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

which nAChR subtype mediated the facilitating effect of nicotine on LTP, recordings were performed in the presence of α7 nAChR agonist and antagonist: choline (10 mM) and MLA (100 nM), respectively. The application of choline (100 s) increased the EPSC and then a single 100 ms/100 Hz burst led to LTP. The co-application of nicotine with MLA (100 s) prevented switching form LTD to LTP. These results suggest that cholinergic stimulation mediated by nAChRs markedly potentiates synaptic transmission and long-term synaptic plasticity along the mossy fibre pathway of the cerebellum.

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2.2

Nicotinic acetylcholine receptors differentially regulate phosphorylation of dopamine target cell proteins in the rat prefrontal cortex

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The prefrontal cortex (PFC) is an executive area of the brain critical for working memory and decision-making. Disorders such as schizophrenia and attention deficit hyperactivity disorder reflect in part an imbalance of prefrontal dopamine. Nicotine, via nicotinic acetylcholine receptors (nAChRs), facilitates dopamine release and enhances executive functions of this region [1], yet the downstream molecular consequences of nAChR-induced dopamine release remain to be fully elucidated. Here we examine the effect of stimulation of nAChRs containing either β2 or α7 subunits upon dopamine receptor D1 signalling pathways *in vitro* in PFC prisms (150 μm) using immunoblotting. Striatum was compared with PFC in parallel, because dopamine and cAMP regulated phosphoprotein of 32 kDa (DARPP-32), are known downstream effectors in this region [2]. Nicotine (100 μM) significantly increased phosphorylation of DARPP-32 at Thr34, indicative of increased cell excitability [2], in striatal but not PFC prisms. Blockade by mecamylamine and insensitivity to α-bungarotoxin (αBgt) supports a mechanism involving β2* nAChRs. This is consistent with comparable changes elicited by the β2* nAChR selective agonist 5-Iodo-A-85380. Activation of α7 nAChRs using choline (3 mM) co-applied with PNU-120596 (10 μM) significantly increased DARPP-32 phospho-Thr34 in PFC and striatum to a similar extent (68 ± 21 % and 75 ± 18 % above basal, respectively), sensitive to αBgt. Increased DARPP-32 phosphoThr34 was also observed in both regions in response to the D1 agonist SKF81297 (10 μM). AMPA receptors are key determinants of cell excitability. AMPA receptors were also significantly phosphorylated following treatment with nicotine, choline plus PNU-120596 or SKF81297 at Ser845, a site associated with increased cell surface trafficking [3], in PFC and striatal prisms. The D1 antagonist SCH23390 (1 μM) blocked all phosphorylation events described (DARPP-32 and $AMPA_R$). Total DARPP-32 and glyceraldehyde 3-phosphate dehydrogenase levels were unaffected by any treatment. These data suggest that PFC α7 nAChR activation facilitates dopamine release that via D1 receptors, increases $AMPA_R$ phospho-Ser845, possibly downstream of DARPP-32 activation. Nicotine-mediated increases in phospho-$AMPA_R$ relies on β2* nAChRs and does not involve DARPP-32. Cellular localization of these changes is under investigation. Elucidation of dopamine signaling in the PFC facilitated by particular nAChR subtypes is critical when viewing these receptors as therapeutic targets.*Acknowledgements*: Supported by BBSRC grant BBS/S/15600 to SW BBSRC Studentship to PL.

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2.3

Nicotine activates a dopamine signal that enables *in vivo* synaptic plasticity of the kind that underlies associative memory

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It has been documented that nicotine induces synaptic plasticity in the hippocampus, which might underlie drug-linked memory that cues continued tobacco use. The hippocampus is an important center for contextual and spatial memory. It receives cholinergic inputs from medial septum and dopaminergic innervation from the midbrain dopaminergic systems. Nicotinic acetylcholine receptors (nAChRs) and dopaminergic receptors (DARs) are co-expressed in hippocampus, including the dentate region. Although DA–ACh interactions in the hippocampus have been reported to be critical for drug-associated memory, it is not known how dopaminergic signaling influences nicotine-induced synaptic plasticity at the physiological level. Here we examined the effects of long-range vs. local manipulation of dopaminergic D1- and D2-type receptors on nicotine-induced synaptic plasticity of the perforant path to the dentate gyrus of awake, freely moving mice. Field recordings from the hilar region of the dentate were measured in response to electrical stimulation in the medial part of the perforant path. The recording and stimulating electrodes were implanted 2-3 weeks before the tests. Acute application of nicotine (i.p.) dose-dependently induced long-term potentiation (LTP) in this pathway. Nicotine (at the biologically relevant dose of 1.0 mg/kg) caused a long-lasting potentiation of the evoked responses. When DA signaling was inhibited by systemic administration of D1-type receptor antagonist SCH23390 or D2-type agonist quinpirole, we found that nicotine-induced LTP was blocked. In contrast, D2-type antagonist eticlopride showed significant enhancement of the LTP induction. To further dissect the local vs. long-range effect of dopaminergic manipulation, SCH23390 was ipsilaterally infused into the dentate gyrus prior to nicotine. The nicotine-induced LTP was completely abolished by the inactivation of D1-type receptors in the dentate. Consistent with the need for an incoming DA signal from the midbrain, local infusion of nicotine in the dentate did not alter the synaptic plasticity along the recording pathway. Inactivation of the midbrain dopaminergic area by tetrodotoxin prevented the nicotine-induced plasticity in the dentate. Together, these results suggest that dopaminergic signaling serves as a functional label of salient events by enabling synaptic plasticity that underlies drug-induced associative memory.

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