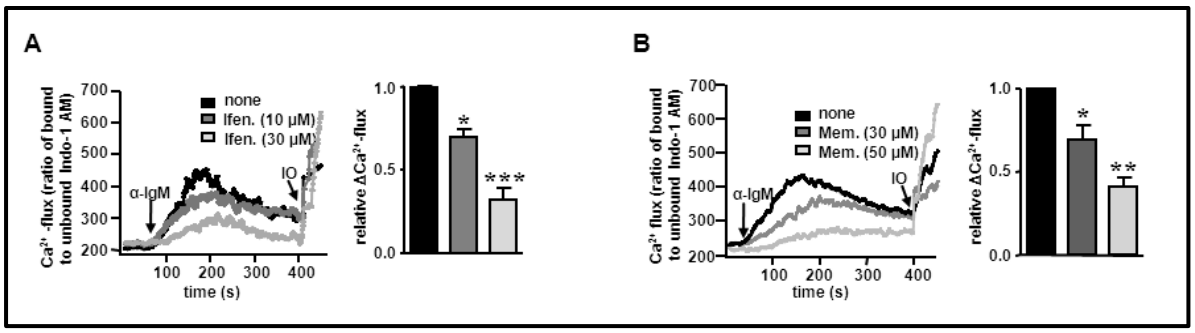


Figure 9. Reduced Ca^{2+}-flux in BCR-activated B cells in the presence of NMDAR antagonists. Indo-1 AM-labelled B cells were stimulated with α-IgM (10 µg/ml) in the presence or absence of (A) ifenprodil (Ifen.) and (B) memantine (Mem.) and Ca^{2+}-flux was determined with flow cytometry. (A) shows the reduction of BCR-induced Ca^{2+}-flux by 10 and 30 µM Ifen. and (B) by 30 and 50 µM Mem. The ΔCa^{2+}-flux from cells activated without Ifen. or Mem. (none) was set as 1. Corresponding graphs represent the ΔCa^{2+}-flux from three experiments each.

5.3 NMDAR Antagonists Modulate the Membrane Potential and Block $K_v1.3$ and $K_{Ca}3.1$ Channels of Lymphocytes

5.3.1 Mouse Primary T Cells and EL-4 Lymphoma Cells

In view of the strong effects of NMDAR pharmacology on Ca^{2+}-flux in lymphocytes, but the elusive expression of NMDARs at protein level, we hypothesized that the inhibitor's targets could involve $K_v1.3$ and $K_{Ca}3.1$ potassium channels (Partiseti et al. 1992; Partiseti et al. 1993; Lewis and Cahalan 1995). These channels regulate T-cell activation by controlling the membrane potential and, hence, the Ca^{2+}-flux into T cells (Desai et al. 2000; Lam and Wulff 2011; Conforti 2012). Indeed, current-clamp recordings of $CD4^+$ T cells activated with CD3+CD28 Abs showed that ifenprodil (30 µM) and memantine (50 µM) depolarized the membrane potential from ~-50 mV to ~-15 mV and ~-20 mV, respectively, as shown in **Figure 10**.

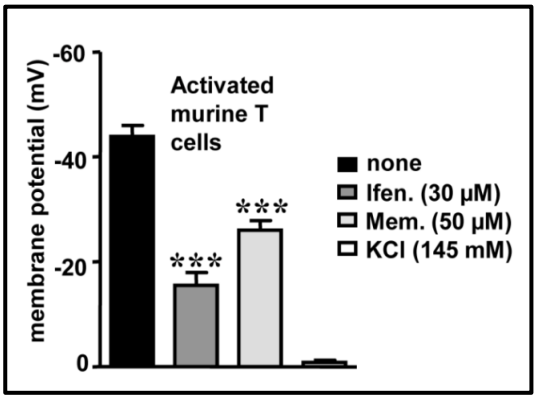


Figure 10. NMDAR antagonists change the membrane potential. The membrane potential of murine $CD4^+$ T cells, activated with CD3+CD28 Abs (3+5 µg/ml) for 24-48 h, was determined with current clamp recording. Addition of ifenprodil or memantine in concentrations as indicated lead to a membrane depolarization. KCl treatment served as a positive control for cell integrity and membrane

depolarization. All data were calculated from 5-6 cells of three experiments in each case and are represented as mean ± SEM.

Since the membrane potential was strongly affected by NMDAR antagonists, I next addressed whether this involves $K_v1.3$ and $K_{Ca}3.1$channels, the major and most abundant K^+-channels in lymphocytes. $K_v1.3$ and $K_{Ca}3.1$ channels currents were recorded by voltage clamp method and dose-response curves were constructed from the transient maximal current amplitudes. The obtained Hill slopes and 50% inhibitory concentrations (IC_{50}) for $K_v1.3$ channels on T cells in the presence of ifenprodil and memantine were ~1.5 and ~1.9 and ~35 µM and ~45 µM, respectively, as shown in **Figure 11 A, B**. For $K_{Ca}3.1$ channels, Hill slope and IC_{50} values were ~1.2 and ~15 µM for ifenprodil and ~1.6 and ~30 µM for memantine, respectively (**Figure 11 C, D**).

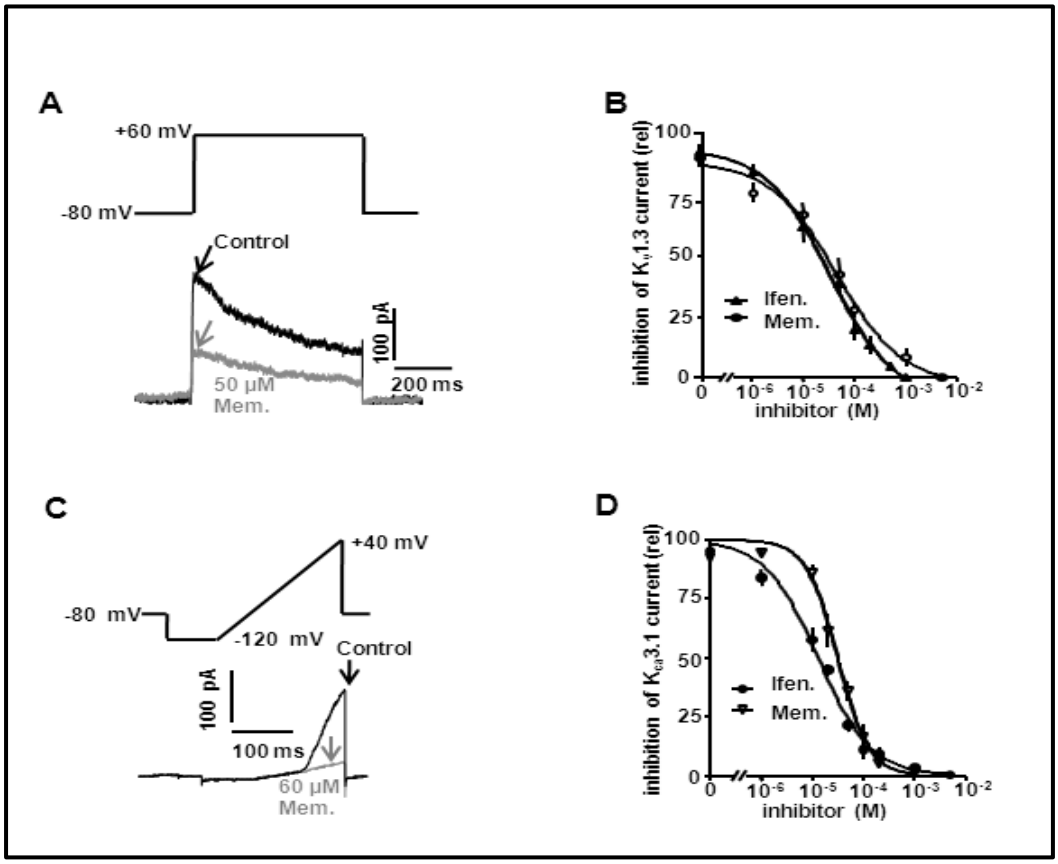


Figure 11. NMDAR antagonists inhibit $K_v1.3$ and $K_{Ca}3.1$ channel activity in activated T cells. $K_v1.3$ and $K_{Ca}3.1$ channel-mediated currents were recorded by voltage-clamp method from T cells activated with CD3+CD28 Abs (3+5 µg/ml) for 24-48 h. Voltage clamp protocol for $K_v1.3$ recording and current traces are shown in (A). (B) shows the curves formed by plotting the transient amplitude of the recorded currents versus the indicated inhibitor concentrations. (C) Ramp protocol for measuring $K_{Ca}3.1$ recorded current and example traces are shown and the dose-response inhibition curves formed by the recorded currents is shown in (D). Each data point in the dose-response inhibition curves represents mean calculated from 5-7 cells and vertical lines show mean ± SEM.

A competitive antagonist can determine the functional expression of a channel as it is competing with the agonist site of the channel. In case of NMDARs, the competitive antagonist frequently used in neuronal systems is D-APV, which is competing with the Glu-binding site. Therefore, the effect of D-APV on $K_v1.3$ channels in activated $CD4^+$ T cells was determined. As shown in **Figure 12A**, D-APV blocked $K_v1.3$ channels, but only at ~20-fold higher concentrations (1 mM) than needed by the other tested inhibitors. In addition to activated primary murine $CD4^+$ T cells, the inhibitory effects of ifenprodil, memantine, MK801, ketamine, and D-APV on $K_v1.3$ channels were analysed in murine EL-4 lymphoma T cells. All NMDAR inhibitors strongly blocked $K_v1.3$ channel currents, but in case of D-APV, ~15-fold higher concentrations were needed to reach an inhibition similar to that obtained with ifenprodil (**Figure 12B**). Thus, the employed concentrations of NMDAR antagonists, which were similar to those used in previous publication by others (Affaticati et al. 2011), non-specifically inhibit two K^+-channels, which reportedly modulate many Ca^{2+}-mediated processes in T cells (Lam and Wulff 2011).

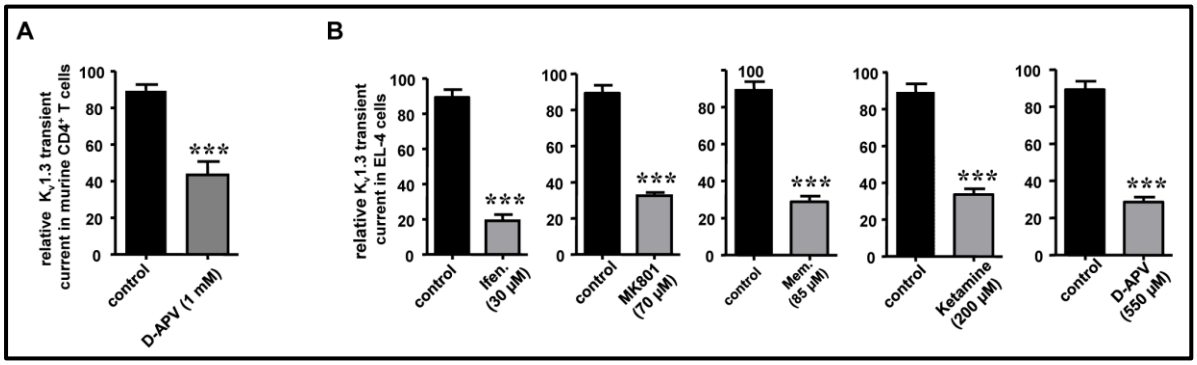


Figure 12. NMDAR antagonists decrease $K_v1.3$ channel activity in activated $CD4^+$ T cells and EL-4 lymphoma cells. $K_v1.3$ channel currents were determined in (A) activated murine $CD4^+$ T cells and (B) EL-4 lymphoma cells in the absence and presence of the indicated NMDAR antagonists. The currents were recorded by voltage-clamp with the protocol given in Figure 11A. The data in the bar graphs represent the relative inhibition of recorded $K_v1.3$ transient currents from 5-6 cells and all data represent mean ± SEM determined by unpaired Student's *t* test.

5.3.2 Murine B Cells

K^+-channels are also expressed on B cells and their inhibition was found to differentially influence B-cell activation and proliferation (Partiseti et al. 1992; Partiseti et al. 1993; Lewis and Cahalan 1995). Since NMDAR inhibitors downregulate BCR-induced Ca^{2+}-flux in B cells, their effects on the membrane potential and $K_v1.3$ and $K_{Ca}3.1$ channels were alalyzed. Ifenprodil (20 µM) and memantine (30 µM) reduced the membrane potential of α-IgM- or LPS/TLR4-activated B cells from ~-40 mV to ~-20 mV and ~-10 mV, respectively as shown in **Figure 13**. Next, $K_v1.3$ channel-mediated currents from activated B cells were recorded

and the dose-response curves in the presence of the inhibitors were calculated from maximal transient current amplitudes. Ifenprodil and memantine markedly reduced the $K_v1.3$ currents of B cells, irrespective of whether they were stimulated with α-IgM or LPS (**Figure 14 A,B**). IC_{50} and Hill slope values for α-IgM-activated B cells were ~20 µM and ~1.3 for ifenprodil and ~40 µM and ~1.8 for memantine. For LPS-activated B cells, IC_{50} and Hill slope values were ~18 µM and ~1.4 for ifenprodil and ~45 µM and ~1.2 for memantine. $K_{Ca}3.1$ channel-mediated currents were also recorded for B cells activated by BCR ligation (**Figure 14B**), whereas $K_{Ca}3.1$ currents were not detected in LPS-stimulated B cells. IC_{50} values for ifenprodil and memantine were ~30 µM and ~50 µM and Hill slopes were ~1.4 and ~1.6. Notably, the competitive NMDAR antagonist D-APV, which blocks neuronal NMDARs at the 1 µM range, had no effect on $K_v1.3$ and $K_{Ca}3.1$ channels, even at 300 µM (**Figure 14C**). Thus, $K_v1.3$ and $K_{Ca}3.1$ channels, whose specific blockade abolishes B-cell activation (Amigorena et al. 1990; Wulff et al. 2004), are at least partially inhibited by the non-competitive NMDAR antagonists ifenprodil and memantine.

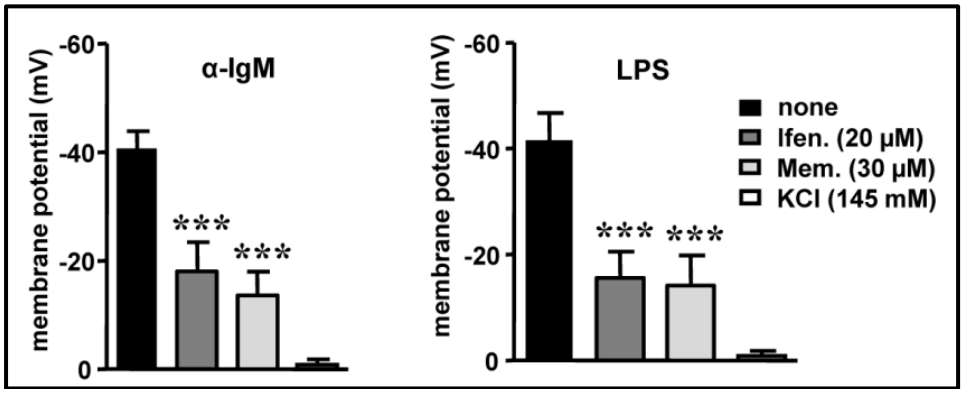


Figure 13. NMDAR antagonists reduce the B cell membrane potential. B cells were activated with α-IgM or LPS (10 µg/ml each) for 24-48 h and analyzed by current-clamp recordings for changes membrane potential upon addition of ifenprodil and memantine in concentrations as indicated. KCl treatment served as a positive control for checking the cell integrity. Each data point contains data from 5-6 cells of four experiments.

