

# Fetal cannabinoid receptors and the “dis-joint-ed” brain

Luigia Cristino & Vincenzo Di Marzo\*

**Microtubule turnover in the growing axons is required for directional axonal growth and synapse formation in the developing brain. In this issue of *The EMBO Journal*, Tortoriello *et al* (2014) show that the microtubule-binding protein SCG10/stathmin-2 is a specific molecular target for a CB<sub>1</sub> receptor-mediated effect of  $\Delta^9$ -tetrahydrocannabinol (THC), the psychoactive ingredient of smoked marijuana, in the fetal brain. Considering the role of CB<sub>1</sub> in modulating the specification and long-distance migration of neurons in the perinatal brain, this study reveals an interesting mechanism potentially accounting for connectivity deficits during cortical development following exposure to CB<sub>1</sub> agonists or THC during pregnancy.**

See also: G Tortoriello *et al* (April 2014)

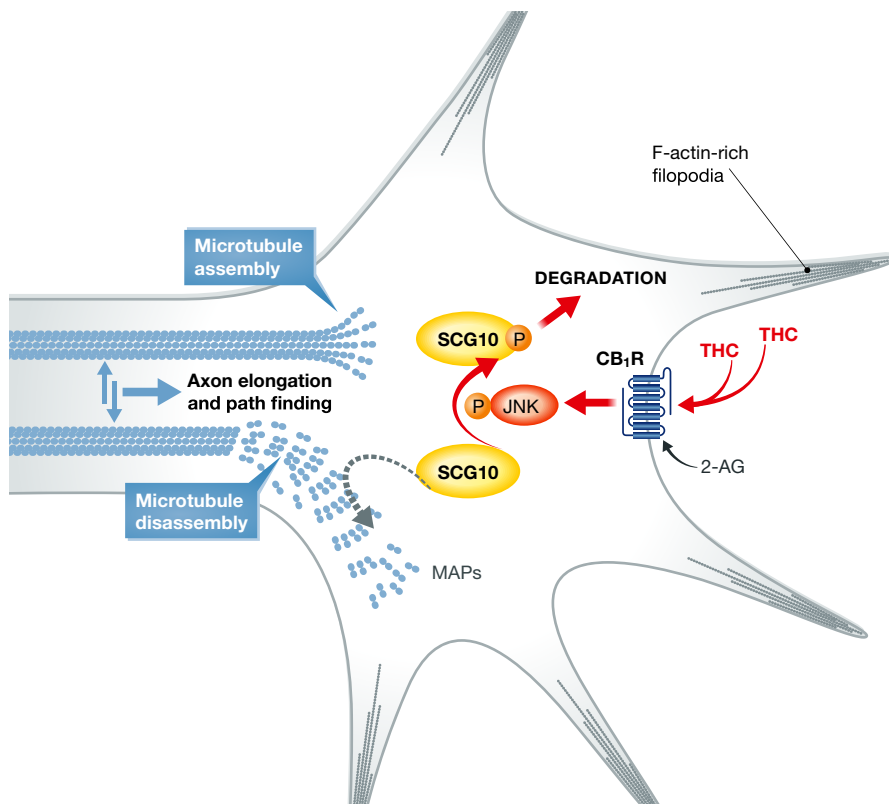
Heavy use of marijuana during pregnancy can impair social behaviors, cognition, and motor functions in the offspring, with impact lasting well into adulthood (Fried *et al*, 2002; Huizink & Mulder, 2006; Passey *et al*, 2014; Shabani *et al*, 2014). Approximately 1/3 of  $\Delta^9$ -tetrahydrocannabinol (THC), the psychoactive constituent of cannabis, and its metabolites can pass through the placental barrier upon marijuana smoking during pregnancy (Hutchings *et al*, 1989). Nonetheless, over 10% of pregnancies in Western countries are associated with maternal cannabis exposure, whereas, during the past decades, selective agriculture of *Cannabis* has resulted in an increased THC content at the expense of the most abundant non-psychoactive component, cannabidiol (Pijlman *et al*,

2005). Therefore, and taking into consideration the increasing awareness of the role of CB<sub>1</sub> receptors in embryonic neuronal differentiation, axonal pathfinding, and target selection (Berghuis *et al*, 2007), intrauterine THC exposure may lead to long-lasting developmental brain defects. However, it is presently unknown whether and how THC triggers CB<sub>1</sub>-driven signaling cascades to disrupt fetal cortical circuitry specification.

