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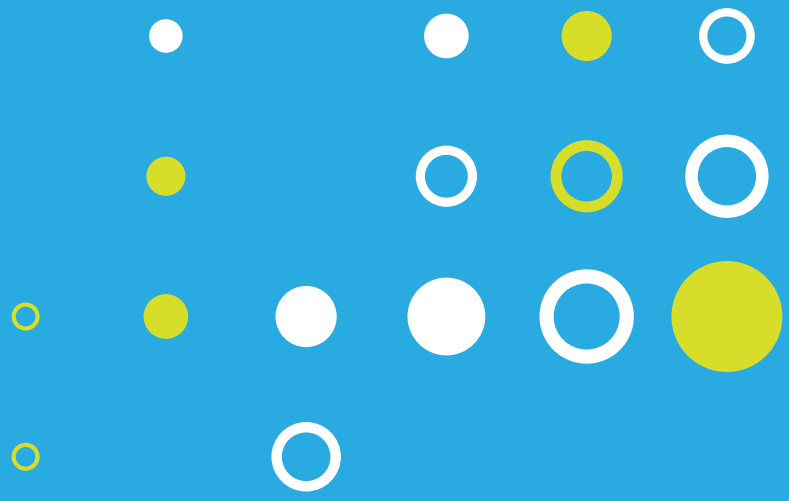

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NS-P09.
LOCALIZATION OF RETINALDEHYDE ISOMERASE IN THE CHICKEN INNER RETINA
Diaz NM, Morera LP, Guido ME.
CIQUIBIC (CONICET)- Dpto. Qca Biol., Fac. Cs. Químicas, Univ. Nac. Córdoba, Córdoba. E-mail: ndiaz@fcq.unc.edu.ar

Retinal cone and rod photoreceptor cells (PRC) are responsible for day and night vision respectively while the inner retina has been mainly involved in the transmission of the nerve impulse from PRC to the brain. However, a third group of PRC has been shown recently to be present in the inner retina, specially in intrinsically photosensitive retinal ganglion cells (reviewed in Guido *et al.*, 2010). Moreover, different non-visual opsins such as Opn4 , Opn5 and RGR were shown to be expressed in the inner retina. However, it is still unknown the mechanism used to regenerate the photopigment chromophore. RPE65 is the main isomerohydrolase in the vertebrate eye expressed in the retinal pigment epithelium but not in the neural retina.The zebra fish has three different isoforms for the RPE65 enzyme of which, RPE65c is expressed in the retina. In the chicken, we observed the expression of the RPE65c homolog in the inner retina only, especially in the inner nuclear and inner plexiform layers. Using specific cell markers we found that expression was mainly restricted to amacrine cells. In addition, we found isomerohydrolase activity in this retinal area. In conclusion, chicken amacrine cells display the expression and activity of this non-typical isomerase. Results provide first evidences on the mechanism by which the inner retina may regenerate the chromophore linked to non-visual opsins.

NS-P10.
NOVEL ISOLATION OF CHICKEN RETINAL HORIZONTAL CELLS
Morera LP, Diaz NM, Guido ME.
CIQUIBIC-Dpto de Química Biológica, Fac. Cs. Qcas, Univ. Nac. Córodba-CONICET, Córdoba, Argentina. E-mail: lmorera@fcq.unc.edu.ar