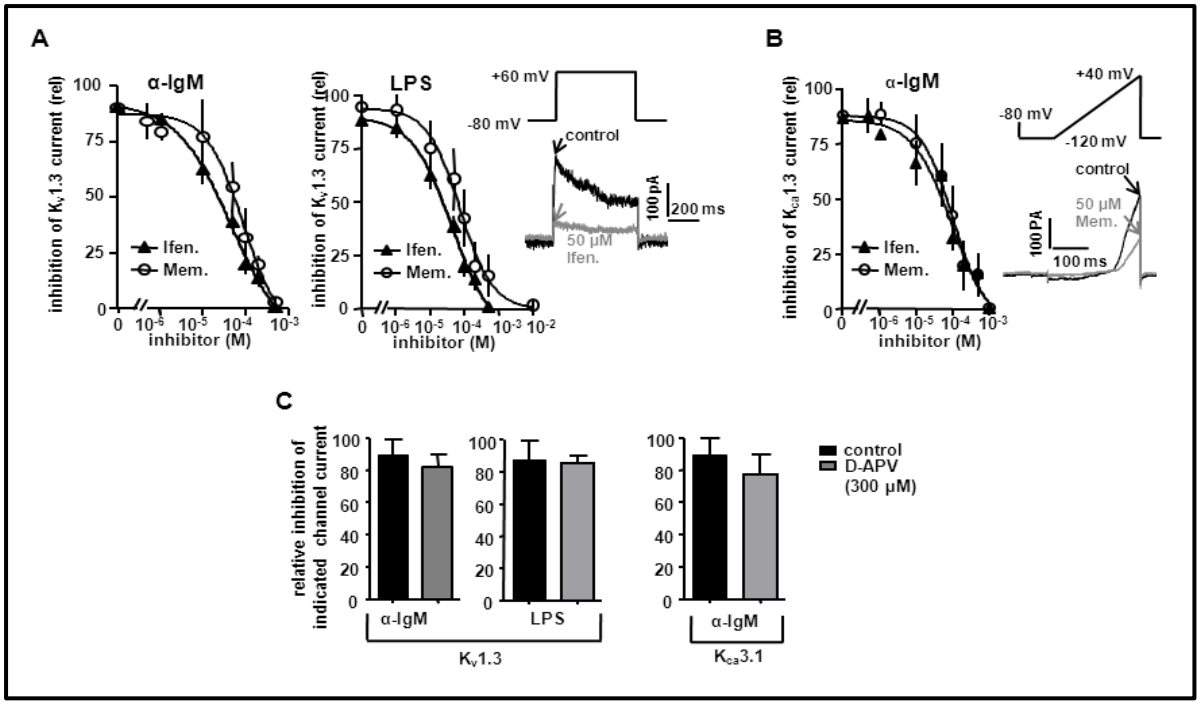


Figure 14. NMDAR antagonists lower K^+-channel activity of B cells. Splenic B cells, activated with α-IgM or LPS, were recorded by voltage-clamp to measure K_v1.3 and K_{Ca}3.1 currents. Dose-inhibition curves of K_v1.3 in (A) and K_{Ca}3.1 in (B) were plotted in the presence of Ifen. or Mem. from the recorded maximal transient currents. Insets show one particular trace of control and inhibited current along with the protocols used for measuring K_v1.3- and K_{Ca}3.1-mediated currents. Data in the graphs in (C) represent the relative inhibition of K_v1.3- and K_{Ca}3.1-mediated currents in the presence of the competitive NMDAR antagonist D-APV in a concentration ~10 times higher than used for the other inhibitors. All data were calculated from 5-6 cells of four experiments.

5.3.3 Human Jurkat T- and Raji B-Lymphoma Cells

As ~2-fold increased plasma Glu levels act as a prognostic marker in certain cancers (e.g. breast cancer and colorectal carcinoma) (Ollenschlager et al. 1989) and the expression of GluN1 subunits seems to correlate with the prognosis of other types of cancers like oral squamous cell carcinoma and retinoblastoma (Choi et al. 2004; Stepulak et al. 2009), it was also interesting to analyze Jurkat and Raji human lymphoma cells. The question was whether lymphoma cells would express NMDARs induced by plasma Glu or whether NMDAR inhibitors would modulate K^+-channels as found for primary murine T and B cells.

5.3.3.1 Jurkat T-Lymphoma Cells

At first, the membrane potential was determined by current clamp analysis. The membrane potential of Jurkat cells was reduced from ~-50 mV to ~-25 mV and ~-20 mV in the presence of ifenprodil (30 µM) and memantine (50 µM), respectively, as shown in **Figure 15**.

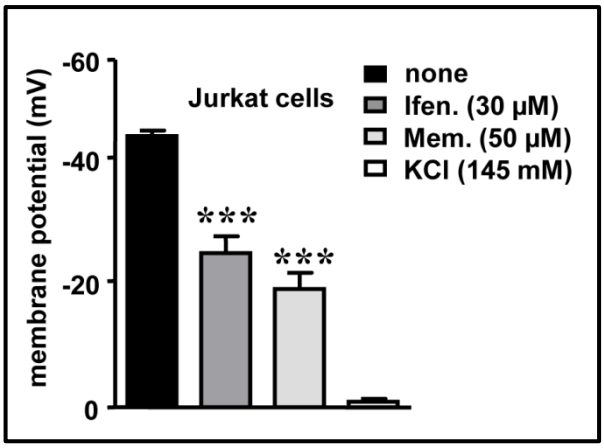


Figure 15. NMDAR antagonists change the membrane potential of Jurkat cells. Human Jurkat T-lymphoma cells were analysed by current clamp for determining the membrane potential. In the presence of Ifen. or Mem., in concentrations as indicated, the membrane potential was reduced. KCl treatment served as a positive control for cell integrity. All data were calculated from 5-6 cells of three experiments and are represented as mean ± SEM.

Next, the recorded currents (I) were plotted versus the used voltage (V) potentials. **Figure 16A** displays the I-V relationship measured at potentials ranging from -80 mV to 60 mV (in 20 mV steps) in control and in presence of ifenprodil (30 µM). The activation of $K_v1.3$ channel differs distinctly in presence of ifenprodil. **Figure 16B** shows the mean current densities (in pA/pF) calculated as maximal current (I_{max}) divided by the cell capacitance (C_{slow}). With this calculation, standardization of the current to the cell size is achieved. Under control condition, $K_v1.3$ value was ~150 pA/pF and it was decreased to ~70 pA/pF in the presence of ifenprodil (30 µM).

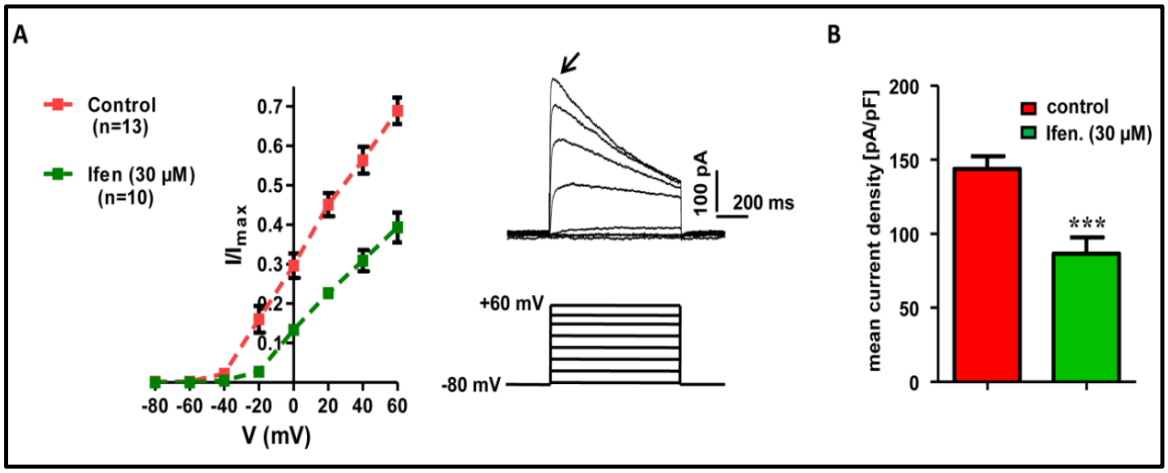


Figure 16. Comparison of recorded $K_v1.3$ current properties between control and Ifen.-treated (30 µM) Jurkat cells. Jurkat cells were patched with the protocol shown in (A, right side) and the maximal transient current amplitude (shown by the arrow) from recorded $K_v1.3$ currents was computed to determine I-V relationships in case of control and Ifen. from 10-13 cells. The recorded currents were normalized to the respective maximal current (arrow in the inset represents I_{max}) to determine the mean current densities as shown in (B). Mean current densities were determined by dividing mean I_{max} with C_{slow} in presence of control or Ifen. (30 µM).

Finally, the major K^+-channels ($K_v1.3$ and $K_{Ca}3.1$) taking part in the depolarizing events of immune cells were shown to inhibit in Jurkat cells in the presence of the tested NMDAR inhibitors ifenprodil and memantine (**Figure 17**). The $K_v1.3$ and $K_{Ca}3.1$ measuring voltage-clamp protocols were mentioned in **Figure 17A** and **Figure 17C** respectively. Furthermore, the current traces measured from this population were depicted here on the lower part of the **Figure 17A** and 17C. As a result, the Hill slope and IC_{50} values for $K_v1.3$ in presence of ifenprodil and memantine were ~2, ~1.5 and ~30 µM, ~45 µM. For $K_{Ca}3.1$, channel Hill slope and IC_{50} values were ~1.2 and ~20 µM for ifenprodil and ~1.4 and ~30 µM for memantine.

In addition to these inhibitors mentioned in **Figure 17**, MK801, ketamine and D-APV were also tested. The constructed curves gave Hill slopes and IC_{50} values for $K_v1.3$ current are respectively ~1.5, ~1.3, ~1.7 and ~70 µM, ~200 µM, ~550 µM for MK801, ketamine and D-APV as mentioned in **Figure 18**. D-APV, a competitive antagonist in case of Jurkat cells needed ~10-fold more concentration to be inhibited in comparison to the other non-competitive inhibitors like ifenprodil and memantine as like murine cells shown before.

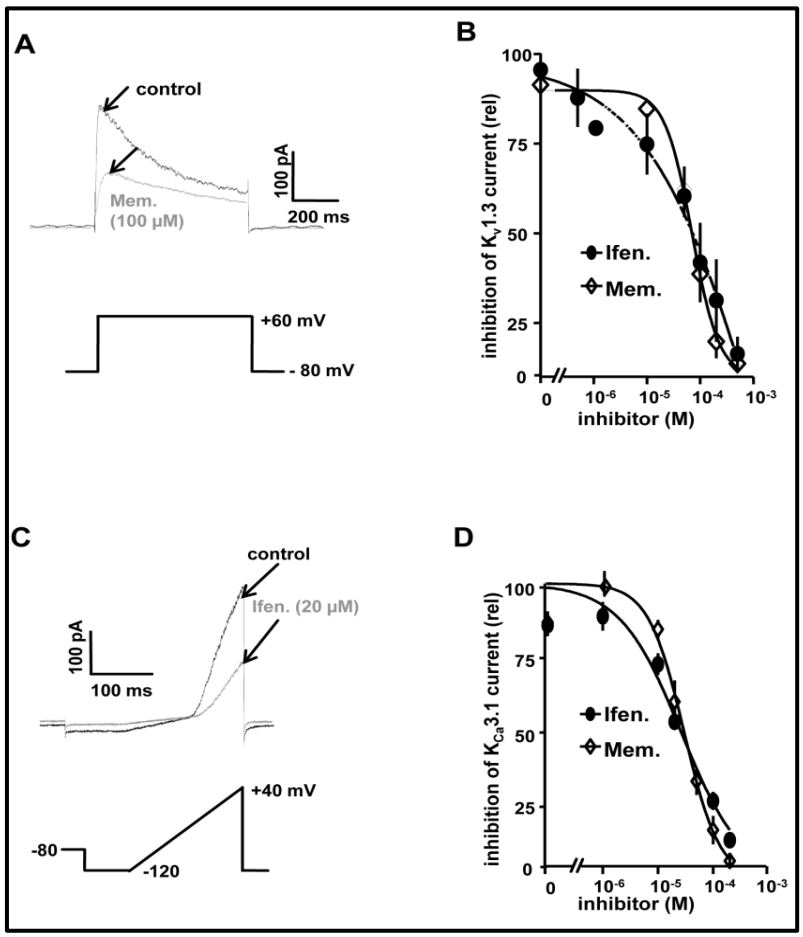


Figure 17.

Electrophysiological analysis in Jurkat lymphoma T cells in the presence of Ifen. and Mem. The Voltage clamp protocol for $K_v1.3$ channel recording and current traces are shown in (A) and the graphs formed by plotting the transient amplitude of the recorded currents versus the indicated inhibitor concentrations are shown in (B). The ramp protocol for $K_{Ca}3.1$-recorded currents and example traces are shown in (C) and the dose-response inhibition curves in (D). Each data point in the dose-response curves represents the mean ± SEM calculated from 5-7 cells.

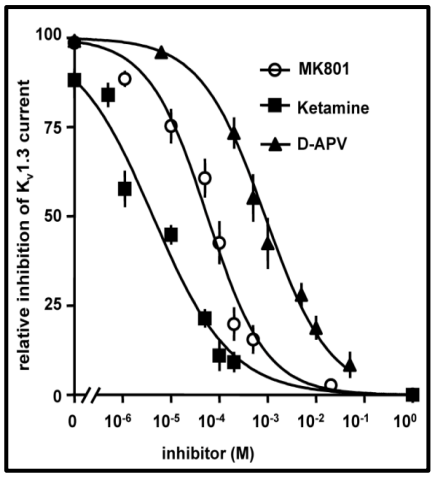


Figure 18. K_v1.3 current recorded from Jurkat cell lines in presence of mentioned inhibitors. K_v1.3 currents from Jurkat cells in the presence of MK801, ketamine and D-APV were recorded according to the voltage-clamp protocol shown in Figure 17A and plotted with the measured maximal amplitude values versus the inhibitor concentrations. Data represent mean ± SEM and each vertical line represents 5-7 cells from 4 experiments.

A very interesting aspect in terms of channel physiology was observed with the two inhibitors ifenprodil and memantine: K_v1.3-mediated tonic currents were also modulated, along with the transient currents (**Figure 19**). The inactivation constant (τ1) for the control population was ~20 ms measured with the 1-exponential model (HEKA FitMaster Program). This was increased to ~60 ms and to ~80 ms in the presence of ifenprodil and memantine, respectively. Thus, these inhibitors increase the inactivation kinetics similar to the other traditional K^+-channel blockers like Tetra Ethyl Ammonium (TEA) or 4-Amino Pyridine (4-AP) (Leung 2012). Thus, it will be interesting to determine whether these inhibitors act at the same amino acid modulating sites as the other K^+-channel blockers. The K_v1.3 channel behavior of Jurkat cells in the presence of different NMDAR inhibitors is summarized in **Table 1**.

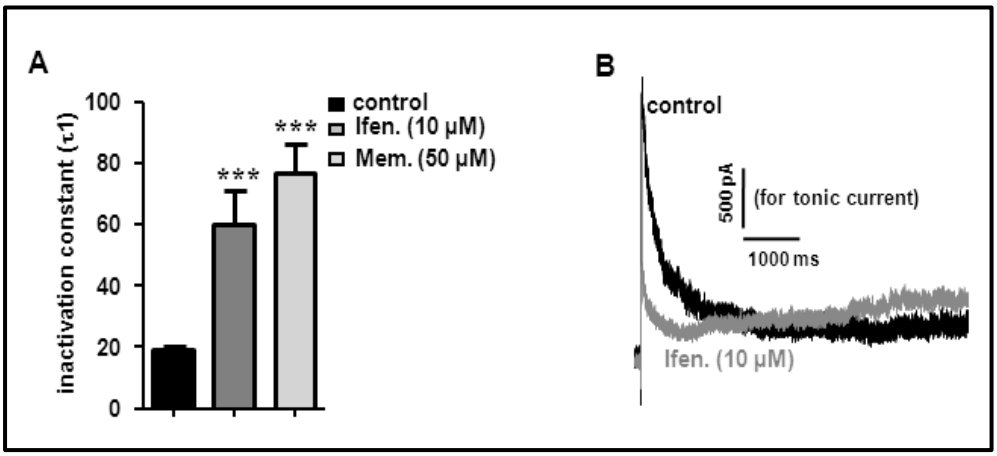


Figure 19. Inactivation current differences in K_v1.3-mediated currents in control and inhibitor population. Steady-state current were differed in case of Ifen. and Mem. as shown in Figure (A) that the τ1 was increased in presence of Ifen. (10 µM) and Mem. (50 µM). For the inactivation protocol, the currents were recorded with a slow ramp of 1 s (as shown in the insets in B). The recorded current traces were shown in (B). The current traces were recorded in HEKA PatchMaster and traces

were developed in IgorPro 5.04B program. Data represent 7-8 cells in each case and mean mean ± SEM.

Table 1: K_v1.3 Channel Behaviour in Jurkat Cells in Presence of the Tested NMDAR Inhibitors

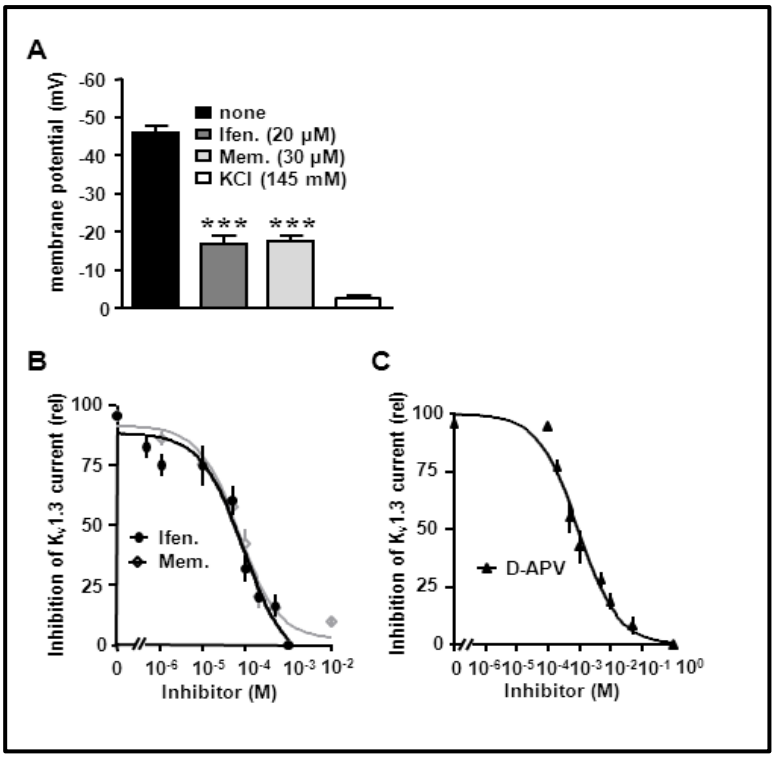
features	ifenprodil	MK801	memantine	ketamine	D-APV
Hill slope	~2	~1.5	~1.4	~1.3	~1.7
IC_{50}(µm)	~30	~70	~45	~200	~550
effect on transient current	yes	yes	yes	yes	yes, only at higher conc.
effect on tonic current	only in low concentration	no	at every concentration (from low-to-high)	no	no
mode of action	non-competitive, GluN2B blocker	uncompetitive, open-channel blocker	uncompetitive, open-channel blocker	uncompetitive and allosteric binding site blocker	competitively inhibits Glu -binding site of NMDARs

5.3.3.2 Raji B-Lymphoma Cells

In parallel to Jurkat T cells, the effects of NMDAR antagonists were analyzed on Raji B-lymphoma cells. For Daudi B-lymphoma cells, it was shown that the B cell lymphoma drug Rituximab can work through K_v1.3 channels (Wang et al. 2012). Thus, modulation of K_v1.3-mediated currents in Raji cells through NMDAR inhibitors would suggest that these drugs can potentially act as an adjuvans in treating B-cell lymphoma.