In this issue of *The EMBO Journal*, Tortoriello *et al* (2014) use a combination of pharmacological methods, organotypic slice preparations, quantitative and qualitative morphometric analysis, and unbiased proteomic profiling to identify SCG10/stathmin-2, a microtubule-binding protein, as the first THC-sensitive molecular effector modulating directional growth of corticofugal axons in the developing cerebrum. In the rodent perinatal brain, cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors and their endogenous agonists, the “endocannabinoids” anandamide and 2-arachidonoylglycerol (2-AG), are present from early developmental stages onwards (Berrendero *et al*, 1999). Evidence shows that this endocannabinoid system plays a key role in determining the synaptic structure and function of the adult brain by correctly “wiring” the fetal cortical circuitries through the strict spatio-temporal dynamics of CB<sub>1</sub> activation.

To investigate its effect on cortical network assembly, Tortoriello and co-authors were careful to administer THC to pregnant mice from embryonic day 5.5 through 17.5 at a “non-intoxicating” daily dose (3 mg/kg, intraperitoneal), that is, a dose that did not alter maternal behavior or

physical measures. They then allowed offspring to mature without postnatal re-introduction of the drug. By postnatal day 10, they observed redistribution of CB<sub>1</sub>-expressing inputs in both the neocortex and hippocampus. This is reminiscent of the long-term structural and functional corticofugal alterations previously observed in CB<sub>1</sub>-“knock-out” mice (Berghuis *et al*, 2007). However, the authors also noted novel features. SCG10 mapping in long-range fore-brain projections was enriched in growth cone-like structures and co-distributed with CB<sub>1</sub> in both the intermediate zone of the cerebral cortex and the primordial hippocampus of vehicle-treated fetuses. While, under normal conditions, SCG10 is proximal to CB<sub>1</sub> in corticofugal axons and could therefore be a downstream target of endocannabinoid actions in particular neurite domains, THC-treated fetuses exhibited a depletion of SCG10 from growing neurites, including the growth cones. Moreover, growth cones underwent morphological reorganization, suggesting their altered rate of motility and reduced outgrowth due to impaired microtubule dynamics. Tortoriello and colleagues also showed that THC induces CB<sub>1</sub>-mediated rapid axonal degradation of SCG10 through its phosphorylation by JNK1 (Fig 1). Consistent with this latter finding, several studies have revealed that JNK1-mediated phosphorylation of SCG10 at Ser62 and Ser73 negatively regulates the activity of SCG10 by promoting its proteasomal degradation in injured axons (Shin *et al*, 2012) and limiting the crucial role of this protein in regulating microtubule dynamics in the developing brain (Grenningloh *et al*, 2004). Finally, to demonstrate that the CB<sub>1</sub>-JNK-SCG10



**Figure 1. THC deregulating effect on microtubule dynamics in growth cones.**

An integrated signaling transduced by  $CB_1$  via JNKs and executed by SCG10 is disrupted by THC during the corticogenesis of prenatal brains exposed to a dose of THC (3 mg/kg/day, embryonic day E5.5–17.5) non-aversive to dams. SCG10 is normally enriched near  $CB_1$  receptors in growth cone-like structures, mainly traversing the pallio-subpallial boundary, where its microtubule-destabilizing action represents an important factor for the dynamic assembly and disassembly of growth cone microtubules during axonal elongation. Phosphorylation at Ser62 and Ser73 by JNK1, the active brain-specific JNK isoform, exerted also through the tonic action of the endocannabinoid 2-AG, promotes SCG10 degradation with ensuing fine regulation of microtubule dynamics and axodendritic morphology. Chronic THC administration during cortical development disrupts this action through both reduced 2-AG biosynthesis and  $CB_1$  expression, and excessive SCG10 phosphorylation and degradation. THC also increases the formation of F-actin-rich filopodia in the distant motile axon segment. 2-AG, 2-arachidonoylglycerol;  $CB_1R$ , cannabinoid receptor type 1; JNK1, c-Jun N-terminal kinase 1; MAPs, microtubule-associated proteins; THC,  $\Delta^9$ -tetrahydrocannabinol; SCG10, superior cervical ganglion 10.

pathway alone is sufficient to account for the THC-induced cytoarchitectural modifications of cortical neurons, the authors over-expressed a pseudophosphorylated SCG10 mutant refractory to phosphorylation by JNK1 and degradation, and found that this process limits neurite outgrowth in competition with endogenous (wild-type) SCG10 and occludes THC effects. Thus, while the “physiological” phosphorylation of SCG10 downstream of 2-AG-activated  $CB_1$  reduces to an essential level the cytoskeletal instability required to maintain the rate of neurite outgrowth, its “pathological” hyperphosphorylation by THC triggers ectopic axonal growth.

