

Importantly, GABA_A receptors act differently during development compared to adulthood; the early exposure leads to excitation rather than inhibition of GABA_A receptors. This excitation, together with NMDA receptor activation, may potentially trigger excess apoptosis affecting neural development.

Conclusions: Specific organic solvents (*n*-hexane; the aromatic hydrocarbons benzene, toluene, styrene, and xylene; the halogenated solvents trichloroethylene and 1-bromopropane; and alcohols *n*-propanol and *n*-butanol) display narcotic effects after acute exposure, NT or narcosis after repeated dosing, and DNT, suggesting a possible link between narcosis and (D)NT. Most of the investigated substances with acute narcotic effects did not show NT in adult animals or DNT, but investigations for DNT appear often lacking, potentially due to lack of identified concern from studies in adult animals or structurally similar substances. It is hypothesised that early interaction with GABA_A and/or NMDA receptors might mechanistically explain (D)NT. However, a clear and undisputed mechanistic link between narcosis/anaesthesia and (D)NT has not been established in the literature as yet. On a general level, it cannot be concluded that narcotic effects exerted by organic solvents after acute or repeated exposure are automatically linked to NT/DNT; it is recognised, though, that available information on effects following early exposure is scarce.

Reference

- [1] Regulation (EC) No 1907/2006 on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

<http://dx.doi.org/10.1016/j.reprotox.2017.06.143>

P-5

Exposure to cannabinoid receptor 1 ligands induces miswiring of GnRH axons in the brain of zebrafish embryos

Patrizia Bovolini^{1,*}, Ilaria D'Atri^{1,2}, Erika Cottone¹, Valentina Pomatto¹, Giulia Zuccarini¹, Yoav Gothilf³, Giorgio R. Merlo⁴

¹ Department Life Science and Systems Biology, University of Torino, Italy

² University of Exeter Medical School, Exeter, UK

³ Department Neurobiology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Israel

⁴ Department Molecular Biotechnology and Health Sciences, University of Torino, Italy

Introduction: CB1 cannabinoid receptors (CB1) are the targets of marijuana (*Cannabis* spp)-derived phytocannabinoids (e.g. Δ⁹-THC) and endocannabinoids (AEA and 2-AG). Children exposed in utero to cannabis present permanent neurobehavioral and cognitive impairments [1]. CB1 cannabinoid receptors are widely expressed in the brain and they have been recently recognized as regulators of brain development, including wiring of neuronal connections. In the zebrafish (*zf*) embryo, previous data showed that CB1 knockdown causes defects in axonal fasciculation in the anterior commissure [2]. Since this area is particularly rich in Gonadotropin Releasing Hormone (GnRH) fibers, we assessed whether pharmacologic modulation of the CB1 receptor could modify GnRH axonal pathfinding and fasciculation in zebrafish embryos.

Methods: We treated transgenic GnRH3::GFP *zf* embryos with increasing concentrations of various CB1 antagonists from 0 to 72 hpf. We also performed morpholino-mediated CB1 knockdown and analyzed several parameters such as survival, hatching time and morphology. Expression levels of key genes potentially involved in CB1-mediated effects were monitored by Real-Time RT-PCR.

Results: Following CB1 antagonist treatment, we found a reduction in GnRH neuropil extension and axon misrouting in the anterior commissure. Morpholino-mediated downregulation of CB1 expression reduced the number of GnRH3::GFP positive cells in the olfactory epithelium while not changing their position. Finally, we observed that CB1 knockdown downregulates the expression of two genes involved in axonal growth and cell migration, namely *Stmn2a/b* and *Sez6a/b*.

Conclusions: Taken together these results indicate that during early zebrafish development, CB1 acts as a regulator of axonal pathfinding on GnRH cells. Future experiments will elucidate if the CB1 miss-regulation also affects GnRH neuron migration from the olfactory placode to the hypothalamus, with consequent effects on sexual maturation and reproduction.

References

- [1] D. Calvigioni, Y.L. Hurd, T. Harkany, E. Keimpema, Neuronal substrates and functional consequences of prenatal cannabis exposure, *Eur. Child. Adolesc. Psychiatry* 23 (2014) 931–941.
[2] S. Watson, D. Chambers, C. Hobbs, P. Doherty, A. Graham, The endocannabinoid receptor CB1 is required for normal axonal growth and fasciculation, *Mol. Cell. Neurosci.* 38 (2008) 89–97.

<http://dx.doi.org/10.1016/j.reprotox.2017.06.144>

P-6

Skeletal examination in a chick embryo model of fetal alcohol syndrome indicates impaired osteogenesis



Deirdre Clissmann, Deirdre Brennan*

School of Medicine, University College Dublin, Belfield, Dublin 4., Ireland

Introduction: Fetal Alcohol Syndrome (FAS) occurs as a direct result of ethanol exposure during the gestational period. The syndrome manifests as a range of developmental defects including aberrant skeletal development [1,2]. Several bone and joint anomalies have previously been reported in both human and animal models of FAS, including radioulnar synostosis [1] and inhibition of long bone development [2]. This study aimed to (1) qualitatively analyse the axial and appendicular skeleton of the chick following early ethanol exposure and (2) quantitatively measure the effects of ethanol on osteogenesis of the skeletal long bone, the radius.

Methods: Fertile chicken eggs (*Gallus gallus domesticus*) were pre-incubated at 37 °C for 24 hours. 10 μl of 0, 1%, 5%, 10% or 20% ethanol solution in chick saline (0.73% NaCl) was injected into the yolk sac via a puncture hole in the eggshell (*n*=5 embryos per group). Nine days post-treatment, embryos were dissected and skeletal staining performed according to standard Alcian Blue/Alizarin Red protocols [2]. For quantitative measurement of the radius, total bone length, percentage ossification and growth plate length were analysed (*n*=10 radii per group).

Results: Ethanol-induced defects were evident in (1) the cranial region with presentation of microcephaly in the 10% ethanol group; (2) the clavicle which demonstrated impaired osteogenesis with increasing dose; and (3) a concentration-dependent decrease in ossification of the metacarpals. From radial measurements, significant reductions in length were found in both the 1% and 5% ethanol groups. Ossification of the radius was also significantly impaired in the 5% group when compared with control counterparts.

Conclusions: Qualitative analysis has demonstrated that perturbation of skeletal development is induced by ethanol exposure, affecting bones of the pectoral girdle and upper limb in particular. Quantitative radial measurement further confirmed an ethanol induced retardation of ossification and total bone length. This work