The membrane potential of Raji cells was found to be reduced from ~-45 mV to ~-20 mV in the presence of ifenprodil (20 µM) and memantine (30 µM) (**Figure 20A**). K_v1.3 currents were recorded from Raji cells in the presence of ifenprodil and memantine (**Figure 20B**) and the competitive blocker D-APV (**Figure 20C**). The IC_{50} and Hill slopes values for ifenprodil, memantine and D-APV were ~25 µM, ~40 µM and ~700 µM and ~1.4, ~1.2 and ~1.7, respectively. Thus, the competitive antagonist D-APV only inhibits at a much higher concentration than the open channel and uncompetitive blockers tested. This strongly suggests that NMDAR inhibitors do not act via NMDARs potentially expressed on (T and B) lymphoma cells but most likely through cross-inhibition of K_v1.3-channels.

Figure 20. Effects of NMDAR antagonists on the membrane potential and K^+-channel activity of Raji B-lymphoma cells. (A) NMDAR antagonists Ifen. and Mem. induce a membrane depolarization as recorded by current-clamp. $K_v1.3$ currents were measured by voltage-clamp in the presence of non-competitive Ifen., uncompetitive Mem. (B) and competitive NMDAR antagonist D-APV (C). Dose-response curves for $K_v1.3$ currents were plotted from the recorded maximal transient currents. All data were calculated from 5-6 cells of four experiments and are represented as mean ± SEM.



5.3.4 Primary Human T Cells from Healthy Donors

Finally, I analysed primary human T cells isolated from peripheral blood of healthy donors. Isolated $CD4^+$ T cells were left untreated or were activated with CD3 Ab (MEM-92) and patched for recording $K_v1.3$ current. It was observed that stimulated T cells needed a higher concentration of antagonist memantine to be inhibited to the same degree as unstimulated T cells. As a result, IC_{50} and Hill slope values are ~20µM, ~40 µM and ~1.2 and ~1.6 for unstimulated and stimulated populations, respectively as shown in **Figure 21A**.

In addition, $CD4^+$ cells isolated from young (20-30 years) and old donors (55-80 years) were patched to verify the differential expression of $K_v1.3$ channels on T cell populations of younger and aged persons as already indicated in the literature before by others (Den Braber I, et al. 2012). Interestingly, it is shown that the expression of $K_v1.3$ was ~20% less in T cells of older population from the younger ones as shown in **Figure 21B**.

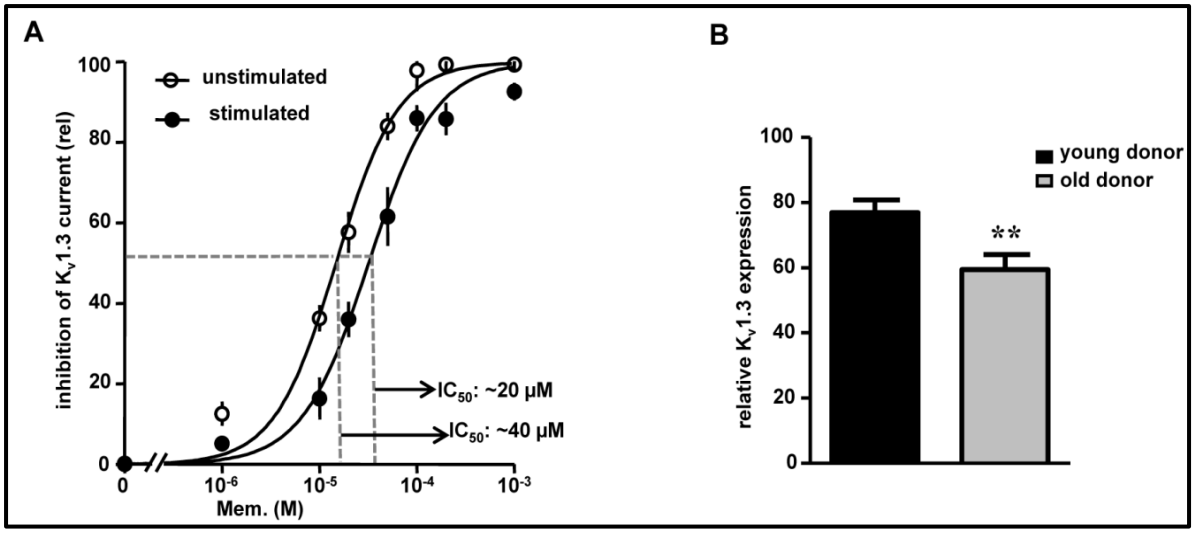


Figure 21. Electrophysiological analysis of primary human T cells. $CD4^+$ T cells from blood of normal healthy human donors, unstimulated or stimulated with MEM-92 Ab, were patched in the presence or absence of Mem. to record $K_v1.3$-mediated currents according to the protocol described in Figure 17A. The amplitude of transient currents was plotted against the inhibitor concentrations to obtain dose-response inhibition curves as shown in (A). IC_{50} values are indicated. The data in (B) represent $K_v1.3$ currents expression recorded from $CD4^+$ T cells isolated from young and old donors. Data represent the mean ± SEM from 4-5 cells recorded from 5-6 cell isolations.

5.4 $K_v1.3$ Channel Expression on Blood T cells from Dementia Patients

Since NMDAR antagonist memantine affected $K_v1.3$ channel activity in primary human T cells, it was highly interesting to investigate how the drug influences T cells of patients diagnosed with Alzheimer's disease, who were treated with memantine. Z1, Z2 and Z3 patients were designated as untreated, treated for 1 week or for 12 weeks with memantine, respectively. The patients were on medication of 10 mg of memantine twice a day.

5.4.1 Altered $K_v1.3$ Channel Expression in Alzheimer's Patients

To analyse whether NMDAR-low-affine inhibitor memantine applied *in vivo* has effects on $K_v1.3$-channels, $CD4^+$ T cells were isolated from the blood of Z1, Z2 and Z3 Alzheimer patients and $K_v1.3$ currents were recorded. Interestingly, in Z2 patients, who had been treated with memantine for only 1 week, the $K_v1.3$ current of T cells was strongly reduced by ~50%. In Z3 patients, the average amplitude values (70%) had returned to the values of untreated patients (77%). Thus, *in vivo* application of memantine, at least transiently, will have a strong effect on $K_v1.3$-channel activity of T cells and, thereby, may alter the activation/differentiation processes of T cells.

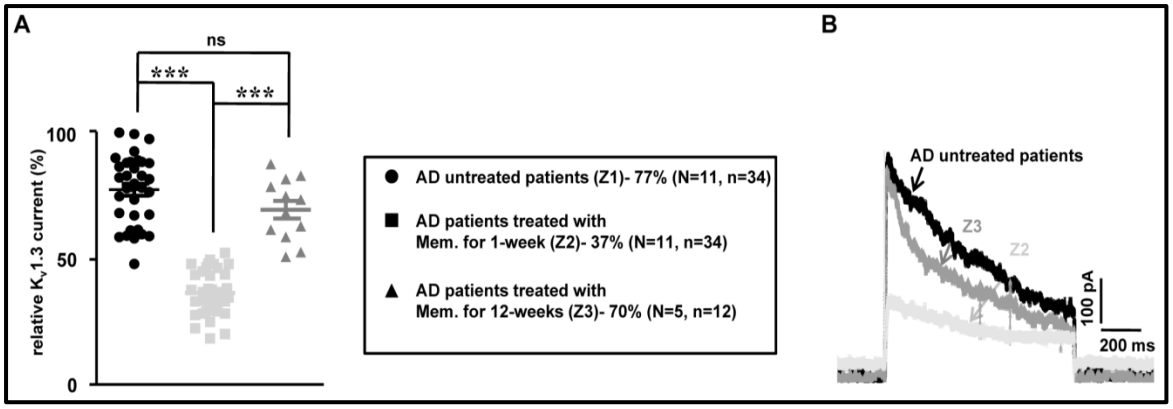


Figure 22. Memantine *in vivo* treatment affects K_v1.3-channel activity of T cells. $CD4^+$ T cells isolated from blood of Alzheimer patients before (Z1) and after the treatment with Mem. for 1 week (Z2) and 12 weeks (Z3) were recorded for measuring the K_v1.3 channel activity. The recorded transient current traces shown in (B) were normalized to the highest recorded amplitude values (set as 100%) and are shown in (A) as mean $\pm$ SEM. The recorded single cells (n) from the number of patients (N) are indicated in the inset.

6. Discussion

6.1 The Expression of Functional NMDARs in Immune Cells is Not Feasible

Several reports have described the presence of NMDARs in immune cells as briefly described in **section 3.7**. As the concentration of Glu in blood is higher than in the CSF, scientists since years have presumed the presence of Glu receptors in immune cells and a role for Glu in regulating immune function. Excited by these observations, we analyzed the function of T and B lymphocytes in the presence of different NMDAR inhibitors used to prove the functional impact of NMDARs in neurons and immune cells (Xiao et al. 2004; Andine et al. 1999). In our collaborative project, we showed that several NMDAR inhibitors reduced antigen-specific T-cell proliferation, cytotoxicity of $CD8^+$ T cells, the migration of lymphocytes towards chemokines and TCR-induced signalling (Kahlfuss et al. 2014). However, the presumptive protein expression of NMDARs in T cells was inconclusive due to the lack of electrophysiological recordings of these receptors and detection of protein bands of the obligatory GluN1 subunit at the exact size (~120 kD) as found in brain protein lysates. The first observation was in line with what was mentioned in the publication by Affaticati (Affaticati et al. 2011*)* and there is no further literature showing the presence of this receptor in lymphocytes at the electrophysiological level.

However, we detected NMDAR expression at the mRNA level (Kahlfuss et al. 2014) and through Ca^{2+}-influx after application of external NMDA (**Figure 6**). There can be at least two explanations for the described NMDA-induced Ca^{2+}-influx. Firstly, NMDAR expression could be very transient, for instance in stress conditions, as the NMDA-induced Ca^{2+}-influx was only observed in HBSS buffer, but not in the cell culture medium RPMI-1640. In connection with this hypothesis, it was observed that GluN1 mRNA expression is increased in the medulla and cortical region of the adrenal gland 24 h after stress exposure (Pirnik et al. 2001). Secondly, NMDA as reagent could cross-react with the pore forming subunit of K^+-channels and, thereby, induce Ca^{2+}-influx in T and B cells. Quantitative RT-PCR analysis should be applied to more clearly determine the differences in NMDAR expression under different cellular conditions.

6.2 NMDAR Inhibitors can be Enlisted in the Therapeutic Arena of K^+-channels of Immune cells

As there was no GluN1 protein expression detectable in wild-type thymocytes when compared with GluN1 ko thymocytes (Kahlfuss et al. 2014), we thought of candidates that could be modulated by the used NMDAR inhibitors. There are a number of ion channels having a role in maintaining the membrane potential of an immune cell as described in **section 3.3.3**.

We have chosen $K_v1.3$- and $K_{Ca}3.1$-channels because of their similar structure with NMDAR subunits and their well-described conductive and gating properties. In addition, there is a similarity between the pore-forming subunit of $K_v1.3$- and $K_{Ca}3.1$-channels between S5 and S6 (as illustrated in **Figure 2A**) and the binding sites between the obligatory GluN1 and GluN2A/B/C subunits of NMDARs, as illustrated in **Figure 4**. Thus, there is a chance of overlap-binding of NMDAR antagonists to these K^+-channels. Inspired by this fact, we measured the membrane potential by current-clamp and recorded $K_v1.3$- and $K_{Ca}3.1$-currents by voltage-clamp method in murine and human lymphocytes. We detected strong inhibitory effects of both non-competitive and uncompetitive NMDAR inhibitors on the overall membrane potential and on the two described K^+-channels of immune cells. My experiments spanned from mouse and human primary T cells to mouse (EL-4) and human (Jurkat) lymphoma cells as shown in **Figure 10-12, 15, 17, 18,** and **21** and primary B-cells and Raji lymphoma B-cells depicted in **Figure 14-15, 20**.

As shown in the following **Table 2**, inhibition of $K_v1.3$- and $K_{Ca}3.1$-channels leads to the amelioration several immune disorders, ranging from transplantation rejection, leukaemia to autoimmune disorders like colitis, Rheumatoid Arthritis and asthma. Thus, based on our results, NMDAR inhibitors can be added in the list K^+-channel inhibitors. As NMDAR antagonists are already in use to treat Alzheimer's disease and are promising candidates for therapy of various other neuronal diseases like Parkinson's disease, depression or stroke (Olivares et al. 2012), the use of these pharmaceuticals also necessitates thorough evaluation of their possible effects on lymphocytes. As NMDAR drugs are administered orally in case of memantine, they will at minimum affect blood lymphocytes while on their way to the brain.

We speculate that NMDAR inhibitors also have side effects on other ion channels. In this respect, specfically the two-pore gated K^+-channels should be studied as they have two-pore forming subunits (P1 and P2 pores as shown in **Figure 2C**), which can simulate the NMDAR tetrameric structure. They are 'background' K^+-channels playing a crucial role in setting the resting membrane potential and regulating cell excitability. They are activated by physical and chemical factors (e.g. lipid, pH, heat, and volatile anaesthetics) and are involved in many physiological processes (Kim 2005). Furthermore, they always remain open, are affected even at low membrane potential and regulate lymphocyte osmotic volume (Bobak et al. 2011; Andronic et al. 2013), apoptosis and tumour genesis (Williams et al. 2013; Patel and Lazdunski 2004).

Table 2. The Disease Pathologies Related with $K_v1.3$ and $K_{Ca}3.1$ Channels

inhibition of ion channel	disease	references
$K_v1.3$	chronic lymphoid leukaemia treatment	Leanza et al. 2013
	allo-transplantation studies	Hautz et al. 2013
	inhibition of T_c cells	Hu et al. 2013
	Rheumatoid arthritis amelioration	Toldi et al. 2013
	experimental autoimmune encephalitis treatment	Li et al. 2012
	immunosuppression strategy in kidney allograft	Grgic et al. 2009b
$K_{Ca}3.1$	ischemia treatment improvement	Chen et al. 2011
	helping in lowering blood pressure	Damkjaer et al. 2012
	T-cell mediated colitis	Di et al. 2010
	renal fibrosis	Grgic et al. 2009a
	immunosuppression strategy in kidney allograft	Grgic et al. 2009b
	prevention of obliterative airway diseases	Hua et al. 2013
	blockage of pancreatic cell growth	Jager et al. 2004
	target for hypertension disease	Kohler et al. 2010
	target for Alzhimer's Disease	Maezawa et al. 2012
	limiting stenosis	Tharp et al. 2008
	blocking atherogenesis	Toyama et al. 2008
	human osteoclast shock recovery	Weskamp et al. 2000
	cardiovascular targets	Wulff and Kohler 2013
	RA amelioration	Toldi et al. 2013
	idiopathic pulmonary fibrosis	Roach et al. 2013
	prevention of allograft vasculopathy	Chen et al. 2013

	increases tumor cell killing	Koshy et al. 2013
	chronic asthma	Girodet et al. 2013

6.3 NMDAR Surface Expression under Tight Control

As discussed, there could be a transient expression of NMDARs in immune cells. It should be considered that even in neuronal cells the expression of NMDARs is hard to detect. In the neuronal synapse, only few NMDARs are expressed, often less than 10 per synapse (Racca et al. 2000). This suggests that few NMDARs expressed on the cell surface are functionally important. In addition, a single NMDAR (30-50 pS) conducts more Ca^{2+} than a single Stim-ORAI1 complex (0.024-0.4 pS) (Hogan et al. 2010; Feske 2007). Thus, the surface expression of NMDARs on immune cells should be under very tight control. In our experiments, we could not reliably detect NMDAR surface expression on live resting or activated T cells or on thymocytes with routine FACS staining and flow cytometry using two different GluN1 Abs (from Synaptic Systems and Alomone labs), which bind to extracellular epitopes of GluN1.

