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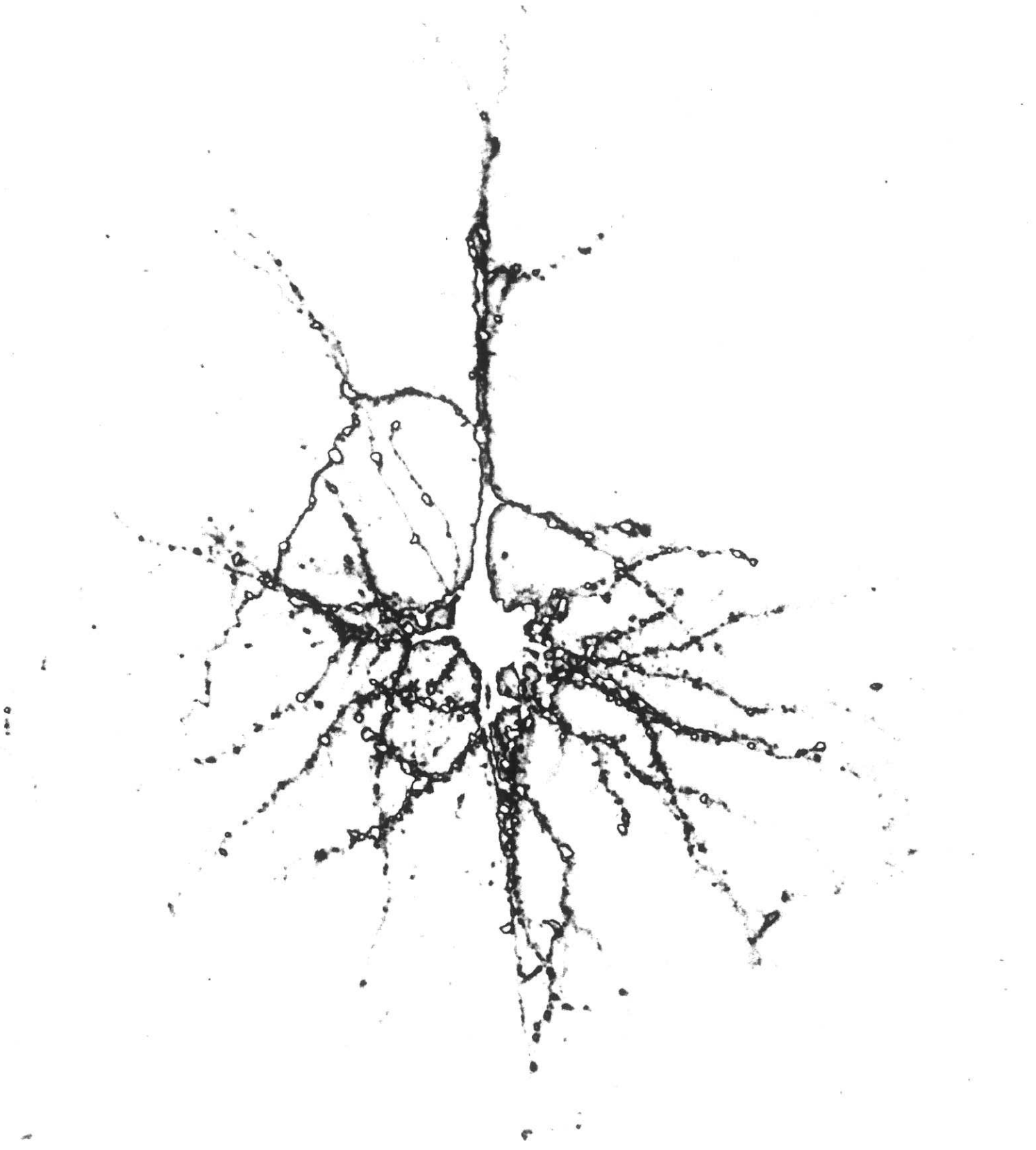

