Rat α4β2		Human α4β2		Human α7	(Enant)-compound
Ki (nM)	EC_{50} (μM)	I_{max} (%ACh)		EC_{50} (μM)	I_{max} (%ACh)
(S)-Isoanatabine	650	0.91	78.8	45.1	76.1
(R)-Isoanatabine	198	0.32	103	51.9	31.2
(S)-Anatabine	282	0.79	39.8	33.0	103
(R)-Anatabine	114	0.48	17.2	41.1	90.3
(S)-Nicotine	5.6	0.21	84.3	56.5	91.7
(S)-Anabasine	1,100	7.91	76.0	18.4	100
Anabaseine	94	~12	~8	18	100

(R)-Isoanatabine (1) is 3-fold more potent than (S)-isoanatabine at α4β2 receptor, but its α7 I_{max} is only about half as great; (2) is a potent α4β2 agonist comparable to (S)-nicotine; (3) displays a much higher efficacy at the α4β2 receptor and a much lower I_{max} at α7 relative to anatabine; (4) relative to anabasine, is a 9-fold more potent α4β2 partial agonist but a 3-fold less efficacious α7 partial agonist; (5) Isoanatabine, relative to anabaseine, is a much more potent and efficacious α4β2 agonist. The 3,4 position double bond in isoanatabine and, to a lesser extent, the 4,5-position double bond in anatabine contribute greatly to their α4β2 agonist activities.

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1.19

Automated two-electrode voltage clamp for medium-throughput studies of ion channels with non-destructive sample analysis

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Electrophysiological recordings using patch clamp systems are important tools for the study of ion channels and receptors. Although conventional patch clamp delivers high-quality data, it does not allow a fast high-throughput screening of drugs on ion channels or receptors, and is mostly limited to record data of maximum 40 cells daily by a skilled scientist. Here, we present a new automated two-electrode voltage clamp, which allows a fast, reliable, and high-quality screening of up to 96 drugs in a single experiment. Our system is equipped with one 96-well plate, which contains the samples (or toxins) using minimal volumes (220 μl). A fact that is especially important when probing effects of samples that are only available in minute amounts such as toxins. Since the automate uses a non-destructive measurement with drugs stored in the 96-well plate, samples can be reused several times to evaluate their effects either at the same channel subtype or to another membrane protein. A second 96-well plate contains the injected oocytes from *Xenopus*. Oocytes are automatically loaded and poked and their properties assessed to determine the membrane quality. As the automate works fully unattended, measurements can be carried continuously over 24 h. Depending on the type of experiment, and therefore the chosen protocol, we can measure from few to hundreds of oocytes daily. The importance of such a tool is illustrated by characterizing several variants of the ImI and ImII toxins. This new system provides a medium-throughput screening platform and expands by orders of magnitudes the number of samples and cells that can be measured in a single day while offering, in addition, the capacity to work with very limited sample size.

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1.20

Novel α7 nAChRs ligands: From virtual screening to functional assays

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Progresses made in the understanding of the tridimensional structure of the nicotinic acetylcholine receptors (nAChRs) allows to exploit new strategies for the finding of novel molecules acting at this class of ligand gated channels. Taking advantage of the crystal structure of the acetylcholine binding protein (AChBP), in silico ligand design from the chemical universe data base (GDB) [1] and virtual screening was performed. This allowed the identification of novel molecules that should display selectivity for the α7 nAChRs. Selected virtual hits were synthesized and their functional properties assessed at human nAChRs expressed in *Xenopus* oocytes. Experimental protocols were designed to probe the putative agonist or antagonist activities of these molecules. 72,945 virtual ligands were investigated using docking (Autodock and Glide) and shape similarity to known α7 ligands (ROCS). Thirty-eight structures among the 10% top-scoring virtual hits were selected for their structural novelty and ease of synthesis, prepared by standard organic synthesis methods, and their properties analyzed. Most of the molecules displayed antagonist properties with IC_{50}'s in the low micromolar range. Thus while this strategy properly identifies ligands that interact with the receptor, further refinement of our model is required for the identification of selective agonists. Altogether these data illustrate the power of combining virtual chemistry, small scale synthesis and electrophysiological approaches.