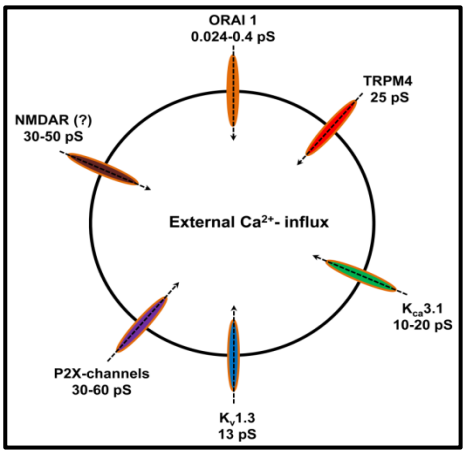


Figure 23. Differential conductivity levels of ion channels present in immune cells. The different ion channels present in immune cells have different conductive properties as indicated. $K_v1.3$- and $K_{Ca}3.1$-channels have more or less the same conductive properties, whereas ORAI1 complex has very less conductivity in comparison with other ion channels. Along with the conductive properties, they have differences in the gating and opening kinetics. NMDARs are hypothetically shown in the figure to illustrate the differences explained in the text.

In addition, experiments for over-expressing these receptors in immune cells using several transfection procedures were not successful in terms of transfection efficiency. Among the different transfection protocols used for transfecting immune cells, the Amaxa Nucleofector procedure was best in immune cells showing in ~5% of cells GluN1-GluN2B double transfection (GluN1-wt from *Paoletti, P.* and GluN2B-SEP from Addgene). Since these two constructs showed the best results among the different tested NMDAR constructs, but still had a very low transfection efficiency, the two constructs were subcloned into another vector, pXJ41, known to carry bigger inserts. Although the subcloning procedure worked with this vector, the transfection efficiency for NMDAR subunits in immune cells was still not improved. This indicates that lentiviral vectors might be needed, which are efficient for gene transfer into human T cells (Verhoeyen et al. 2009).

6.4 NMDAR Inhibitors Modulate the Steady-state Current of K_v1.3-channels

K_v1.3- and K_{Ca}3.1-channels are similar with regard to their conductance properties as both channels are highly selective and have similar single-channel conductance in the order of 10-14 pS as shown in **Figure 23**. However, they are remarkably different in their gating and blocker sensitivity. K_v1.3-channels are activated by depolarization of the plasma membrane. The activation threshold of the channel is close to the resting potential of the immune cells of ~-50 mV. This current is quickly activated and then inactivated by a slow C-type inactivation. On the contrary, K_{Ca}3.1-channels are solely activated by the increase of cytosolic Ca^{2+}-concentration and they are not deactivated after prolonged exposure to increased cytosolic Ca^{2+}-concentration (Cahalan and Chandy 2009; Panyi et al. 2006).

Ifenprodil blocks pre-synaptic L-type Ca^{2+} channels at a concentration of >10 µM (Delaney et al. 2012). We saw ifenprodil at lower concentration and memantine from low to high concentration has substantial effects on both the amplitude and steady-state parameters of K_v1.3-channel kinetics as described in **Table 1** for Jurkat cells. It was shown in **Figure 19** that ifenprodil and memantine have quantitative effects on the tonic current properties of Jurkat cells. This can hypothetically be simulated as shown **Figure 24**, where MK801 block in immune cells can easily be released as it is only affecting the amplitude of K_v1.3 channel transient current. In contrast, a block with ifenprodil is difficult to release immediately as the steady-state or tail current parameters are also affected along with the amplitude. Although, 20 min wash-out with the extracellular solution can reverse back the normal K_v1.3 current in an experimental situation.

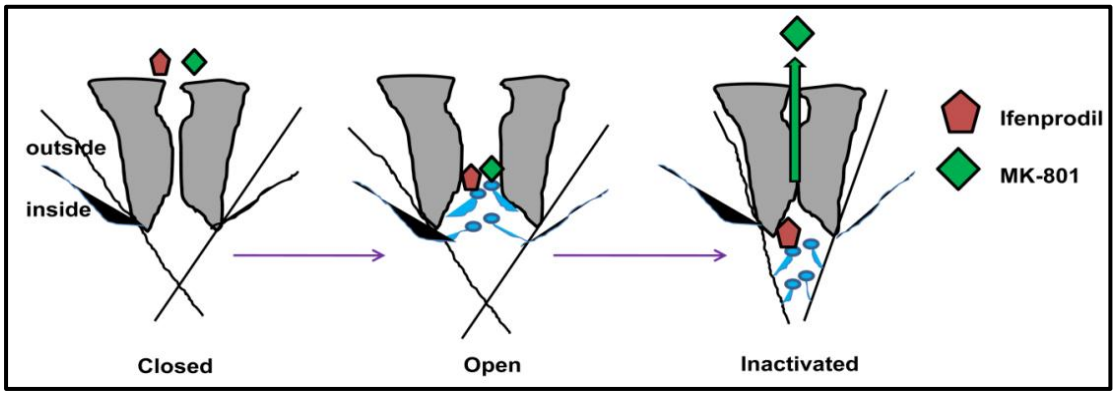


Figure 24. Hypothetical model for the regulation of K_v1.3-channel by ifenprodil and memantine. As explained in **section 3.6.1**, K_v1.3 generally follows C-type inactivation, which is characterized by a slow type of inactivation. Ifenprodil blocks the pore region so that the channel can not be inactivated anymore. A channel generally follows three steps of opening and closing: open, inactivated, deactivated and closed. It is easy to change an inactivated state of a channel to a closed and open stage, but it is hard to close a channel completely when it reaches a deactivated stage. In case of ifenprodil, K_v1.3 hypothetically reaches to a deactivated stage. Thus, this inhibitor is hard to re-

lease from the pore region of the bound channel. In contrast, MK801 block can easily be released as the state reached after its release is an inactivated stage (modified from *Barros, F., 2012)* (Barros et al. 2012).

The inactivation parameters of K_v1.3-channels are interesting to study in terms of K_v1.3-channel inhibitors. Increasing amount of evidence suggests that there are certain compounds which inhibit K_v-currents not directly by the K^+-conduction pathway (S4), but by accelerating the selectivity filter destabilization (between S5 and S6) once the channel opens (as illustrated in **Figure 25**). This mode represents an alternative mechanism of K_v-channel inhibition. Mutation of these amino acids to arginine, lysine, alanine or glutamate accelerates C-type inactivation, while mutation to valine or tyrosine residues retards it. In addition, the interaction via hydrogen bonds between Shaker 438 and 447 is important in controlling the C-type inactivation gate. However, how pivotal these residues determine the C-type inactivation rate is still unknown. Notably, the role of T449, a residue at the external mouth has been studied in details for this kind of inactivation (Cordero-Morales et al. 2011; Lopez-Barneo et al. 1993). The traditional K^+-channel blocker Tetra-ethyl ammonium (TEA) and 4-Aminopyridine (4-AP) have been shown to hasten the slow C-type inactivation. It was already shown that TEA possibly binds to T449 preventing the selectivity filter constriction in a 'foot-in-the-door' fashion (Leung 2012). Thus, it would be interesting to study whether the NMDAR inhibitors acting on the tonic current also modulate the T449 site like traditional K^+-channel blockers or any other site of the K_v1.3-channel unknown until now. This inactivity phenomenon of shaker K^+-channels by NMDAR antagonists could be very complex as they could also interplay with accessory β subunits as shown for K_v1.5- channels (Decher et al. 2005).

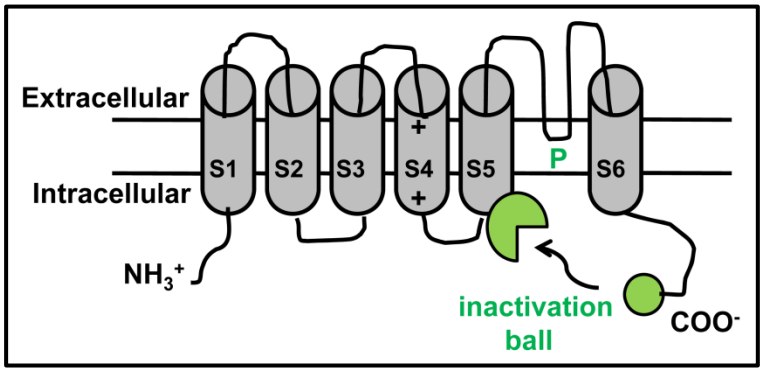


Figure 25. The K_v1.3-channel inactivation pathway. Shaker K^+-channels (K_v1.1-1.9) and K_{Ca} channels have a 6-transmembrane topology along with a one pore-forming subunit (forming the gate) and a charge-transfer subunit (S4). It is shown how the C-terminal is occluding the intracellular mouth of the ion-conducting pore in case of K_v1.3 inactivation (modified from *Buckley, C,2005* and *Barros, F., 2012)* (Barros et al. 2012; Buckley and Vincent 2005).

6.5 NMDAR Inhibitors Affect B-Cell Activation

Since there exist Abs against different ion channels present in neuronal cells as described in **Table 3**, it is clear that B cells take part in neuronal disorders like MS (Meinl et al. 2006; Ritchie et al. 2004). Therefore, we also analysed B-cell activation in the presence of different NMDAR inhibitors. Notably, they inhibited BCR-induced Ca^{2+}-flux as shown in **Figure 9**, lowered the total membrane potential and inhibited K^+-channels in activated primary murine B cells and Raji lymphoma cells (**Figure 13, 14, 20**).

In general, B cells contribute to neuronal disorders via four mechanisms: by production of Abs causing tissue damage either via complement activation or Ab-dependent-cell-mediated cytotoxicity; by acting as APCs for the expansion of cytotoxic T cells and Th cell cytokine production; by production of pro-inflammatory cytokines like IL-6 and TNF, which activate macrophages and T cells enhancing tissue damage; and by formation of ectopic germinal centres in the intermeningeal place in a process called neolymphogenesis (Dalakas 2008). In specific, Abs against NMDARs were detected in women with ovarian tumour, young children and men without tumour (Dalmau et al. 2007; Novillo-Lopez et al. 2008). The major symptoms in women were memory loss, seizures and decreased consciousness, whereas children and men showed a diffuse lymphocytic meningoencephalitis and acute juvenile non-herpetic encephalitis (Dale et al. 2009; Iizuka et al. 2008). The main epitope targeted by the Abs lies in the extracellular N-terminal domain of the GluN1 subunit (Dalmau et al. 2008). Thus, application of non-competitive NMDAR antagonists during chronic treatments of neurological disorders like Morbus Alzheimer may not only involve neuronal NMDARs, but may have additive side-effects by targeting B cells, in addition to T cells, which are assumed to contribute to these disorders (Yanaba et al. 2008; Danysz and Parsons 2012; Cunningham 2013; Frodl and Amico 2014). The inhibitory side-effects of NMDAR inhibitors on B cells might also be beneficial in treating sepsis (Roger et al. 2009) as we have seen a reduction in LPS/TLR4-induced B-cell signalling and proliferation (**Figure 9** and data not shown but in revision for publication) in the presence of memantine and other NMDAR inhibitors.

B cells express $K_v1.3$ and $K_{Ca}3.1$ channels and their mode of Ca^{2+}-influx is through Stim-ORAI complexes as in T cells. Blockade of these K^+-channels by specific K^+-channel inhibitors results in immunosuppression (Wulff et al. 2004; Matsumoto et al. 2011). The $K_v1.3$- and $K_{Ca}3.1$-specific blockers Shk and TRAM-34 are used in treating vasculopathy or kidney allograft rejection (Chen et al. 2013; Grgic et al. 2009a). $K_v1.3$ inhibitor Shk-186 had been started to use in human trial stage I for treating diseases by Kineta. In addition to BCR- and LPS-induced activation of primary B cells, we found that memantine reduced $K_v1.3$-channel currents recorded from Raji lymphoma cells (**Figure 20**). In this respect, memantine may

therapeutically compete with Rituximab, which has been shown to act through the inhibition of $K_v1.3$ channels in human B lymphoma cells (Wang et al. 2012). Further studies are required to determine the drug's suitability in diverse *in vivo* settings.

Table 3. Autoimmune Disorders or Abs Related with Neuronal Channels

channel	disease	references
voltage-gated Ca^{2+} channel	Lambert Eaton Myasthenic Syndrome (LEMS)	Pellkofer et al. 2008
Voltage-gated K^+ channel	Acquired Neuromyotonia, Morvan's Syndrome (MoS), Limbic Encephalitis (LE), Epilepsy	Hart et al. 1997; Liguori et al. 2001; Bataller et al. 2007; Dedek et al. 2001
α-3 ganglionic acetylcholine receptor	antibodies to α-3 AchR containing α3 and β4	Vernino et al. 2000
Glu and GABA receptors	Encephalitis associated with NMDAR, AMPAR and GABAR	Novillo-Lopez et al. 2008; Iizuka et al. 2008; Dale et al. 2009; Lai et al. 2009; Rogers et al. 1994; Lancaster et al. 2010
Aquaporin-4	Neuromyelitic Optica (NMO) or Devic's Syndrome	Lennon et al. 2005

6.6 Surveillance of Immune Status is Required during Memantine Treatment

A new therapeutic concept generally passes through a long journey of clinical trials to accomplish the final verification for using it in humans. NMDAR antagonists like memantine may have a wide range of potential therapeutic applications, spanning from acute neurodegeneration in stroke and trauma, chronic neurodegeneration in Morbus Parkinson and Alzheimer, amyotrophic lateral sclerosis, and Huntington's disease to symptomatic treatments in epilepsy, anxiety, or chronic pain (Parsons et al. 1995; Meldrum 1992a, b; Muller et al. 1996). The high affinity competitive inhibitor D-APV cannot be used *in viv*o as it impairs normal synaptic transmission creating serious side-effects. Therefore, the major challenge was to develop an antagonist that prevents the pathological activation of NMDARs but maintains their physiological activity. Memantine (1-amino-3, 5-dimethyl-adamantane) as an uncompetitive low-affinity inhibitor has fulfilled the criteria. It was registered in Germany for a variety of CNS indications in 1978, but its most likely therapeutic mechanism was only discovered 10 years later (Bormann 1989; Kornhuber et al. 1989; Kornhuber et al. 1991). It

was first synthesized by researchers at Eli Lilly company as an agent to lower blood sugar levels (Gerzon et al. 1963). But, since it is devoid of such activity, Merz & Co. applied for a patent in 1972 for the treatment of several cerebral disorders. Since then, several *in vivo* studies were performed to determine its effects and Merz filed an international application in 1989 claiming memantine as ideal for the treatment of cerebral ischemia and Alzheimer's dementia. Since then, clinical research has focused on the treatment of dementia (Parsons et al. 1999).

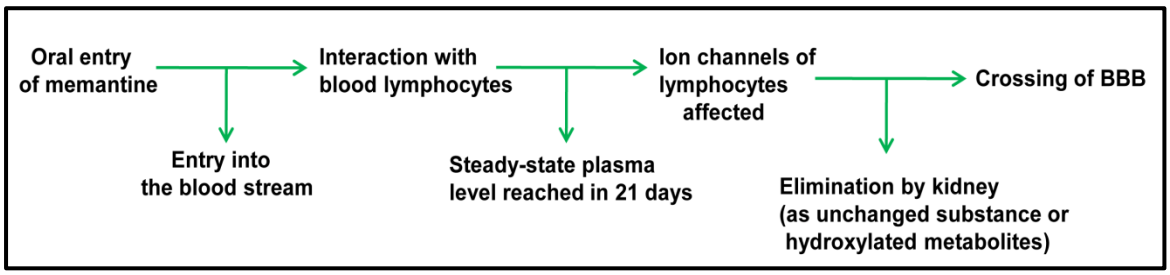


Figure 26. Scheme of memantine trafficking in the body. A schematic pathway of the traversing route of memantine when administered in patients. Memantine is mainly administered orally because of stability issues. It is taken up by the gut and passes into the blood stream where it can act on blood lymphocytes. Memantine then crosses the BBB, gets absorbed into the liver or is eliminated by the kidney as unchanged substance or hydroxylated metabolites. Bioavailability is thought to reach ~100% in case of this drug.

Memantine is prescribed orally as a tablet or solution. In our experiments, we have analysed T cells from patients who are taking a 10 mg tablet twice a day. We found that $K_v1.3$-mediated currents were lowered by 50% on T cells isolated from patients treated for 1 week with memantine, compared to T cells from those patients before drug treatment. $K_v1.3$-mediated currents were nearly normal in T cells from patients treated for 12 weeks with the drug. Orally uptaken memantine crosses the BBB to act on neurons and is metabolized in the liver through first-pass metabolism or is excreted through the kidney as an unchanged substance or hydroxylated metabolites. Thus, it is obvious that memantine could affect blood lymphocytes. No major side-effects of memantine on lymphocytes were reported until the Food and Drug Administration (FDA)'s October 2013 survey summary. This summary describes memantine's adverse reactions on blood cells and the lymphatic system as post-marketing drug exposure experience.

In this context, our results with the attenuation of $K_v1.3$-channel activity in Z2 patients is very striking. Although $K_v1.3$-channel activity was almost "normal" in Z3 patients, T cells from Z3 patients are non-reactive to stimulation (personal communication of unpublished results by T. Lowinus/U. Bommhardt).