To make their study relevant to a condition potentially occurring in marijuana

smoking pregnant mothers, Tortoriello and colleagues assessed the distribution and density of SCG10 mRNA in the primordial hippocampus and parahippocampal gyrus of electively aborted second-trimester human fetuses exposed prenatally to cannabis *vs.* age-matched controls. They found that, also in the human developing brain, SCG10 mRNA levels were significantly reduced in cannabis-exposed subjects during the temporal window coincident with the formation of intra- and extracortical axonal trajectories (Kostovic' & Judaš, 2010). Indeed, retrospective longitudinal studies have documented potential long-term behavioral abnormalities in marijuana-exposed offspring—including exaggerated startle response and poor habituation to novel

stimuli in infants, hyperactivity, inattention, and cognitive retardation in adolescents (Fried *et al*, 2002; Huizink & Mulder, 2006). Experimental studies substantiate these observations and link behavioral and cognitive deficits and emotional responsiveness to pharmacological interference with endocannabinoid signals during fetal development (Passey *et al*, 2014; Shabani *et al*, 2014). Increased incidence of schizophrenia, depression, and predisposition to substance abuse in offspring prenatally exposed to cannabis (Substance Abuse and Mental Health Service Administration, 2012) might also be due, in part, to  $CB_1$ -induced altered developmental synaptic organization, which, even when remaining latent for long periods, might be prone to failure if challenged by environmental cues.

In conclusion, the important findings by Tortoriello *et al* provide a mechanistic explanation as to why marijuana abuse should be avoided during pregnancy. Future investigations should address the effect of THC on axon targeting processes in neonates and developing pups, thus allowing us to understand whether or not these observations are relevant also to the ever-increasing use of cannabidiol-enriched, but still THC-containing, cannabis extracts for pediatric indications, such as epilepsy (Porter & Jacobson, 2013).

### Conflict of interest

Vicenzo Di Marzo is a consultant for GW Pharmaceuticals, UK, and receives research funds from the same company. The other author, Luigia Cristino, declares that she has no conflict of interest.

### References

- Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urbán GM, Monory K, Marsicano G, Matteoli M, Cauty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T (2007) Hardwiring the brain: endocannabinoids shape neuronal connectivity. *Science* 316: 1212–6
- Berrendero F, Sepe N, Ramos JA, Di Marzo V, Fernández-Ruiz JJ (1999) Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. *Synapse* 33: 181–91
- Fried P, Watkinson B, James D, Gray R (2002) Current and former marijuana use: preliminary findings of a longitudinal study of effects on IQ in young adults. *CMAJ* 166: 887–91

- Grenningloh G, Soehrman S, Bondallaz P, Ruchti E, Cadas H (2004) Role of the microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. *J Neurobiol* 58: 60–9
- Huizink AC, Mulder EJ (2006) Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neurosci Biobehav Rev* 30: 24–41
- Hutchings DE, Hutchings DE, Martin BR, Gamagaris Z, Miller N, Fico T (1989) Plasma concentrations of  $\Delta^9$ -tetrahydrocannabinol in dams and fetuses following acute or multiple prenatal dosing in rats. *Life Sci* 44: 697–701
- Kostovic' I, Judoš M (2010) The development of the subplate and thalamocortical connections in the human foetal brain. *Acta Paediatr* 99: 1119–1127
- Passey ME, Sanson-Fisher RW, D'Este CA, Stirling JM (2014) Tobacco, alcohol and cannabis use during pregnancy: clustering of risks. *Drug Alcohol Depend* 134: 44–50
- Pijlman FT, Rigter SM, Hoek J, Goldschmidt HM, Niesink RJ (2005) Strong increase in total delta-THC in cannabis preparations sold in Dutch coffee shops. *Addict Biol* 10: 171–80
- Porter BE, Jacobson C (2013) Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. *Epilepsy Behav* 29: 574–7
- Substance Abuse and Mental Health Services Administration (2012) *Mental Health, United States, 2010*. HHS Publication No. (SMA) 12-4681. Rockville, MD: Substance Abuse and Mental Health Services Administration.
- Shabani M, Mahnam A, Sheibani V, Janahmadi M (2014) Alterations in the intrinsic burst activity of Purkinje neurons in offspring maternally exposed to the CB1 Cannabinoid agonist WIN 55212-2. *J Membr Biol* 247: 63–72
- Shin JE, Miller BR, Babetto E, Cho Y, Sasaki Y, Qayum S, Russler EV, Cavalli V, Milbrandt J, DiAntonio A (2012) SCG10 is a JNK target in the axonal degeneration pathway. *Proc Natl Acad Sci USA* 109: E3696–705
- Tortoriello G, Morris CV, Alpar A, Fuzik J, Shirran SL, Calvigioni D, Keimpema E, Botting CH, Reinecke K, Herdegen T, Courtney M, Hurd YL, Harkany T (2014) Miswiring the brain:  $\Delta^9$ -tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway. *EMBO J* 33: 668–685