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122 258 **DARK–REARING AFFECTS THE NUMBER BUT NOT THE SUBUNIT COMPOSITION OF HETEROMERIC NICOTINIC RECEPTOR EXPRESSED DURING THE RETINA DEVELOPMENT IN RAT**

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Acting through nicotinic receptors (nAChRs), acetylcholine plays an important role in retinal development and the formation of retinal connections to target tissues, but very little is known about the nAChR subtypes expressed in vertebrate retina during neuronal development and how are regulated by sensory stimuli. We used immunoprecipitation and nicotinic ligand binding to study the expression of retinal heteromeric nAChRs during development and adulthood, and found that it is strictly developmentally regulated, reaching a peak on postnatal day 21. At all developmental stages, more than 80% of the high affinity 3H-Epibatidine binding receptors contained the beta2 subunit, whereas the expression pattern of the alpha subunits was more heterogeneous and changed with development: the alpha4 subunit was present at all stages and accounted for more than 50% of the receptors, whereas the levels of the alpha2 and alpha6 subunits increased more than 30 times from P1 to P21, when they were present in 25-30% of the receptors. Raising rats in complete darkness from birth until the age of 21 days resulted in a significant increase (25%) in the expression of 3H-Epibatidine receptors in retina. Moreover, parallel immunoprecipitation studies of retina obtained from control and dark-reared animals using subunit- specific polyclonal Abs showed that the expression ratios of the different nicotinic subtypes is not affected by light deprivation.

123 105 **DEVELOPMENT OF PRIMARY VISUAL CORTEX: ROLE OF NICOTINIC RECEPTORS AND VISUAL INPUT.**

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Alpha4 and a7 subunits compose the most abundant subtypes of nicotinic receptors in mammalian cortex. In order to study the participation of these two cholinergic subunits in the formation of cortical circuitry, we have sampled different rat postnatal ages starting from postnatal day 12 (P12), when eyes have not been opened yet, to adulthood. Additionally, we have studied the effect of light input on the expression levels of these subunit transcripts. To address this matter, rat pups were reared in darkness from P10 to P23, which corresponds with the peak of the critical period. We have found that before eye opening, the abundance of the a4 transcript is comparable to that of a7, while in the adulthood the relative amount of a4 mRNA is ~30% higher than that of a7. We have determined as well that both, a4 and a7 expression levels, undergo a slow but constant increment that, in the case of a4 reaches a plateau by P35, roughly corresponding to the end of the critical period, and remains unchanged in adulthood. On the other hand, a7 transcript level peaks its expression at P35 to finally decrease to a level comparable to that found at P23. Finally, we found no significant changes in the level of expression of a4 or a7 mRNA in dark reared animals. Taken together, these results suggest that the primary visual cortex organization or refinement could be related, at least in part, to the expression of these two cholinergic subunits, as their expression profile undergoes a constant increment along early postnatal development. Particularly, the a7 increment could reflect its putative involvement in different developmental phenomena like gene expression and/or activation of intracellular cascades. Finally, as shown by the light deprivation experiments, visual input appears to be unessential for maturation of nicotinic AChR expression in primary visual cortex.