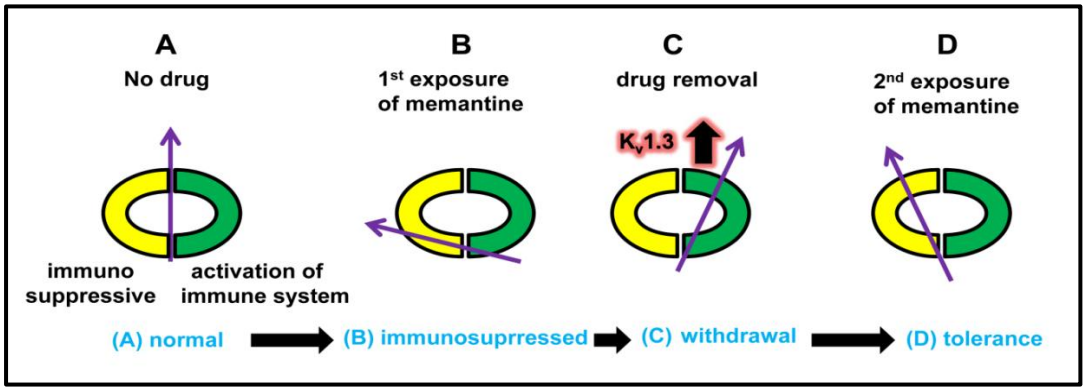


Figure 27. Hypothetical model of K_v1.3 counter-adaptive action upon memantine exposure. A hypothetical model to determine the action of K_v1.3 channels during first and second drug exposure. During first drug exposure the immune cells become suppressed, whereas during continued or second exposure lymphocytes regain their function through the increase of K_v1.3 channel density (*modified from Ghezzi, A.)* (Ghezzi et al. 2010).

The observed "re-expression" of K_v1.3 currents in Z3 patients thus may represent some counter-adaptive role of K_v-channels to resist to the drug's long-term exposure. Such counter-adaptive drug tolerance was described as triggering adaptive homeostatic changes that oppose the drug's effects to produce drug tolerance (Koob and Bloom 1988). Upon drug clearance, the same changes become counter-adaptive and produce symptoms of dependence. Our idea of memantine-induced K_v-channel tolerance can hypothetically be described by a model as illustrated in **Figure 27**. In this case, the immune system or lymphocytes become suppressed with the first exposure to memantine after 7 days (**Figure 27B**). Then, there is thought to be the induction of higher K_v1.3 transcription in the drug removal phase (**Figure 27C**). Thus, the immune system reaches to a tolerance level in the 2nd exposure of the drug after 12-weeks (**Figure 27D**). In addition, there should be a time point recorded in between these two extreme time points (1 and 12 weeks) to validate the hypothesis of increased channel expression described in **Figure 27C**. Furthermore, single channel current should be recorded to support our idea of K_v channel counter-adaptive effect.

6.7 Good News for Neurons

The physiological role of K^+-channels is evident in microglia, resident macrophages in CNS (Villalonga et al. 2010). Expression of K_v1.3 and K_{Ca}3.1 is similar in macrophages to peripheral T and B cells along with the Ca^{2+}-influx through CRAC channels. Microglia has dichotomous functions when activated, either causing neuronal damage by releasing cytotoxic substances and pro-inflammatory cytokines or being neuro-protective by releasing neurotrophic factors and clearing amyloid aggregates and debris from degenerated neurons

(McGeer and McGeer 2010; El Khoury and Luster 2008; Cameron and Landreth 2010; Hickman et al. 2008). $K_{Ca}3.1$-mediated Ca^{2+}-entry has been shown to be neurodegenerative in case of microglia when induced by amyloid aggregates causing oxidative burst, nitric oxide production, and neuronal death (Kaushal et al. 2007; Schilling et al. 2004; Khanna et al. 2001). Soluble Aβ oligomers (AβO) are small and early stage amyloid aggregates (Maezawa et al. 2011). In experimental conditions, AβO was found to initiate an increase of intracellular Ca^{2+} of microglia either directly by forming a membrane pore or indirectly through interaction with an unknown membrane receptor. This leads to a hyperpolarisation of the membrane potential by effluxing K^+ through $K_{Ca}3.1$ channel. This hyperpolarisation provides the driving force of Ca^{2+}-entry through store-operated inward-rectifier CRAC channels, thus sustaining the Ca^{2+}-signals necessary for selective Ca^{2+}-activated pathways (Maezawa et al. 2012). In this case, $K_{Ca}3.1$ channel inhibitors should be neuro-protective by deactivating the microglial functions involved in degenerating neurons. Indeed, the $K_{Ca}3.1$ inhibitor TRAM-34 inhibits AβO-induced microglial activation and microglia-mediated neuronal toxicity (Maezawa et al. 2011). In conclusion, if memantine would inhibit microglial $K_{Ca}3.1$ currents as well as pathologic NMDAR functions, the drug would have a double benefit for dementia patients. However, this interesting hypothesis needs further experimentation.

7. References

7.1 Books

Goldsby RA, Kindt TJ, Osborne BA, Kuby J (2003) Immunology. W. H. Freeman, NY.

Hille B (2001) Ionic Channels of Excitable Membranes. Sinauer Sunderland, MA.
Rouzaire-Dubois B, Benoit E, Dubois JM (2002) Ion channels and Physiopathologies of Nerve Conduction and Cell Proliferation. Research Signpost, IN.

Murphy K, Janeway CA Jr, Travers P, Walport M (2012) Janeway's Immunobiology. Garland Science, NY.

Nelson DL, Cox MM (2004) Lehninger Principles of Biochemistry. W.H. Freeman and Company, NY.

Kandel ER, Schwartz JH, Jessell TM (2000) Principles of Neural Science. McGraw-Hill, NY.

7.2 Journals

Abernethy DR, Schwartz JB (1999) Calcium-antagonist drugs. The New England journal of medicine 341 (19):1447-1457.

Adinolfi E, Callegari MG, Ferrari D, Bolognesi C, Minelli M, Wieckowski MR, Pinton P, Rizzuto R, Di Virgilio F (2005) Basal activation of the P2X7 ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth. Molecular biology of the cell 16 (7):3260-3272.

Affaticati P, Mignen O, Jambou F, Potier MC, Klingel-Schmitt I, Degrouard J, Peineau S, Gouadon E, Collingridge GL, Liblau R, Capiod T, Cohen-Kaminsky S (2011) Sustained calcium signalling and caspase-3 activation involve NMDA receptors in thymocytes in contact with dendritic cells. Cell death and differentiation 18 (1):99-108.

Amador M, Dani JA (1991) MK-801 inhibition of nicotinic acetylcholine receptor channels. Synapse 7 (3):207-215.

Amigorena S, Choquet D, Teillaud JL, Korn H, Fridman WH (1990) Ion channel blockers inhibit B cell activation at a precise stage of the G1 phase of the cell cycle. Possible involvement of K^+ channels. Journal of immunology 144 (6):2038-2045.

Andine P, Widermark N, Axelsson R, Nyberg G, Olofsson U, Martensson E, Sandberg M (1999) Characterization of MK-801-induced behavior as a putative rat model of psychosis. The Journal of pharmacology and experimental therapeutics 290 (3):1393-1408.

Andronic J, Bobak N, Bittner S, Ehling P, Kleinschnitz C, Herrmann AM, Zimmermann H, Sauer M, Wiendl H, Budde T, Meuth SG, Sukhorukov VL (2013) Identification of two-pore domain potassium channels as potent modulators of osmotic volume regulation in human T lymphocytes. Biochimica et biophysica acta 1828 (2):699-707.

Antonov SM, Johnson JW (1996) Voltage-dependent interaction of open-channel blocking molecules with gating of NMDA receptors in rat cortical neurons. The Journal of physiology 493 (Pt 2):425-445.

Aracava Y, Pereira EF, Maelicke A, Albuquerque EX (2005) Memantine blocks alpha7* nicotinic acetylcholine receptors more potently than n-methyl-D-aspartate receptors in rat hippocampal neurons. The Journal of pharmacology and experimental therapeutics 312 (3):1195-1205.

Barann M, Bonisch H, Urban BW, Gothert M (1998) Inhibition of 5-HT3 receptor cation channels by ifenprodil in excised patches of N1E-115 cells. Naunyn-Schmiedeberg's archives of pharmacology 358 (2):145-152.

Baricordi OR, Ferrari D, Melchiorri L, Chiozzi P, Hanau S, Chiari E, Rubini M, Di Virgilio F (1996) An ATP-activated channel is involved in mitogenic stimulation of human T lymphocytes. Blood 87 (2):682-690.

Barnden MJ, Allison J, Heath WR, Carbone FR (1998) Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. Immunology and cell biology 76 (1):34-40.

Barros F, Dominguez P, de la Pena P (2012) Cytoplasmic domains and voltage-dependent potassium channel gating. Frontiers in pharmacology 3:49.

Bataller L, Kleopa KA, Wu GF, Rossi JE, Rosenfeld MR, Dalmau J (2007) Autoimmune limbic encephalitis in 39 patients: immunophenotypes and outcomes. Journal of neurology, neurosurgery, and psychiatry 78 (4):381-385.

Beeton C, Wulff H, Barbaria J, Clot-Faybesse O, Pennington M, Bernard D, Cahalan MD, Chandy KG, Beraud E (2001) Selective blockade of T lymphocyte K^+ channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. Proceedings of the National Academy of Sciences of the United States of America 98 (24):13942-13947.

Beeton C, Wulff H, Standifer NE, Azam P, Mullen KM, Pennington MW, Kolski-Andreaco A, Wei E, Grino A, Counts DR, Wang PH, LeeHealey CJ, B SA, Sankaranarayanan A, Homerick D, Roeck WW, Tehranzadeh J, Stanhope KL, Zimin P, Havel PJ, Griffey S, Knaus HG, Nepom GT, Gutman GA, Calabresi PA, Chandy KG (2006) $K_v1.3$ channels are a therapeutic target for T cell-mediated autoimmune diseases. Proceedings of the National Academy of Sciences of the United States of America 103 (46):17414-17419.

Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH (2000) Antidepressant effects of ketamine in depressed patients. Biological psychiatry 47 (4):351-354.

Berridge MJ (1995) Calcium signalling and cell proliferation. BioEssays:news and reviews in molecular, cellular and developmental biology 17 (6):491-500.

Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. Nature reviews Molecular cell biology 1 (1):11-21.

Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM, Bockaert J (1993) Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. European journal of pharmacology 237 (1):45-50.

Bezanilla F (2008) How membrane proteins sense voltage. Nature reviews Molecular cell biology 9 (4):323-332.

Bianchi ME (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. Journal of leukocyte biology 81 (1):1-5.

Bobak N, Bittner S, Andronic J, Hartmann S, Muhlpfordt F, Schneider-Hohendorf T, Wolf K, Schmelter C, Gobel K, Meuth P, Zimmermann H, Doring F, Wischmeyer E, Budde T, Wiendl H, Meuth SG, Sukhorukov VL (2011) Volume regulation of murine T lymphocytes relies on voltage-dependent and two-pore domain potassium channels. Biochimica et biophysica acta 1808 (8):2036-2044.

Boldyrev AA, Kazey VI, Leinsoo TA, Mashkina AP, Tyulina OV, Johnson P, Tuneva JO, Chittur S, Carpenter DO (2004) Rodent lymphocytes express functionally active glutamate receptors. Biochemical and biophysical research communications 324 (1):133-139.

Bormann J (1989) Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. European journal of pharmacology 166 (3):591-592.

Bresink I, Benke TA, Collett VJ, Seal AJ, Parsons CG, Henley JM, Collingridge GL (1996) Effects of memantine on recombinant rat NMDA receptors expressed in HEK 293 cells. British journal of pharmacology 119 (2):195-204.

Buckley C, Vincent A (2005) Autoimmune channelopathies. Nature clinical practice Neurology 1 (1):22-33.

Cahalan MD (2009) STIMulating store-operated Ca^{2+} entry. Nature cell biology 11 (6):669-677.

Cahalan MD, Chandy KG (2009) The functional network of ion channels in T lymphocytes. Immunological reviews 231 (1):59-87.

Cahalan MD, Chandy KG, DeCoursey TE, Gupta S (1985) A voltage-gated potassium channel in human T lymphocytes. The Journal of physiology 358:197-237.

Cameron B, Landreth GE (2010) Inflammation, microglia, and Alzheimer's disease. Neurobiology of disease 37 (3):503-509.

Chandy KG, Wulff H, Beeton C, Pennington M, Gutman GA, Cahalan MD (2004) K^+ channels as targets for specific immunomodulation. Trends in pharmacological sciences 25 (5):280-289.

Chen HS, Lipton SA (1997) Mechanism of memantine block of NMDA-activated channels in rat retinal ganglion cells: uncompetitive antagonism. The Journal of physiology 499 (Pt 1):27-46.

Chen HS, Pellegrini JW, Aggarwal SK, Lei SZ, Warach S, Jensen FE, Lipton SA (1992) Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. The Journal of neuroscience:the official journal of the Society for Neuroscience 12 (11):4427-4436.

Chen YJ, Lam J, Gregory CR, Schrepfer S, Wulff H (2013) The Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ as a potential new target for the prevention of allograft vasculopathy. PloS one 8 (11):e81006.

Chen YJ, Raman G, Bodendiek S, O'Donnell ME, Wulff H (2011) The $K_{Ca}3.1$ blocker TRAM-34 reduces infarction and neurological deficit in a rat model of ischemia/reperfusion stroke. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism 31 (12):2363-2374.

Choi SW, Park SY, Hong SP, Pai H, Choi JY, Kim SG (2004) The expression of NMDA receptor 1 is associated with clinicopathological parameters and prognosis in the oral squamous cell carcinoma. Journal of oral pathology & medicine:official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology 33 (9):533-537.

Clapham DE, Runnels LW, Strubing C (2001) The TRP ion channel family. Nature reviews Neuroscience 2 (6):387-396.

Cognet L, Groc L, Lounis B, Choquet D (2006) Multiple routes for glutamate receptor trafficking: surface diffusion and membrane traffic cooperate to bring receptors to synapses. Science's STKE: signal transduction knowledge environment 2006 (327):pe13.

Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels. Neuropharmacology 56 (1):2-5.

Conforti L (2012) The ion channel network in T lymphocytes, a target for immunotherapy. Clin Immunol 142 (2):105-106.

Cordero-Morales JF, Jogini V, Chakrapani S, Perozo E (2011) A multipoint hydrogen-bond network underlying KcsA C-type inactivation. Biophysical journal 100 (10):2387-2393.

Crabtree GR (1999) Generic signals and specific outcomes: signaling through Ca^{2+}, calcineurin, and NF-AT. Cell 96 (5):611-614.

Cunningham C (2013) Microglia and neurodegeneration: the role of systemic inflammation. Glia 61 (1):71-90.

Daggett A, Yang XW (2013) Huntington's disease: easing the NMDAR traffic jam. Nature medicine 19 (8):971-973.

Dalakas MC (2008) B cells as therapeutic targets in autoimmune neurological disorders. Nature clinical practice Neurology 4 (10):557-567.

Dale RC, Irani SR, Brilot F, Pillai S, Webster R, Gill D, Lang B, Vincent A (2009) N-methyl-D-aspartate receptor antibodies in pediatric dyskinetic encephalitis lethargica. Annals of neurology 66 (5):704-709.

Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, Dessain SK, Rosenfeld MR, Balice-Gordon R, Lynch DR (2008) Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet neurology 7 (12):1091-1098.

Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR (2007) Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Annals of neurology 61 (1):25-36.

Damkjaer M, Nielsen G, Bodendiek S, Staehr M, Gramsbergen JB, de Wit C, Jensen BL, Simonsen U, Bie P, Wulff H, Kohler R (2012) Pharmacological activation of $K_{Ca}3.1$/$K_{Ca}2.3$ channels

produces endothelial hyperpolarization and lowers blood pressure in conscious dogs. British journal of pharmacology 165 (1):223-234.

Danysz W, Parsons CG (2003) The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer's disease: preclinical evidence. International journal of geriatric psychiatry 18 (Suppl 1):S23-32.

Danysz W, Parsons CG (2012) Alzheimer's disease, beta-amyloid, glutamate, NMDA receptors and memantine--searching for the connections. British journal of pharmacology 167 (2):324-352.

Davis CN, Mann E, Behrens MM, Gaidarova S, Rebek M, Rebek J, Jr., Bartfai T (2006) MyD88-dependent and -independent signaling by IL-1 in neurons probed by bifunctional Toll/IL-1 receptor domain/BB-loop mimetics. Proceedings of the National Academy of Sciences of the United States of America 103 (8):2953-2958.

Decher N, Kumar P, Gonzalez T, Renigunta V, Sanguinetti MC (2005) Structural basis for competition between drug binding and $K_v$beta 1.3 accessory subunit-induced N-type inactivation of K_v1.5 channels. Molecular pharmacology 68 (4):995-1005.

Dedek K, Kunath B, Kananura C, Reuner U, Jentsch TJ, Steinlein OK (2001) Myokymia and neonatal epilepsy caused by a mutation in the voltage sensor of the KCNQ2 K^+ channel. Proceedings of the National Academy of Sciences of the United States of America 98 (21):12272-12277.

