N-methyl-D-aspartate (NMDA) receptors are a subtype of ionotropic glutamate receptors, which mediate fast synaptic transmission in the vertebrate central nervous system and play a key role in synaptic plasticity. NMDA receptor overexcitation leads to cell death that underlies many serious neurological and psychiatric disorders. The aim of our research was to characterize novel drugs that modulate NMDAR activity.

We have tested a series of newly synthesized steroidal compounds for their activity on recombinant receptors. We have demonstrated a relationship between the structure of the analogues of a naturally occurring neurosteroid pregnanolone sulfate and their ability to modulate NMDA receptors. The results of our experiments characterize the role of substituents at the steroid A and D ring. We have found specific substituents on carbons C3 and C17 of the steroidal skeleton that lead to a substantial increase of steroid efficacy and the ability to positively affect the ratio between the inhibition of stationary and non-stationary receptor activation. These two states represent receptors long-term activated (tonically) and synaptically activated (phasically), respectively.

Using electrophysiological and optical methods in combination with mathematical modeling we have shown that the plasma membrane plays an important role in the steroid access to the NMDA receptor. We have proposed a model which indicates that there is a local increase in the concentration of steroid monomers on the surface of the plasma membrane that can exceed the critical micellar concentration.

We have identified the binding site of inhibitory steroids at the NMDA receptor. The binding site is located in the extracellular vestibule of the ion channel pore in the highly conserved SYTANLAAF motif. We have proposed a model of the open ion channel configuration of the NMDA receptor by taking advantage of the current-voltage relationship of mutated NMDA receptors in the presence of neurosteroids, in combination with molecular modeling methods.

Neurosteroids and their synthetic derivatives are a promising class of NMDA antagonists. We reveal structural determinants of the steroid molecule that are crucial for the inhibitory effect and furthermore elucidate the molecular mechanism of the steroid modulation of NMDA receptors. We are convinced that these findings will lead to the design and characterization of new efficacious drugs that will prove to be useful in clinical practice for the treatment of diseases linked to NMDA receptor dysfunction.