We described that the non-visual photopigment melanopsin X is expressed mainly in horizontal cells of the chicken retina (Verra *et al*., 2011). In this work our aim was to purify and culture horizontal cells (HC´s) from the chicken embryonic retina for further characterization.
Disaggregated retinas of chicken embryos at day 14 were subjected to a bovine serum albumin (BSA) discontinuous gradient of concentrations ranging from 1 to 5%. After centrifugation, cells collected from the different phases were cultured for 4 days and characterized by immunochemistry and cell morphology. Phases were examined with specifics antibodies against HC markers: PROX-1 and Islet-1. Results showed that only the fraction corresponding to 2.5% of BSA was highly enriched in PROX-1 positive cells ($\geq$ 95%) displaying a typical HC morphology. In fact, some cells in this fraction resembled axon-less candelabrum-shaped HC's. Preliminary results showed that around 50% of the PROX-1 cells in this phase were positive for Islet-1. Moreover, Western blot assays demonstrated that mainly the phase at 2.5% BSA, exhibited positive PROX-1 immunoreactivity (MW: 83kDa).
In conclusion the BSA gradient proved to be the most effective method to separate different retinal cell populations, and particularly HC´s which will allow us to characterize them as potential photoreceptors by biochemical and pharmacological studies.

NS-P11.
RESPONSE OF FLY MUTANTS FOR THE METABOLISM OF N-ß-ALANYLDERIVATIVES TO CROWDING STRESS
Rossi FA, Sabio G, Quesada-Allué LA, Pérez MM.
IIBBA-CONICET, QB-FCEyN-UBA and FIL. Av. Patricias Argentinas 435, Buenos Aires, Argentina (1405). E-mail: frossi@leloir.org.ar

In *Drosophila melanogaster*, Ebony and Tan proteins are responsible for the synthesis and hydrolysis, respectively, of N- -alanylderivatives like N- -alanyldopamine (NBAD). Together, they establish a system that regulates dopamine (DA) and other neurotransmitter levels in insects, maintaining central nervous system (CNS) homeostasis; which is altered in null-function mutants. *ebony* presents reduced levels of NBAD and excess of DA; the opposite is true for *tan*. In *Drosophila* DA plays central regulatory roles controlling sleep and wakefulness. In a crowded environment, a high release of neurotransmitters occurs, so flies need to recycle DA in order to avoid potential oxidative stress in the CNS. Our aim was to compare wild type and mutants response to crowding stress. Main parameters studied were sleep patterns (recorded in activity monitors), lipid peroxidation (indicative of oxidative stress) and NBAD metabolism (indicator of DA recycling). Both *ebony* and *tan* mutant strains exhibited differences in sleep patterns. *ebony* flies showed a decrease in total sleeping time at night. *tan* showed difficulties maintaining sleep, with a decreased in the duration of sleep bouts, but an increase in their frequencies. Differences in lipid peroxidation in response to stress were observed between the strains. These results suggest a role of the Ebony-Tan system in the maintenance of CNS homeostasis.

NS-P12.
NOT JUST ANY FREE FATTY ACID INHIBITS THE NICOTINIC ACETYLCHOLINE RECEPTOR
Perillo VL, Vallés AS, Barrantes FJ, Antollini SS.
INIBIBB, CONICET – Universidad Nacional del Sur, B. Blanca, Argentina. E-mail: vperillo@criba.edu.ar

To elucidate the mechanism involved in the non-competitive inhibition of the nicotinic acetylcholine receptor (AChR) caused by free fatty acids (FFAs), we studied the effect of FFAs with a single double-bond at different positions (6, 9, 11 and 13, *cis*-18:1) on different AChR properties. Two FFAs (6 and 9) reduced the duration of the channel open-state. The briefest component of the closed-time distribution remained unaltered, suggesting that 6 and 9 do not behave as typical open-channel blockers but rather as allosteric blockers. Fluorescence resonance energy transfer studies showed that all FFAs locate at the lipid-AChR interface, 6 being restricted to annular sites and all others occupying non-annular sites. Fluorescence quenching studies of pyrene-labeled AChR indicate that all *cis*-FFAs produce AChR conformational changes at the transmembrane level. Using the AChR conformational-sensitive probe crystal violet, we observed that all unsaturated FFAs increase its K_D in the AChR desensitized state, but only 9, 11 and 13 *cis*-18:1 decrease its K_D in the resting state. In conclusion, some FFAs appear to directly inhibit AChR function probably by localizing at superficial sites inside the membrane, whereas other FFAs modulate the receptor´s conformational states by a different mechanism.