DeHaven WI, Smyth JT, Boyles RR, Putney JW, Jr. (2007) Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. The Journal of biological chemistry 282 (24):17548-17556.

Delaney AJ, Power JM, Sah P (2012) Ifenprodil reduces excitatory synaptic transmission by blocking presynaptic P/Q type calcium channels. Journal of neurophysiology 107 (6):1571-1575.

Demo SD, Yellen G (1991) The inactivation gate of the Shaker K^+ channel behaves like an open-channel blocker. Neuron 7 (5):743-753.

Den Braber I, Mugwagwa T, Vrisekoop N, Westera L, Mögling R, Bregje de Boer A, Willems N, H.R. Schrijver E, Spierenburg G, Gaiser K, Mul E, Otto A.S., Ruiter F.C.A., Ackermans M.T., Miedema F, Borghans A.M. J, J. de Boer R, Tesselaar K (2012) MAintanance of peripheral naïve T cells is sustained by thymus output in mice but not humans. Immunity 36(2):288-297.

Desai R, Peretz A, Idelson H, Lazarovici P, Attali B (2000) Ca^{2+}-activated K^+ channels in human leukemic Jurkat T cells. Molecular cloning, biochemical and functional characterization. The Journal of biological chemistry 275 (51):39954-39963.

Di L, Srivastava S, Zhdanova O, Ding Y, Li Z, Wulff H, Lafaille M, Skolnik EY (2010) Inhibition of the K^+ channel K_{Ca}3.1 ameliorates T cell-mediated colitis. Proceedings of the National Academy of Sciences of the United States of America 107 (4):1541-1546.

Diem R, Hobom M, Grotsch P, Kramer B, Bahr M (2003) Interleukin-1 beta protects neurons via the interleukin-1 (IL-1) receptor-mediated Akt pathway and by IL-1 receptor-independent decrease of transmembrane currents in vivo. Molecular and cellular neurosciences 22 (4):487-500.

Dubyak GR (2004) Ion homeostasis, channels, and transporters: an update on cellular mechanisms. Advances in physiology education 28 (1-4):143-154.

El Khoury J, Luster AD (2008) Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications. Trends in pharmacological sciences 29 (12):626-632.

Fakler B, Adelman JP (2008) Control of K_{Ca} channels by calcium nano/microdomains. Neuron 59 (6):873-881.

Fanger CM, Neben AL, Cahalan MD (2000) Differential Ca^{2+} influx, K_{Ca} channel activity, and Ca^{2+} clearance distinguish Th1 and Th2 lymphocytes. Journal of immunology 164 (3):1153-1160.

Fanger CM, Rauer H, Neben AL, Miller MJ, Rauer H, Wulff H, Rosa JC, Ganellin CR, Chandy KG, Cahalan MD (2001) Calcium-activated potassium channels sustain calcium signaling in T lymphocytes. Selective blockers and manipulated channel expression levels. The Journal of biological chemistry 276 (15):12249-12256.

Fasth AE, Cao D, van Vollenhoven R, Trollmo C, Malmstrom V (2004) CD28nullCD4^{+} T cells-characterization of an effector memory T-cell population in patients with rheumatoid arthritis. Scandinavian journal of immunology 60 (1-2):199-208.

Feske S (2007) Calcium signalling in lymphocyte activation and disease. Nature reviews Immunology 7 (9):690-702.

Feske S (2009) ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca^{2+} entry in the immune system and beyond. Immunological reviews 231 (1):189-209.

Feske S (2013) Ca^{2+} influx in T cells: how many Ca^{2+} channels? Frontiers in immunology 4:99.

Feske S, Giltnane J, Dolmetsch R, Staudt LM, Rao A (2001) Gene regulation mediated by calcium signals in T lymphocytes. Nature immunology 2 (4):316-324.

Feske S, Picard C, Fischer A (2010) Immunodeficiency due to mutations in ORAI1 and STIM1. Clin Immunol 135 (2):169-182.

Feske S, Skolnik EY, Prakriya M (2012) Ion channels and transporters in lymphocyte function and immunity. Nature reviews Immunology 12 (7):532-547.

Finke C, Kopp UA, Scheel M, Pech LM, Soemmer C, Schlichting J, Leypoldt F, Brandt AU, Wuerfel J, Probst C, Ploner CJ, Pruss H, Paul F (2013) Functional and structural brain changes in anti-N-methyl-D-aspartate receptor encephalitis. Annals of neurology.

Friedrich M, Krammig S, Henze M, Docke WD, Sterry W, Asadullah K (2000) Flow cytometric characterization of lesional T cells in psoriasis: intracellular cytokine and surface antigen expression indicates an activated, memory/effector type 1 immunophenotype. Archives of dermatological research 292 (10):519-521.

Frodl T, Amico F (2014) Is there an association between peripheral immune markers and structural/functional neuroimaging findings? Progress in neuro-psychopharmacology & biological psychiatry 48:295-303.

Gadsby DC (2009) Ion channels versus ion pumps: the principal difference, in principle. Nature reviews Molecular cell biology 10 (5):344-352.

Ganor Y, Besser M, Ben-Zakay N, Unger T, Levite M (2003) Human T cells express a functional ionotropic glutamate receptor GluR3, and glutamate by itself triggers integrin-mediated adhesion to laminin and fibronectin and chemotactic migration. Journal of immunology 170 (8):4362-4372.

Gao YD, Hanley PJ, Rinne S, Zuzarte M, Daut J (2010) Calcium-activated K^{+} channel ($K_{Ca}3.1$) activity during Ca^{2+} store depletion and store-operated Ca^{2+} entry in human macrophages. Cell calcium 48 (1):19-27.

Gerzon K, Krumkalns EV, Brindle RL, Marshall FJ, Root MA (1963) The Adamantyl Group in Medicinal Agents. I. Hypoglycemic N-Arylsulfonyl-N'-Adamantylureas. Journal of medicinal chemistry 6:760-763.

Ghanshani S, Wulff H, Miller MJ, Rohm H, Neben A, Gutman GA, Cahalan MD, Chandy KG (2000) Up-regulation of the $IK_{Ca}1$ potassium channel during T-cell activation. Molecular mechanism and functional consequences. The Journal of biological chemistry 275 (47):37137-37149.

Ghezzi A, Pohl JB, Wang Y, Atkinson NS (2010) BK channels play a counter-adaptive role in drug tolerance and dependence. Proceedings of the National Academy of Sciences of the United States of America 107 (37):16360-16365.

Gibson FC, 3rd, Yumoto H, Takahashi Y, Chou HH, Genco CA (2006) Innate immune signaling and Porphyromonas gingivalis-accelerated atherosclerosis. Journal of dental research 85 (2):106-121.

Gilhar A, Bergman R, Assay B, Ullmann Y, Etzioni A (2011) The beneficial effect of blocking $K_v1.3$ in the psoriasiform SCID mouse model. The Journal of investigative dermatology 131 (1):118-124.

Gouaux E, Mackinnon R (2005) Principles of selective ion transport in channels and pumps. Science 310 (5753):1461-1465.

Grgic I, Kiss E, Kaistha BP, Busch C, Kloss M, Sautter J, Muller A, Kaistha A, Schmidt C, Raman G, Wulff H, Strutz F, Grone HJ, Kohler R, Hoyer J (2009a) Renal fibrosis is attenuated by targeted disruption of $K_{Ca}3.1$ potassium channels. Proceedings of the National Academy of Sciences of the United States of America 106 (34):14518-14523.

Grgic I, Wulff H, Eichler I, Flothmann C, Kohler R, Hoyer J (2009b) Blockade of T-lymphocyte $K_{Ca}3.1$ and $K_v1.3$ channels as novel immunosuppression strategy to prevent kidney allograft rejection. Transplantation proceedings 41 (6):2601-2606.

Groc L, Heine M, Cousins SL, Stephenson FA, Lounis B, Cognet L, Choquet D (2006) NMDA receptor surface mobility depends on NR2A-2B subunits. Proceedings of the National Academy of Sciences of the United States of America 103 (49):18769-18774.

Hannaert-Merah Z, Combettes L, Coquil JF, Swillens S, Mauger JP, Claret M, Champeil P (1995) Characterization of the co-agonist effects of strontium and calcium on myo-inositol trisphosphate-dependent ion fluxes in cerebellar microsomes. Cell calcium 18 (5):390-399.

Harmar AJ, Hills RA, Rosser EM, Jones M, Buneman OP, Dunbar DR, Greenhill SD, Hale VA, Sharman JL, Bonner TI, Catterall WA, Davenport AP, Delagrange P, Dollery CT, Foord SM, Gutman GA, Laudet V, Neubig RR, Ohlstein EH, Olsen RW, Peters J, Pin JP, Ruffolo RR, Searls DB, Wright MW, Spedding M (2009) IUPHAR-DB: the IUPHAR database of G protein-coupled receptors and ion channels. Nucleic acids research 37 (Database issue):D680-685.

Hart IK, Waters C, Vincent A, Newland C, Beeson D, Pongs O, Morris C, Newsom-Davis J (1997) Autoantibodies detected to expressed K^+ channels are implicated in neuromyotonia. Annals of neurology 41 (2):238-246.

Hautz T, Krapf C, Grahammer J, Zelger B, Hickethier T, Seger C, Eberhart N, Wallner C, Messner F, Kotsch K, Griesmacher A, Brandacher G, Lee WP, Margreiter R, Pratschke J, Glossmann H, Schneeberger S (2013) Targeting the $K_v1.3$ potassium channel for immunosuppression in vascularized composite allotransplantation- a pilot study. Transplant international: official journal of the European Society for Organ Transplantation 26 (5):552-561.

Hickenbottom SL, Grotta J (1998) Neuroprotective therapy. Seminars in neurology 18 (4):485-492.

Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. The Journal of neuroscience: the official journal of the Society for Neuroscience 28 (33):8354-8360.

Hinoi E, Takarada T, Ueshima T, Tsuchihashi Y, Yoneda Y (2004) Glutamate signaling in peripheral tissues. European journal of biochemistry/FEBS 271 (1):1-13.

Hitchcock IS, Skerry TM, Howard MR, Genever PG (2003) NMDA receptor-mediated regulation of human megakaryocytopoiesis. Blood 102 (4):1254-1259.

Hogan PG, Lewis RS, Rao A (2010) Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. Annual review of immunology 28:491-533.

Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR (1994) T cell receptor antagonist peptides induce positive selection. Cell 76 (1):17-27.

Hoshi T, Zagotta WN, Aldrich RW (1991) Two types of inactivation in Shaker K^+ channels:effects of alterations in the carboxy-terminal region. Neuron 7 (4):547-556.

Hoth M, Penner R (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 355 (6358):353-356.

Hu L, Wang T, Gocke AR, Nath A, Zhang H, Margolick JB, Whartenby KA, Calabresi PA (2013) Blockade of $K_v1.3$ potassium channels inhibits differentiation and granzyme B secretion of human $CD8^+$ T effector memory lymphocytes. PloS one 8 (1):e54267.

Hua X, Deuse T, Chen YJ, Wulff H, Stubbendorff M, Kohler R, Miura H, Langer F, Reichenspurner H, Robbins RC, Schrepfer S (2013) The potassium channel $K_{Ca}3.1$ as new therapeutic target for the prevention of obliterative airway disease. Transplantation 95 (2):285-292.

Huettner JE, Bean BP (1988) Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. Proceedings of the National Academy of Sciences of the United States of America 85 (4):1307-1311.

Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ (2000) Functional requirement for class I MHC in CNS development and plasticity. Science 290 (5499):2155-2159.

Iizuka T, Sakai F, Ide T, Monzen T, Yoshii S, Iigaya M, Suzuki K, Lynch DR, Suzuki N, Hata T, Dalmau J (2008) Anti-NMDA receptor encephalitis in Japan: long-term outcome without tumor removal. Neurology 70 (7):504-511.

Iravani MM, Muscat R, Kruk ZL (1999) MK-801 interaction with the 5-HT transporter: a real-time study in brain slices using fast cyclic voltammetry. Synapse 32 (3):212-224.

Isacoff EY, Jan YN, Jan LY (1991) Putative receptor for the cytoplasmic inactivation gate in the Shaker K^+ channel. Nature 353 (6339):86-90.

Jager H, Dreker T, Buck A, Giehl K, Gress T, Grissmer S (2004) Blockage of intermediate-conductance Ca^{2+}-activated K^+ channels inhibit human pancreatic cancer cell growth in vitro. Molecular pharmacology 65 (3):630-638.

Janeway CA, Jr., Medzhitov R (2002) Innate immune recognition. Annual review of immunology 20:197-216.

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325 (6104):529-531.

Johnson JW, Kotermanski SE (2006) Mechanism of action of memantine. Current opinion in pharmacology 6 (1):61-67.

Joly E, Mucke L, Oldstone MB (1991) Viral persistence in neurons explained by lack of major histocompatibility class I expression. Science 253 (5025):1283-1285.

Junger WG (2011) Immune cell regulation by autocrine purinergic signalling. Nature reviews Immunology 11 (3):201-212.

Kahlfuss S, Simma N, Mankiewicz J, Bose T, Lowinus T, Klein-Hessling S, Sprengel R, Schraven B, Heine M, Bommhardt U (2014) Immunosuppression by N-methyl-D-aspartate receptor antagonists is mediated through inhibition of $K_v1.3$ and $K_{Ca}3.1$ channels in T cells. Molecular and cellular biology 34 (5):820-831.

Kaushal V, Koeberle PD, Wang Y, Schlichter LC (2007) The Ca^{2+}-activated K^+ channel KCNN4/$K_{Ca}3.1$ contributes to microglia activation and nitric oxide-dependent neurodegeneration. The Journal of neuroscience : the official journal of the Society for Neuroscience 27 (1):234-244.

Kemp JA, McKernan RM (2002) NMDA receptor pathways as drug targets. Nature neuroscience 5 Suppl:1039-1042.

Kew JN, Trube G, Kemp JA (1996) A novel mechanism of activity-dependent NMDA receptor antagonism describes the effect of ifenprodil in rat cultured cortical neurones. The Journal of physiology 497 (Pt 3):761-772.

Khanna R, Roy L, Zhu X, Schlichter LC (2001) K^+ channels and the microglial respiratory burst. American journal of physiology Cell physiology 280 (4):C796-806.

Kim D (2005) Physiology and pharmacology of two-pore domain potassium channels. Current pharmaceutical design 11 (21):2717-2736.

Kleopa KA (2011) Autoimmune channelopathies of the nervous system. Current neuropharmacology 9 (3):458-467

Kobayashi T, Washiyama K, Ikeda K (2006) Inhibition of G protein-activated inwardly rectifying K^+ channels by ifenprodil. Neuropsychopharmacology:official publication of the American College of Neuropsychopharmacology 31 (3):516-524.

Kohler R, Kaistha BP, Wulff H (2010) Vascular K_{Ca}-channels as therapeutic targets in hypertension and restenosis disease. Expert opinion on therapeutic targets 14 (2):143-155.

Koob GF, Bloom FE (1988) Cellular and molecular mechanisms of drug dependence. Science 242 (4879):715-723.

Kornhuber J, Bormann J, Hubers M, Rusche K, Riederer P (1991) Effects of the 1-amino-adamantanes at the MK-801-binding site of the NMDA-receptor-gated ion channel:a human postmortem brain study. European journal of pharmacology 206 (4):297-300.

Kornhuber J, Bormann J, Retz W, Hubers M, Riederer P (1989) Memantine displaces [3H]MK-801 at therapeutic concentrations in postmortem human frontal cortex. European journal of pharmacology 166 (3):589-590.

Kornhuber J, Quack G (1995) Cerebrospinal fluid and serum concentrations of the N-methyl-D-aspartate (NMDA) receptor antagonist memantine in man. Neuroscience letters 195 (2):137-139.

Koroshetz WJ, Moskowitz MA (1996) Emerging treatments for stroke in humans. Trends in pharmacological sciences 17 (6):227-233.

Koshy S, Wu D, Hu X, Tajhya RB, Huq R, Khan FS, Pennington MW, Wulff H, Yotnda P, Beeton C (2013) Blocking $K_{Ca}3.1$ channels increases tumor cell killing by a subpopulation of human natural killer lymphocytes. PloS one 8 (10):e76740.

Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, Mata S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J (2009) AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. Annals of neurology 65 (4):424-434.

Lam J, Wulff H (2011) The Lymphocyte Potassium Channels $K_v1.3$ and $K_{Ca}3.1$ as Targets for Immunosuppression. Drug development research 72 (7):573-584.

Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, Friedman D, Skeen MB, Grisold W, Kimura A, Ohta K, Iizuka T, Guzman M, Graus F, Moss SJ, Balice-Gordon R, Dalmau J (2010) Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. Lancet neurology 9 (1):67-76.

Lauger P (1979) A channel mechanism for electrogenic ion pumps. Biochimica et biophysica acta 552 (1):143-161.

Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet JP (2004) TRPM4 regulates calcium oscillations after T cell activation. Science 306 (5700):1374-1377.

Leanza L, Trentin L, Becker KA, Frezzato F, Zoratti M, Semenzato G, Gulbins E, Szabo I (2013) Clofazimine, Psora-4 and PAP-1, inhibitors of the potassium channel $K_v1.3$, as a new and selective therapeutic strategy in chronic lymphocytic leukemia. Leukemia 27 (8):1782-1785.

