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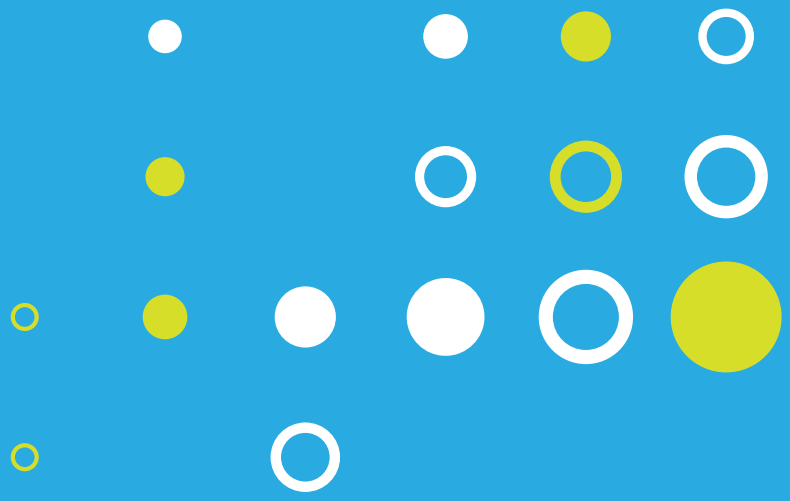

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NS-P01.
PARTITION OF NICOTINIC ACETYLCHOLINE RECEPTOR IN MODEL MEMBRANES
Bermúdez V, Antollini SS, Fernández Nievas GA, Aveldaño MI, Barrantes FJ.
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Nicotinic acetylcholine receptor (AChR) affinity-purified from *T. californica* membranes and a synthetic peptide corresponding to the M4 transmembrane region (ãM4) of the receptor were reconstituted into synthetic liposomes with lipid compositions resembling that of raft domains (PC:SM:Chol, 1:1:1). The preferential localization of AChR or ãM4 in such synthetic membranes was analyzed using detergent-resistant (DRM) or detergent-soluble domains (DSM) obtained by treatment with 1% Triton X-100 at 4°C followed by SDS-PAGE and Western blotting. The influence of the ganglioside GM1 on AChR was also analyzed with this techniques. The efficiency of the Förster resonance energy transfer (E) between the protein intrinsic fluorescence and dehydroergosterol (fluorescent cholesterol mimetic) served as a measure of the protein location in the membrane. Purified AChR displayed no preferential partition between raft and non-raft domains whereas the ãM4 peptide preferentially partitioned into ordered domains. The presence of GM1 increased the proportion of AChR in the DRM fractions, and antibody-mediated crosslinking of the ganglioside induced a significant redistribution of the AChR towards the DSM fraction. Thus, the preferential localization of the AChR protein in the membrane may not only be governed by its lipid-protein interface.

NS-P02.
INTRACELLULAR GSH MEDIATES THE DENITROSYLATION OF PROTEIN NITROSOTHIOLS IN THE RAT SPINAL CORD
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Protein S-nitrosothiols (PrSNOs) have been implicated in the pathophysiology of neuroinflammatory disorders characterized by extensive nitrosative stress. Using rat spinal cord slices we have previously established that S-nitrosoglutathione (GSNO) is a viable intercellular S-nitrosylating agent. Moreover, generation of PrSNOs with GSNO occurs exclusively via a S-transnitrosylation mechanism. Although the metabolical instability of PrSNOs is well known, there is little understanding of the factors involved in the cleavage of S-NO linkages in intact cells. To address this issue, we conducted chase experiments in spinal cord slices incubated with GSNO. The results show that removal of GSNO leads to a rapid decreasing of PrSNOs ($t_{1/2}$ ~ 2h), which is greatly accelerated when glutathione (GSH) levels are raised with the permeable analogue GSH ethyl ester, suggesting that GSH plays a key role in the denitrosylation process. Inhibition of both GSH-dependent enzymes and enzymes that could mediate denitrosylation do not alter the rate of PrSNO decomposition. The lack of protein glutathionylation during the chase suggest that most proteins are denitrosylated via rapid transnitrosylation with GSH. The differences in the denitrosylation rate of individual proteins would indicate the existence of additional structural factors in this process.
Supported by NIH grant NS 47448.

NS-P03.
MODULATION OF ACH RELEASE AT THE EFFERENT-IHC SYNAPSE BY THE GABAERGIC SYSTEM
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Before the onset of hearing, inner hair cells (IHC) of the mammalian cochlea are transiently innervated by medial olivocochlear (MOC) efferent fibers. This synapse is cholinergic, inhibitory and mediated by the á9á10 nicotinic receptor. Although ACh is the main transmitter released at this synapse, there is evidence showing that the GABA is present at MOC synaptic terminals and that GABAA receptors are expressed in outer hair cells (OHC). However, no GABAergic currents have been recorded so far in IHC or OHC. Moreover, the possibility that synaptically released GABA could be modulating the cholinergic input at MOC-synapses by acting on presynaptic GABAB receptors has not been investigated yet. In the present work, we therefore evaluated the effects of a GABAB antagonist (CGP55845) on the quantal content of transmitter release at the MOC-IHC synapse. Postsynaptic cholinergic currents, evoked by electrically stimulating the efferent fibers, were recorded in voltage-clamped (-90 mV) IHCs from acutely isolated mouse organs of Corti. The quantal content of evoked release was significantly increased by 0.3 ì M CGP55845 (60 ± 25 %). The lack of effect of CGP55845 on spontaneous synaptic current amplitude indicates that the site of action of this drug is presynaptic. Our results suggest that GABA might be exerting a negative feedback control on the release of ACh at this synapse.

NS-P04.
FIRST SNAIL EGG PROTEIN WITH A NEUROTOXIC EFFECT ON MICE
Frassa MV[1], Fernández PE[2], Dreon MS[3], Galosi CM[4], Gimeno EJ[3], Heras H[3].
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While many invertebrates sequester toxic compounds to endow eggs with chemical defences, here we show, for the first time to our knowledge, the identification of a neurotoxin of proteinaceous nature localized inside an egg.
Egg extracts from the freshwater apple snail *Pomacea canaliculata* displayed a neurotoxic effect in mice upon intraperitoneal injection (i.p.) (LD50, 96 h 2.3 mg/kg). Egg protein and total lipids were analysed separately and the only fraction displaying a highly toxic effect (LD50, 96 h 0.25 mg/kg, i.p.) was further purified to homogeneity as an oligomeric glyco-lipoprotein of 400 kDa indistinguishable from the previously described perivitellin PV2. The neurotoxin was heat sensitive and there was evidence of circulating antibody response to sublethal i.p. doses on mice. Clinical signs, histopathological and immunocytochemical studies revealed damage mostly in mice spinal cord. Experiments showed chromatolysis and a decreased response to calbindin D-28K associated with a significant increase of TUNEL-positive cells (terminal deoxynucleotidyl transferase (TdT)–UTP–biotin nick end labelling) in the dorsal horn neurons.
These results suggest that calcium buffering and apoptosis may play a role in the neurological disorders induced by the toxin in mammalian central nervous system.