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1.21

Homology models of the alpha7 acetylcholine receptor based upon bacterial receptors: Comparison of experimental and *in silico* derived data

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Unraveling the mechanistic link between agonist binding and ion permeation in ligand-gated channels remains a challenge for modern biophysics. The recent high-resolution crystallization of

bacterial homologs of cys-loop receptor family of ion channels has provided atomic-level detail of these proteins and led to several proposed mechanisms of receptor gating. We used structures of two related bacterial channels to construct homology models of the chick alpha7 nicotinic receptor in putative closed and open states [1,2]. Profiles 3D and PROCHECK were used as an initial means to validate these models. We then compared our models with those constructed from multiple structural templates, such as bacterial ACh-Binding Proteins and the *Torpedo* AChR. While the closed and open alpha7 models share a great degree of global similarity to these related structures, we observed conformational variability in the agonist-binding site(s) that would appear to preclude binding of cholinergic agonists and antagonists. This observation is not unexpected, as neither of the bacterial homologs are acetylcholine-activated. We next asked if the models could be used to predict experimental data and perhaps lead to the development of testable hypotheses for gating. We compared rates of MTS modification of introduced cysteines [3] to *in silico* measurements of side chain solvent accessibility, local electrostatic potential, and pH. Our comparisons suggest that homology models such as these are likely to require an iterative process of refinement based upon experimental data before they can be used for molecular dynamics and predictive approaches.

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1.22

Heterogenity in release probability and depression dynamics at a nicotinic CNS connection

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The axo-axonic contact(s) between the Mauthner (M-) axon and cranial relay neuron (CRN) of the goldfish are readily accessible *in vivo* and provide a unique opportunity to study fast cholinergic synaptic transmission in the central nervous system (CNS). Using variance-mean analysis we have demonstrated that steady state frequency-dependent depression at CRN connections mediated by α7 nicotinic acetylcholine receptors (nAChRs) is largely due to a decreased release probability, *p*, under stationary conditions. Development of depression for α7 nAChR mediated CRNs under non-stationary conditions is shown here to also be due to a decrease in release probability, as explored with MPFA and corroborated with covariance analysis. The variance-mean data are well fit by a modified parabolic function and no correlation is observed between successive EPSP amplitudes (EPSPn + 1 vs EPSPn). Interestingly, latency increases with depression, which we attribute to the refractory state of the release machinery and not to a change in conduction velocity. The composite variance-mean data from M-axon/CRN connections mediated by α7/α3β2* or α7/α3β4* nAChRs often are not well fit by a single modified parabolic function, but are best approximated by two parabolic functions that represent different values of *p*, quantal size, *q*, and vesicles ready for release, *n*. These fits of composite variance-mean data are corroborated by determining the variance-mean data for each component, α7 and α3β*, separately. This is achieved by stripping the α7 component from the composite EPSP to yield the α3β* either with antagonists or by subtracting the α7 component, approximated from curve fitting and latency analysis. The resulting α3β* component data enables variance-mean, covariance and latency analysis. Based on non-stationary as well as steady state depression data we conclude that contacts mediated by different nAChRs, namely α7 and α3β2* or α3β4*, exhibit a wide range of release probability. Composite variance-mean data well fit by one modified parabola reflect M-axon/CRN connections mediated by α7 nAChR and by α3β* contacts that have similar release probabilities. However, composite variance-mean data better fit by two modified parabolas reflect contributions from α7 and α3β* contacts that exhibit release probabilities quite different from one another.

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Section 2. Cognition/cognitive deficits

2.1

Nicotinic receptor activation increases glutamatergic transmission and plasticity in the rat cerebellum

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Neuromodulatory systems of the brain have been suggested to profoundly impact on neurotransmission and long-term synaptic plasticity, the cellular correlate for learning and memory. The cerebellum, involved in procedural memory, receives abundant cholinergic innervation and shows a dense nicotinic acetylcholine receptor (nAChRs) expression. However, the functional effects of nAChRs in the cerebellum are still largely unknown. To address this issue we have performed voltage-clamp recordings in whole-cell configuration in the granular layer of acute slices obtained from the cerebellar vermis of P18-P22 rats. A 100-sec application of nicotine (1 μM) significantly enhanced glutamatergic EPSCs. The effect was transient, suggesting that nAChR were progressively desensitizing. As nAChRs are often located in the presynaptic terminals where they modulate other neurotransmitter release we have therefore investigated whether a similar mechanism could operate in the cerebellum. EPSCs mediated by AMPA receptors were elicited in pairs with an interpulse interval of 20 ms. Nicotine exposure readily caused a reduction of the pair pulse ratio (PPR). Moreover, a high calcium buffer concentration in the intracellular solution was still accompanied by a significant PPR decrease during nicotine application supporting its presynaptic origin. EPSCs mediated by NMDA receptors were not influenced by nicotine. Interestingly, when a high calcium buffer concentration was added to the intracellular solution, the effect of nicotine was restored and NMDA EPSCs increased. Therefore, nicotine could act both pre- and postsynaptically. The enhancement of neurotransmission caused by nicotine suggested that nicotine could also enhance the induction of LTP. We therefore tested whether a single 100 ms/100 Hz burst, which determines a long-term depression of EPSC peak could turn into LTP induction in the presence of nicotine. Exposure to 1 μM nicotine led the development of LTP of the EPSCs following the 100 ms/100 Hz burst. To explore