Lee MS, Kim YJ (2007) Signaling pathways downstream of pattern-recognition receptors and their cross talk. Annual review of biochemistry 76:447-480.

Lefranc F, Kiss R (2008) The sodium pump alpha1 subunit as a potential target to combat apoptosis-resistant glioblastomas. Neoplasia 10 (3):198-206.

Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. The Journal of experimental medicine 202 (4):473-477.

Leonard RJ, Garcia ML, Slaughter RS, Reuben JP (1992) Selective blockers of voltage-gated K^+ channels depolarize human T lymphocytes: mechanism of the antiproliferative effect of charybdotoxin. Proceedings of the National Academy of Sciences of the United States of America 89 (21):10094-10098.

Leung YM (2012) Involvement of C-type inactivation gating in the actions of voltage-gated K^+ channel inhibitors. Pharmacology & therapeutics 133 (2):151-158.

Levite M, Hermelin A (1999) Autoimmunity to the glutamate receptor in mice--a model for Rasmussen's encephalitis? Journal of autoimmunity 13 (1):73-82.

Lewis RS (2001) Calcium signaling mechanisms in T lymphocytes. Annual review of immunology 19:497-521.

Lewis RS, Cahalan MD (1995) Potassium and calcium channels in lymphocytes. Annual review of immunology 13:623-653.

Li Z, Chen L, Niu X, Liu J, Ping M, Li R, Xie X, Guo L (2012) Immunomodulatory synergy by combining atorvastatin and rapamycin in the treatment of experimental autoimmune encephalomyelitis (EAE). Journal of neuroimmunology 250 (1-2):9-17.

Liguori R, Vincent A, Clover L, Avoni P, Plazzi G, Cortelli P, Baruzzi A, Carey T, Gambetti P, Lugaresi E, Montagna P (2001) Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. Brain:a journal of neurology 124 (Pt 12):2417-2426.

Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE, Jr., Meyer T (2005) STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. Current biology:CB 15 (13):1235-1241.

Lipton SA (1992) Memantine prevents HIV coat protein-induced neuronal injury in vitro. Neurology 42 (7):1403-1405.

Lipton SA (1993) Prospects for clinically tolerated NMDA antagonists:open-channel blockers and alternative redox states of nitric oxide. Trends in neurosciences 16 (12):527-532.

Lipton SA (2004) Paradigm shift in NMDA receptor antagonist drug development:molecular mechanism of uncompetitive inhibition by memantine in the treatment of Alzheimer's disease and other neurologic disorders. Journal of Alzheimer's disease:JAD 6 (6 Suppl):S61-74.

Lipton SA (2006) Paradigm shift in neuroprotection by NMDA receptor blockade:memantine and beyond. Nature reviews Drug discovery 5 (2):160-170.

Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. The New England journal of medicine 330 (9):613-622.

Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, Fleig A, Penner R (2007) CRACM1, CRACM2, and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties. Current biology: CB 17 (9):794-800.

Liu Y, Jurman ME, Yellen G (1996) Dynamic rearrangement of the outer mouth of a K^+ channel during gating. Neuron 16 (4):859-867.

Lombardi G, Dianzani C, Miglio G, Canonico PL, Fantozzi R (2001) Characterization of ionotropic glutamate receptors in human lymphocytes. British journal of pharmacology 133 (6):936-944.

Lopez-Barneo J, Hoshi T, Heinemann SH, Aldrich RW (1993) Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. Receptors & channels 1 (1):61-71.

Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS (2008) Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. Nature 454 (7203):538-542.

Lutsep HL, Clark WM (1999) Neuroprotection in acute ischaemic stroke. Current status and future potential. Drugs in R&D 1 (1):3-8.

Machado-Vieira R, Salvadore G, DiazGranados N, Ibrahim L, Latov D, Wheeler-Castillo C, Baumann J, Henter ID, Zarate CA, Jr. (2010) New therapeutic targets for mood disorders. The Scientific World Journal 10:713-726.

Mackay CR (1999) Dual personality of memory T cells. Nature 401 (6754):659-660.

MacKenzie AB, Surprenant A, North RA (1999) Functional and molecular diversity of purinergic ion channel receptors. Annals of the New York Academy of Sciences 868:716-729.

Maezawa I, Jenkins DP, Jin BE, Wulff H (2012) Microglial $K_{Ca}3.1$ Channels as a Potential Therapeutic Target for Alzheimer's Disease. International journal of Alzheimer's disease 2012:868972.

Maezawa I, Zimin PI, Wulff H, Jin LW (2011) Amyloid-beta protein oligomer at low nanomolar concentrations activates microglia and induces microglial neurotoxicity. The Journal of biological chemistry 286 (5):3693-3706.

Malinow R (2012) New developments on the role of NMDA receptors in Alzheimer's disease. Current opinion in neurobiology 22 (3):559-563.

Matsumoto M, Fujii Y, Baba A, Hikida M, Kurosaki T, Baba Y (2011) The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. Immunity 34 (5):703-714.

Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. Nature 309 (5965):261-263.

McGeer EG, McGeer PL (2010) Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy. Journal of Alzheimer's disease:JAD 19 (1):355-361.

McGeer PL, Akiyama H, Itagaki S, McGeer EG (1989) Activation of the classical complement pathway in brain tissue of Alzheimer patients. Neuroscience letters 107 (1-3):341-346.

Meier T, Polzer P, Diederichs K, Welte W, Dimroth P (2005) Structure of the rotor ring of F-Type Na^+-ATPase from Ilyobacter tartaricus. Science 308 (5722):659-662.

Meinl E, Krumbholz M, Hohlfeld R (2006) B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation. Annals of neurology 59 (6):880-892.

Meldrum BS (1992a) Excitatory amino acid receptors and disease. Current opinion in neurology and neurosurgery 5 (4):508-513.

Meldrum BS (1992b) Excitatory amino acids in epilepsy and potential novel therapies. Epilepsy research 12 (2):189-196.

Meuth SG, Bittner S, Meuth P, Simon OJ, Budde T, Wiendl H (2008) TWIK-related acid-sensitive K^+ channel 1 (TASK1) and TASK3 critically influence T lymphocyte effector functions. The Journal of biological chemistry 283 (21):14559-14570.

Mijatovic T, Roland I, Van Quaquebeke E, Nilsson B, Mathieu A, Van Vynckt F, Darro F, Blanco G, Facchini V, Kiss R (2007) The alpha1 subunit of the sodium pump could represent a novel target to combat non-small cell lung cancers. The Journal of pathology 212 (2):170-179.

Mori H, Masaki H, Yamakura T, Mishina M (1992) Identification by mutagenesis of a Mg^{2+}-block site of the NMDA receptor channel. Nature 358 (6388):673-675.

Morrell CN, Sun H, Ikeda M, Beique JC, Swaim AM, Mason E, Martin TV, Thompson LE, Gozen O, Ampagoomian D, Sprengel R, Rothstein J, Faraday N, Huganir R, Lowenstein CJ (2008) Glutamate mediates platelet activation through the AMPA receptor. The Journal of experimental medicine 205 (3):575-584.

Mothet JP, Parent AT, Wolosker H, Brady RO, Jr., Linden DJ, Ferris CD, Rogawski MA, Snyder SH (2000) D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proceedings of the National Academy of Sciences of the United States of America 97 (9):4926-4931.

Muller WE, Pergande G, Ushijima H, Schleger C, Kelve M, Perovic S (1996) Neurotoxicity in rat cortical cells caused by N-methyl-D-aspartate (NMDA) and gp120 of HIV-1: induction and pharmacological intervention. Progress in molecular and subcellular biology 16:44-57.

Murata T, Yamato I, Kakinuma Y, Leslie AG, Walker JE (2005) Structure of the rotor of the V-Type Na+-ATPase from Enterococcus hirae. Science 308 (5722):654-659.

Murphy JB, Sturm E (1923) Conditions Determining the Transplantability of Tissues in the Brain. The Journal of experimental medicine 38 (2):183-197.

Nakanishi S (1992) Molecular diversity of glutamate receptors and implications for brain function. Science 258 (5082):597-603.

Nordstrom T, Nevanlinna HA, Andersson LC (1992) Mitosis-arresting effect of the calcium channel inhibitor SK&F 96365 on human leukemia cells. Experimental cell research 202 (2):487-494.

Novillo-Lopez ME, Rossi JE, Dalmau J, Masjuan J (2008) Treatment-responsive subacute limbic encephalitis and NMDA receptor antibodies in a man. Neurology 70 (9):728-729.

O'Neill LA, Bowie AG (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nature reviews Immunology 7 (5):353-364.

Oh-Hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A (2008) Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. Nature immunology 9 (4):432-443.

Olivares D, Deshpande VK, Shi Y, Lahiri DK, Greig NH, Rogers JT, Huang X (2012) N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. Current Alzheimer research 9 (6):746-758.

Ollenschlager G, Karner J, Karner-Hanusch J, Jansen S, Schindler J, Roth E (1989) Plasma glutamate--a prognostic marker of cancer and of other immunodeficiency syndromes? Scandinavian journal of clinical and laboratory investigation 49 (8):773-777.

Orgogozo JM, Rigaud AS, Stoffler A, Mobius HJ, Forette F (2002) Efficacy and safety of memantine in patients with mild to moderate vascular dementia: a randomized, placebo-controlled trial (MMM 300). Stroke; a journal of cerebral circulation 33 (7):1834-1839.

Orser BA, Pennefather PS, MacDonald JF (1997) Multiple mechanisms of ketamine blockade of N-methyl-D-aspartate receptors. Anesthesiology 86 (4):903-917.

Osborne NN (1999) Memantine reduces alterations to the mammalian retina, in situ, induced by ischemia. Visual neuroscience 16 (1):45-52.

Owsianik G, Talavera K, Voets T, Nilius B (2006) Permeation and selectivity of TRP channels. Annual review of physiology 68:685-717.

Pacheco R, Gallart T, Lluis C, Franco R (2007) Role of glutamate on T-cell mediated immunity. Journal of neuroimmunology 185 (1-2):9-19.

Padeh S, Cohen A, Roifman CM (1991) ATP-induced activation of human B lymphocytes via P2-purinoceptors. Journal of immunology 146 (5):1626-1632.

Palmer GC (2001) Neuroprotection by NMDA receptor antagonists in a variety of neuropathologies. Current drug targets 2 (3):241-271.

Panyi G, Possani LD, Rodriguez de la Vega RC, Gaspar R, Varga Z (2006) K^+ channel blockers: novel tools to inhibit T cell activation leading to specific immunosuppression. Current pharmaceutical design 12 (18):2199-2220.

Paoletti P, Bellone C, Zhou Q (2013) NMDA receptor subunit diversity:impact on receptor properties, synaptic plasticity and disease. Nature reviews Neuroscience 14 (6):383-400.

Parsons CG, Danysz W, Quack G (1999) Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist--a review of preclinical data. Neuropharmacology 38 (6):735-767.

Parsons CG, Quack G, Bresink I, Baran L, Przegalinski E, Kostowski W, Krzascik P, Hartmann S, Danysz W (1995) Comparison of the potency, kinetics and voltage-dependency of a series of uncompetitive NMDA receptor antagonists in vitro with anticonvulsive and motor impairment activity in vivo. Neuropharmacology 34 (10):1239-1258.

Parsons CG, Stoffler A, Danysz W (2007) Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. Neuropharmacology 53 (6):699-723.

Partiseti M, Choquet D, Diu A, Korn H (1992) Differential regulation of voltage- and calcium-activated potassium channels in human B lymphocytes. Journal of immunology 148 (11):3361-3368.

Partiseti M, Korn H, Choquet D (1993) Pattern of potassium channel expression in proliferating B lymphocytes depends upon the mode of activation. Journal of immunology 151 (5):2462-2470.

Patel AJ, Lazdunski M (2004) The 2P-domain K^+ channels: role in apoptosis and tumorigenesis. Pflugers Archiv : European journal of physiology 448 (3):261-273.

Pellegrini JW, Lipton SA (1993) Delayed administration of memantine prevents N-methyl-D-aspartate receptor-mediated neurotoxicity. Annals of neurology 33 (4):403-407.

Pellkofer HL, Armbruster L, Krumbholz M, Titulaer MJ, Verschuuren JJ, Schumm F, Voltz R (2008) Lambert-eaton myasthenic syndrome differential reactivity of tumor versus non-tumor patients to subunits of the voltage-gated calcium channel. Journal of neuroimmunology 204 (1-2):136-139.

Pin JP, Acher F (2002) The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. Current drug targets CNS and neurological disorders 1 (3):297-317.

Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34 (1):1-26.

Pirnik Z, Schwendt M, Jezova D (2001) Single dose of morphine influences plasma corticosterone and gene expression of main NMDA receptor subunit in the adrenal gland but not in the hippocampus. Endocrine regulations 35 (4):187-193.

Poltorak M, Arndt B, Kowtharapu BS, Reddycherla AV, Witte V, Lindquist JA, Schraven B, Simeoni L (2013) TCR activation kinetics and feedback regulation in primary human T cells. Cell communication and signaling : CCS 11:4.

Prakriya M (2009) The molecular physiology of CRAC channels. Immunological reviews 231 (1):88-98.

Preussat K, Beetz C, Schrey M, Kraft R, Wolfl S, Kalff R, Patt S (2003) Expression of voltage-gated potassium channels $K_v1.3$ and $K_v1.5$ in human gliomas. Neuroscience letters 346 (1-2):33-36.

Putney JW, Jr. (1986) A model for receptor-regulated calcium entry. Cell calcium 7 (1):1-12

Putney JW, Jr., Broad LM, Braun FJ, Lievremont JP, Bird GS (2001) Mechanisms of capacitative calcium entry. Journal of cell science 114 (Pt 12):2223-2229.

Putney JW, Jr., McKay RR (1999) Capacitative calcium entry channels. BioEssays:news and reviews in molecular, cellular and developmental biology 21 (1):38-46.

Racca C, Stephenson FA, Streit P, Roberts JD, Somogyi P (2000) NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. The Journal of neuroscience: the official journal of the Society for Neuroscience 20 (7):2512-2522.

Rammes G, Rupprecht R, Ferrari U, Zieglgansberger W, Parsons CG (2001) The N-methyl-D-aspartate receptor channel blockers memantine, MRZ 2/579 and other amino-alkyl-cyclohexanes antagonise 5-HT(3) receptor currents in cultured HEK-293 and N1E-115 cell systems in a non-competitive manner. Neuroscience letters 306 (1-2):81-84.

Ramsey IS, Delling M, Clapham DE (2006) An introduction to TRP channels. Annual review of physiology 68:619-647.

Rao A (2009) Signaling to gene expression:calcium, calcineurin and NFAT. Nature immunology 10 (1):3-5.

Remillard CV, Yuan JX (2004) Activation of K^+ channels: an essential pathway in programmed cell death. American journal of physiology Lung cellular and molecular physiology 286 (1):L49-67.

Rezzani R, Corsetti G, Rodella L, Angoscini P, Lonati C, Bianchi R (2003) Cyclosporine-A treatment inhibits the expression of metabotropic glutamate receptors in rat thymus. Acta histochemica 105 (1):81-87.

Ritchie AM, Gilden DH, Williamson RA, Burgoon MP, Yu X, Helm K, Corboy JR, Owens GP (2004) Comparative analysis of the $CD19^+$ and $CD138^+$ cell antibody repertoires in the cerebrospinal fluid of patients with multiple sclerosis. Journal of immunology 173 (1):649-656.

Roach KM, Duffy SM, Coward W, Feghali-Bostwick C, Wulff H, Bradding P (2013) The K^+ channel $K_{Ca}3.1$ as a novel target for idiopathic pulmonary fibrosis. PloS one 8 (12):e85244.

Rogawski MA (2000) Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents--toward an understanding of their favorable tolerability. Amino acids 19 (1):133-149.

Rogawski MA, Wenk GL (2003) The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. CNS drug reviews 9 (3):275-308.

Roger T, Froidevaux C, Le Roy D, Reymond MK, Chanson AL, Mauri D, Burns K, Riederer BM, Akira S, Calandra T (2009) Protection from lethal gram-negative bacterial sepsis by targeting Toll-like receptor 4. Proceedings of the National Academy of Sciences of the United States of America 106 (7):2348-2352.

Rogers J, Luber-Narod J, Styren SD, Civin WH (1988) Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. Neurobiology of aging 9 (4):339-349.

Rogers SW, Andrews PI, Gahring LC, Whisenand T, Cauley K, Crain B, Hughes TE, Heinemann SF, McNamara JO (1994) Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. Science 265 (5172):648-651.

Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA (2005) STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. The Journal of cell biology 169 (3):435-445.

Rush LJ, Raval A, Funchain P, Johnson AJ, Smith L, Lucas DM, Bembea M, Liu TH, Heerema NA, Rassenti L, Liyanarachchi S, Davuluri R, Byrd JC, Plass C (2004) Epigenetic profiling in chronic lymphocytic leukemia reveals novel methylation targets. Cancer research 64 (7):2424-2433.

Sakurada K, Masu M, Nakanishi S (1993) Alteration of Ca^{2+} permeability and sensitivity to Mg^{2+} and channel blockers by a single amino acid substitution in the N-methyl-D-aspartate receptor. The Journal of biological chemistry 268 (1):410-415.