124 148 **DEVELOPMENTAL REGULATION OF NEUROTROPHIN-4 IN CENTRAL NERVOUS SYSTEM**

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Neurotrophin family of proteins (NGF, BDNF, NT3, NT4/5) has been shown to be involved in neuronal differentiation, survival and plasticity in CNS. The least studied one, NT4/5, binds to TrkB like BDNF to elicit cellular responses. Data on the expression of this protein in CNS are lacking. Here we studied the expression and quantification of the NT4/5 in the mouse visual cortex and hippocampus. We performed a sandwich ELISA to quantify the amount of NT4/5. We used four different age groups of animals. The age group consisted of embryonic stage (E14), P10 (postnatal days 10, before eye opening), P20 (peak of the critical period) and P60 (adult stage). We have used NT4/5 knock out mice as control for specificity of the antibody. The cross reactivity of the antibody with other neurotrophins was also tested. We found the expression of protein from the embryonic age of the mice. The protein levels are around 11 to 18 picograms. We performed an immunohistochemistry to show the cellular expression of NT4/5. In the visual cortex, NT4/5 is expressed in pyramidal neurons of layers 4 and 5, whereas it could be detected at a very low level in the other cortical layers. In the hippocampus we found it to be expressed in the interneurons. To be sure that the immunoreactivity for NT4/5 corresponded to the synthesized protein and not to protein taken up by cells possessing TrkB receptor we investigated the expression of NT4/5 mRNA by using non-isotopic in situ hybridization. The riboprobe that we used was synthesized from the cDNA containing the coding region of the mouse NT4. We found that this probe show labeling also in layer II-III in addition to layer V neurons; labeling of layer II-III neurons was present to some extent in knockouts mice. We have also designed probes from the 3'untranslated regions of the alternatively splice isoform containing the exon 1 and from the 5'untranslated regions, to see the specific expression of the different NT4/5 isoforms.

125 239 **DIFFERENT ROUTS OF DRUG ADMINISTRATION IN THE PERINATAL PERIOD: EFFECTS ON PREGNANCY AND ON OFFSPRING'S BEHAVIOUR.**

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It is well established that pregnancy and neurobehavioral development of the offspring may be affected by prenatal or postnatal maternal stress. In the present study we investigated whether drugs administration by gavage, a procedure commonly considered as 'stressful', might exert different effects on reproductive performance of CD-1 Swiss female mice and on the behaviour of the offspring, compared to drug self-administration. From gestational day (GD) 6 to postnatal day (PND) 21 corn oil (0.1 ml/50 g body weight/day) was administered daily to dams intragastrically (gavage group) using a 3-mm in diameter, 3-cm long curved feeding needles attached to a 1-cm3 syringe, or by a procedure that allowed oral administration without disturbing the animal (non-gavage group). This consisted of introducing through the cage top a modified syringe (without the needle and with a larger hole) and letting the animal drink the oil from it. A further group of dams was left undisturbed (control group). Pregnancy duration, number, sex ratio and body weight of pups on PND 1 were not affected by the different administration procedure. Nevertheless, the offspring from dams in the gavage group had a lower body weight in comparison with pups of both non-gavage and control groups from PND 8 to PND 18. This difference disappeared starting on PND 20. Offspring of the three groups underwent an open field test at different ages (PND 34, 60, 90, and 120) and results did not show any difference in all the behavioural parameters considered (thigmotaxis, number of crossings, number of rearings and wall rearing). In conclusion, our data suggest that gavage procedure may not be as stressful for the offspring as commonly assumed. Further studies are needed to assess the long-term effects of early administration procedures on behavioural and hormonal parameters, as well as the interaction between route of administration and pharmacological treatment.

126 326 **DIFFERENTIAL EXPRESSION OF CHAT IMMUNOREACTIVE NEURONS DURING DEVELOPMENT IN DARK REARED RETINAS OF THE CHICK .**

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The purpose of this study was to evaluate the influence of light on the morpho-functional development of cholinergic neurons in the chick retina. White Leghorn chicken eggs were incubated at 38°C and 60% relative humidity for the desired period of time. The eggs were incubated in two different conditions. A series of several stages of incubation (E7, E9, E12, E15, E17, E19, E21: the day of hatching) was maintained under constant darkness (treated embryos); a second series was incubated under a diurnal cycle of illumination and was used as control at the same ages as treated animals. The day of experiments embryos and hatchings were removed under dim light illumination heavily anaesthetized with ether, decapitated and quickly immersed in the fixative (PAF 4% in PBS). The eyes were removed, maintained for a maximum of 1h in the fixative. For cry protection prior to cryostatic sectioning the cup eyes were immersed in sucrose 15% in saline buffer 0.1M pH 7.2 Chat immunoreactivity was detected by subsequent steps of biotin and extravidina FITC conjugated. Modifications have been found at the level of the IPL and of the presumptive cholinergic amacrine cells in the embryonic retinas incubated in complete darkness. compared to the control or to the results described in the bibliography (Spira et al., 1987. J. Comp. Neurol., 260: 526-538). Since the chick retina is considered a mainly cholinergic nervous structure, development in acute condition of darkness could imply several modifications in the retinal circuitry