Samelson LE, Harford JB, Klausner RD (1985) Identification of the components of the murine T cell antigen receptor complex. Cell 43 (1):223-231.

Sarchielli P, Di Filippo M, Candeliere A, Chiasserini D, Mattioni A, Tenaglia S, Bonucci M, Calabresi P (2007) Expression of ionotropic glutamate receptor GLUR3 and effects of glutamate on MBP- and MOG-specific lymphocyte activation and chemotactic migration in multiple sclerosis patients. Journal of neuroimmunology 188 (1-2):146-158.

Schenk U, Westendorf AM, Radaelli E, Casati A, Ferro M, Fumagalli M, Verderio C, Buer J, Scanziani E, Grassi F (2008) Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels. Science signaling 1 (39):ra6.

Schilling T, Stock C, Schwab A, Eder C (2004) Functional importance of Ca^{2+}-activated K^+ channels for lysophosphatidic acid-induced microglial migration. The European journal of neuroscience 19 (6):1469-1474.

Seeman P, Caruso C, Lasaga M (2008) Dopamine partial agonist actions of the glutamate receptor agonists LY 354,740 and LY 379,268. Synapse 62 (2):154-158.

Serfling E, Berberich-Siebelt F, Avots A (2007) NFAT in lymphocytes:a factor for all events? Science's STKE : signal transduction knowledge environment 2007 (398):pe42.

Shieh CC, Coghlan M, Sullivan JP, Gopalakrishnan M (2000) Potassium channels:molecular defects, diseases, and therapeutic opportunities. Pharmacological reviews 52 (4):557-594.

Shleper M, Kartvelishvily E, Wolosker H (2005) D-serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. The Journal of neuroscience: the official journal of the Society for Neuroscience 25 (41):9413-9417.

Shohami E, Biegon A (2013) Novel Approach to the Role of NMDA Receptors in Traumatic Brain Injury. CNS & neurological disorders drug targets.

Shuttleworth TJ (1996) Arachidonic acid activates the noncapacitative entry of Ca^{2+} during $[Ca^{2+}]_i$ oscillations. The Journal of biological chemistry 271 (36):21720-21725.

Sprengel R, Single FN (1999) Mice with genetically modified NMDA and AMPA receptors. Annals of the New York Academy of Sciences 868:494-501.

Srivastava S, Ko K, Choudhury P, Li Z, Johnson AK, Nadkarni V, Unutmaz D, Coetzee WA, Skolnik EY (2006) Phosphatidylinositol-3 phosphatase myotubularin-related protein 6 negatively regulates CD4 T cells. Molecular and cellular biology 26 (15):5595-5602.

Stepulak A, Luksch H, Gebhardt C, Uckermann O, Marzahn J, Sifringer M, Rzeski W, Staufner C, Brocke KS, Turski L, Ikonomidou C (2009) Expression of glutamate receptor subunits in human cancers. Histochemistry and cell biology 132 (4):435-445.

Storey NM, Gomez-Angelats M, Bortner CD, Armstrong DL, Cidlowski JA (2003) Stimulation of $K_v1.3$ potassium channels by death receptors during apoptosis in Jurkat T lymphocytes. The Journal of biological chemistry 278 (35):33319-33326.

Striessnig J, Grabner M, Mitterdorfer J, Hering S, Sinnegger MJ, Glossmann H (1998) Structural basis of drug binding to L Ca^{2+} channels. Trends in pharmacological sciences 19 (3):108-115.

Sucher NJ, Lipton SA, Dreyer EB (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. Vision research 37 (24):3483-3493.

Szabo I, Gulbins E, Apfel H, Zhang X, Barth P, Busch AE, Schlottmann K, Pongs O, Lang F (1996) Tyrosine phosphorylation-dependent suppression of a voltage-gated K^+ channel in T lymphocytes upon Fas stimulation. The Journal of biological chemistry 271 (34):20465-20469.

Takuwa N, Iwamoto A, Kumada M, Yamashita K, Takuwa Y (1991) Role of Ca^{2+} influx in bombesin-induced mitogenesis in Swiss 3T3 fibroblasts. The Journal of biological chemistry 266 (3):1403-1409.

Takuwa N, Zhou W, Kumada M, Takuwa Y (1992) Ca^{2+}/calmodulin is involved in growth factor-induced retinoblastoma gene product phosphorylation in human vascular endothelial cells. FEBS letters 306 (2-3):173-175.

Tharp DL, Wamhoff BR, Wulff H, Raman G, Cheong A, Bowles DK (2008) Local delivery of the $K_{Ca}3.1$ blocker, TRAM-34, prevents acute angioplasty-induced coronary smooth muscle phenotypic modulation and limits stenosis. Arteriosclerosis, thrombosis, and vascular biology 28 (6):1084-1089.

Toldi G, Bajnok A, Dobi D, Kaposi A, Kovacs L, Vasarhelyi B, Balog A (2013) The effects of $K_v1.3$ and $IK_{Ca}1$ potassium channel inhibition on calcium influx of human peripheral T lymphocytes in rheumatoid arthritis. Immunobiology 218 (3):311-316.

Toyama K, Wulff H, Chandy KG, Azam P, Raman G, Saito T, Fujiwara Y, Mattson DL, Das S, Melvin JE, Pratt PF, Hatoum OA, Gutterman DD, Harder DR, Miura H (2008) The intermediate-

conductance calcium-activated potassium channel $K_{Ca}3.1$ contributes to atherogenesis in mice and humans. The Journal of clinical investigation 118 (9):3025-3037.

Tsan MF, Gao B (2004) Endogenous ligands of Toll-like receptors. Journal of leukocyte biology 76 (3):514-519.

Tsien RW, Lipscombe D, Madison D, Bley K, Fox A (1995) Reflections on Ca^{2+}-channel diversity, 1988-1994. Trends in neurosciences 18 (2):52-54.

Van Der Velden J, Sum G, Barker D, Koumoundouros E, Barcham G, Wulff H, Castle N, Bradding P, Snibson K (2013) $K_{Ca}3.1$ channel-blockade attenuates airway pathophysiology in a sheep model of chronic asthma. PloS one 8 (6):e66886.

Venkatachalam K, Montell C (2007) TRP channels. Annual review of biochemistry 76:387-417.

Vennekens R, Nilius B (2007) Insights into TRPM4 function, regulation and physiological role. Handbook of experimental pharmacology (179):269-285.

Verhoeyen E, Costa C, Cosset FL (2009) Lentiviral vector gene transfer into human T cells. Methods in molecular biology 506:97-114.

Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA (2000) Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. The New England journal of medicine 343 (12):847-855.

Vezzani A, Maroso M, Balosso S, Sanchez MA, Bartfai T (2011) IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. Brain, behavior, and immunity 25 (7):1281-1289.

Vidaver GA (1966) Inhibition of parallel flux and augmentation of counter flux shown by transport models not involving a mobile carrier. Journal of theoretical biology 10 (2):301-306.

Villalonga N, David M, Bielanska J, Vicente R, Comes N, Valenzuela C, Felipe A (2010) Immunomodulation of voltage-dependent K^+ channels in macrophages: molecular and biophysical consequences. The Journal of general physiology 135 (2):135-147.

Vorwerk CK, Lipton SA, Zurakowski D, Hyman BT, Sabel BA, Dreyer EB (1996) Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. Investigative ophthalmology & visual science 37 (8):1618-1624.

Wang LH, Wang N, Lu XY, Liu BC, Yanda MK, Song JZ, Dai HM, Sun YL, Bao HF, Eaton DC, Ma HP (2012) Rituximab inhibits $K_v1.3$ channels in human B lymphoma cells via activation of FcgammaRIIB receptors. Biochimica et biophysica acta 1823 (2):505-513.

Wasserman JK, Koeberle PD (2009) Development and characterization of a hemorrhagic rat model of central post-stroke pain. Neuroscience 161 (1):173-183.

Wei AD, Gutman GA, Aldrich R, Chandy KG, Grissmer S, Wulff H (2005) International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. Pharmacological reviews 57 (4):463-472.

Weiss A (2009) TCR signal transduction: opening the black box. Journal of immunology 183 (8):4821-4827.

Weiss A (2010) The right team at the right time to go for a home run: tyrosine kinase activation by the TCR. Nature immunology 11 (2):101-104.

Wenning AS, Neblung K, Strauss B, Wolfs MJ, Sappok A, Hoth M, Schwarz EC (2011) TRP expression pattern and the functional importance of TRPC3 in primary human T-cells. Biochimica et biophysica acta 1813 (3):412-423.

Weskamp M, Seidl W, Grissmer S (2000) Characterization of the increase in $[Ca^{2+}]_i$ during hypotonic shock and the involvement of Ca^{2+}-activated K^+ channels in the regulatory volume decrease in human osteoblast-like cells. The Journal of membrane biology 178 (1):11-20.

Wienands J, Engels N (2001) Multitasking of Ig-alpha and Ig-beta to regulate B cell antigen receptor function. International reviews of immunology 20 (6):679-696.

Williams S, Bateman A, O'Kelly I (2013) Altered expression of two-pore domain potassium (K_{2P}) channels in cancer. PloS one 8 (10):e74589.

Winblad B, Poritis N (1999) Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). International journal of geriatric psychiatry 14 (2):135-146.

Wisden W, Seeburg PH (1993) Mammalian ionotropic glutamate receptors. Current opinion in neurobiology 3 (3):291-298.

Wittebole X, Castanares-Zapatero D, Laterre PF (2010) Toll-like receptor 4 modulation as a strategy to treat sepsis. Mediators of inflammation 2010:568396.

Woehrle T, Yip L, Elkhal A, Sumi Y, Chen Y, Yao Y, Insel PA, Junger WG (2010) Pannexin-1 hemichannel-mediated ATP release together with P2X1 and P2X4 receptors regulate T-cell activation at the immune synapse. Blood 116 (18):3475-3484.

Wolosker H, Blackshaw S, Snyder SH (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proceedings of the National Academy of Sciences of the United States of America 96 (23):13409-13414.

Wulff H, Calabresi PA, Allie R, Yun S, Pennington M, Beeton C, Chandy KG (2003) The voltage-gated $K_v1.3$ K^+ channel in effector memory T cells as new target for MS. The Journal of clinical investigation 111 (11):1703-1713.

Wulff H, Castle NA, Pardo LA (2009) Voltage-gated potassium channels as therapeutic targets. Nature reviews Drug discovery 8 (12):982-1001.

Wulff H, Knaus HG, Pennington M, Chandy KG (2004) K^+ channel expression during B cell differentiation: implications for immunomodulation and autoimmunity. Journal of immunology 173 (2):776-786.

Wulff H, Kohler R (2013) Endothelial small-conductance and intermediate-conductance K_{Ca} channels: an update on their pharmacology and usefulness as cardiovascular targets. Journal of cardiovascular pharmacology 61 (2):102-112.

Xia XM, Fakler B, Rivard A, Wayman G, Johnson-Pais T, Keen JE, Ishii T, Hirschberg B, Bond CT, Lutsenko S, Maylie J, Adelman JP (1998) Mechanism of calcium gating in small-conductance calcium-activated potassium channels. Nature 395 (6701):503-507.

Xiao F, Pardue S, Arnold T, Carden D, Alexander JS, Monroe J, Sharp CD, Turnage R, Conrad S (2004) Effect of ifenprodil, a polyamine site NMDA receptor antagonist, on brain edema formation following asphyxial cardiac arrest in rats. Resuscitation 61 (2):209-219.

Xu HP, Chen H, Ding Q, Xie ZH, Chen L, Diao L, Wang P, Gan L, Crair MC, Tian N (2010) The immune protein CD3zeta is required for normal development of neural circuits in the retina. Neuron 65 (4):503-515.

Yanaba K, Bouaziz JD, Matsushita T, Magro CM, St Clair EW, Tedder TF (2008) B-lymphocyte contributions to human autoimmune disease. Immunological reviews 223:284-299.

Yeh MW, Kaul M, Zheng J, Nottet HS, Thylin M, Gendelman HE, Lipton SA (2000) Cytokine-stimulated, but not HIV-infected, human monocyte-derived macrophages produce neurotoxic levels of l -cysteine. Journal of immunology 164 (8):4265-4270.

Yellen G (2002) The voltage-gated potassium channels and their relatives. Nature 419 (6902):35-42.

Yip L, Woehrle T, Corriden R, Hirsh M, Chen Y, Inoue Y, Ferrari V, Insel PA, Junger WG (2009) Autocrine regulation of T-cell activation by ATP release and P2X7 receptors. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 23 (6):1685-1693.

Yoshimoto T, Nakanishi K (2006) Roles of IL-18 in basophils and mast cells. Allergology international: official journal of the Japanese Society of Allergology 55 (2):105-113.

Zunszain PA, Horowitz MA, Cattaneo A, Lupi MM, Pariante CM (2013) Ketamine: synaptogenesis, immunomodulation and glycogen synthase kinase-3 as underlying mechanisms of its antidepressant properties. Molecular psychiatry 18 (12):1236-1241.

Zweifach A, Lewis RS (1993) Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. Proceedings of the National Academy of Sciences of the United States of America 90 (13):6295-6299.

8. Abbreviations

aa: *amino acid*
Abs: *antibodies*
AchR: *acetyl choline receptor*
AD: *Alzheimer's disease*
APC: *antigen-presenting cell*
AMPA: *α-amino-3-hydroxy-5-methylisoxasole-4-propionate*
BBB*: blood-brain barrier*
BCR: *B-cell receptor*
BSA: *bovine serum albumin*
Ca_v: *voltage-activated calcium channel*
CRAC: *calcium release-activated* Ca^{2+}*-channels*
CD: *cluster of differentiation*
CLL: *chronic lymphoid leukemia*
CNS: *central nervous system*
CSF: *cerebro-spinal fluid*
DC: *dendritic cell*
DMSO: *Dimethylsulfoxide*
DP: *double positive*
DN: *double negative*
ER: *endoplasmic reticulum*
EAE: *experimental autoimmune encephalomyelitis*
FACS: *fluorescence activated cell sorter*
FBS: *fetal bovine serum*
FITC: *Fluorescein IsoThio Cyanate*
Glu: *glutamate*
HBSS: *Hanks' Balanced Salt Solution*
5-HT3: *serotonin receptor*
Ifen.: *ifenprodil*
iGluR: *ionotropic glutamate receptor*
KO: *knockout*
K_v: *voltage-activated potassium channel*
K_{Ca}: *calcium-activated potassium channel*
LN: *lymph node*

LPS: *lipopolysaccharide*

Mem.: *memantine*

MEM: *minimum essential medium eagle*

MAPK: *mitogen-activated protein kinase*

MOG: *myelin oligodendrocyte glycoprotein*

mGluR: *metabotropic glutamate receptor*

mTOR: *mammalian target of rapamycin*

NMDAR: *N-methyl D-aspartate receptor*

NEA: *non-essential amino acids*

NF-κB: *nuclear factor-κB*

NFAT: *nuclear factor of transcription of activated T-cells*

OVA: *ovalbumin*

PBS: *phosphate buffered saline*

PBL: *peripheral blood lymphocytes*

PFA: *paraformaldehyde*

PBMCs: *peripheral blood mononuclear cells*

rpm: *revolutions per minute*

RACC: *receptor-activated calcium channel*

RT: *room temperature*

RPMI: *Roswell Park Memorial Institute Medium*

SOCC: *store-operated calcium channel*

SOCE: *store-operated Ca^{2+}-entry*

SMOCC: *second messenger-operated calcium channel*

STIM: *stromal interaction molecule*

tg: *transgenic*

T_H: *T helper cell*

T_C: *cytotoxic T cell*

TCR: *T-cell receptor*

TLR: *Toll-like receptor*

TRP: *transient receptor potential*

T_{CM}: *central memory T cell*

T_{EM}: *effector memory T cells*

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11. Erklärung

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Erklärung

Hiermit erkläre ich, dass ich die von mir eingereichte Dissertation zum dem Thema

'Crosstalk between NMDAR Antagonists and Potassium Channels in Murine and Human Lymphocytes'

selbständig verfasst, nicht schon als Dissertation verwendet habe und die benutzen Hilfsmittel und Quellen vollständig angegeben wurden.

Weiterhin erkläre ich, dass ich werder diese noch eine andere Arbeit zur Erklangung des akademischen Grades Dr. rer. nat. an anderen Einrichtungen eingereicht habe.

Unterschrift

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Marks secured	85.6%	71.2%	74.6%	Aggregate: 5.24 CGPA (out of 6)

Publications

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Public Appearance

Poster Presentation

- 'NMDA receptor antagonists: effective modulators of T cell signaling and effector function.' N. Simma*****, T. Bose*****, S. Kahlfuß*****, J. Mankiewicz, T. Lowinus, R. Sprengel, B. Schraven, M. Heine, U. Bommhardt**+**

- 'Insight into the molecular basis of the unique T cell signaling induced by the CD28 superagonistic antibody TGN1412.' Rudolph J.M., Wiechec-Los E., Bartholomäus P., Merten C., Ramonat A., Steiner M., Bose T., Heine M., Fischer K.-D., Kliche S., Kalinke U., Schraven B. - selected for oral presentation**+**

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