127 473 **DISTROGLICANO E SVILUPPO DEL SISTEMA NERVOSO CENTRALE**

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Il distroglicano è un membro del complesso multiproteico di glicoproteine associate alla distrofina, presente sia nel muscolo sia nel sistema nervoso centrale. Questo recettore proteico transmembrana, altamente glicosilato, svolge un importante ruolo strutturale, legando componenti della matrice extracellulare a proteine citoscheletriche. La maggior parte delle distrofie finora conosciute è associata a mutazioni su proteine del complesso sopracitato, però nessuna patologia distrofica sembra essere dovuta a mutazioni dirette del gene del distroglicano, infatti in modelli animali, è stato dimostrato che è un gene essenziale nei primi stadi dello sviluppo e che la sua assenza provoca difetti che risultano letali già 6.5 giorni dopo il concepimento. Dati recenti dimostrano che alcuni tipi di distrofie muscolari congenite quali la Fukuyama, la MEB e la sindrome di Walker-Warburg, nelle quali i pazienti evidenziano anche forti difetti nello sviluppo del sistema nervoso centrale (microftalmia, riduzione delle cellule gangliari retiniche, distacco della retina, lissencefalia, fusione degli emisferi, errata migrazione cellulare in corteccia ecc.), sono implicabili a mutazioni di geni (Fukutin, POMGnT1) responsabili dei processi di glicosilazione del distroglicano. Al fine di indagare sul ruolo del gene del distroglicano durante le fasi precoci dello sviluppo del sistema nervoso centrale, abbiamo isolato tale gene da Xenopus laevis, e ne abbiamo analizzato l'espressione mediante tecniche di ibridazione in situ. Il distroglicano di Xenopus è altamente omologo a quello umano; la presenza del suo mRNA può essere messa in evidenza già a stadio 13-15 a livello della notocorda e della piastra neurale anteriore, successivamente il pattern di espressione a livello del sistema nervoso centrale è molto complesso e dinamico, suggerendo un diretto coinvolgimento nella differenziazione di specifiche strutture. Attualmente stiamo effettuando esperimenti di perdita di funzione, tramite la tecnica del morpholino, per impedire la produzione di distroglicano endogeno e microiniettare forme mutanti di distroglicano ricombinante, in modo da studiare la funzione di specifiche porzioni della proteina.

128 418 **ENHANCED NEUROGENESIS IN NF-KAPPAB P50 NULL MICE**

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The adult brain has little capacity to generate new cells. One exception is the subventricular zone (SVZ) where some stem cells persist and continue to divide throughout life. Although the vast majority of newly generated cells die, some of these migrate along the rostral migratory stream (RMS) and differentiate into the olfactory bulb. The combination of genes that regulates the proliferation and cell fate determination of subventricular zone precursors remains largely unknown. We have shown previously that distinct rel/NF-kB proteins are selectively expressed both postnatally and throughout adulthood in two neurogenic regions of the telencephalon: the SVZ and the RMS. All cell types expressing NF-kB family members belong to the lineage of olfactory interneurons. These results suggest that NF-kB complexes of various composition (homo and heterodimers) may act as transcriptional regulators of neurogenesis and/or migration and cell fate determination. In this work, we have analyzed the phenotye of mice carrying a homologous deletion of the NF-kB1 gene. We have used different protocols of BrdU administration to label cell populations with different cell cycle length, in particular the rapidly dividing population and the