

EFFECTS OF MATERNAL SMOKING ON NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT mRNA EXPRESSION

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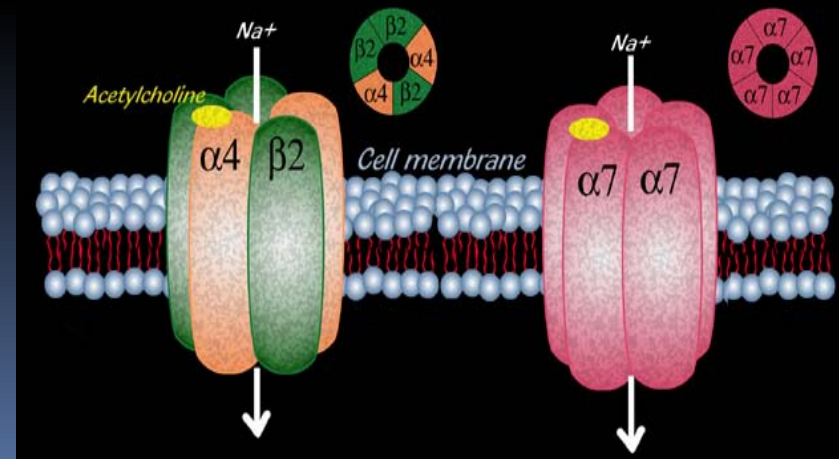
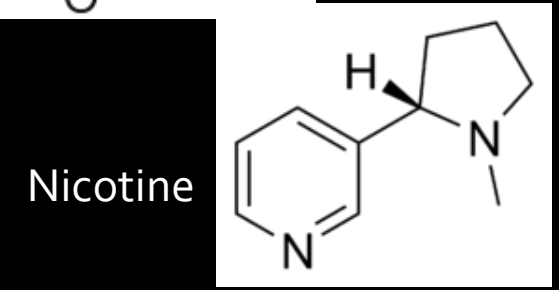
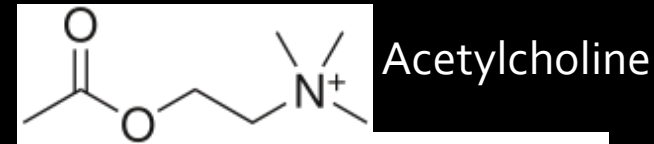
Children born to mothers that smoke are at higher risk of behavioral and cognitive deficits



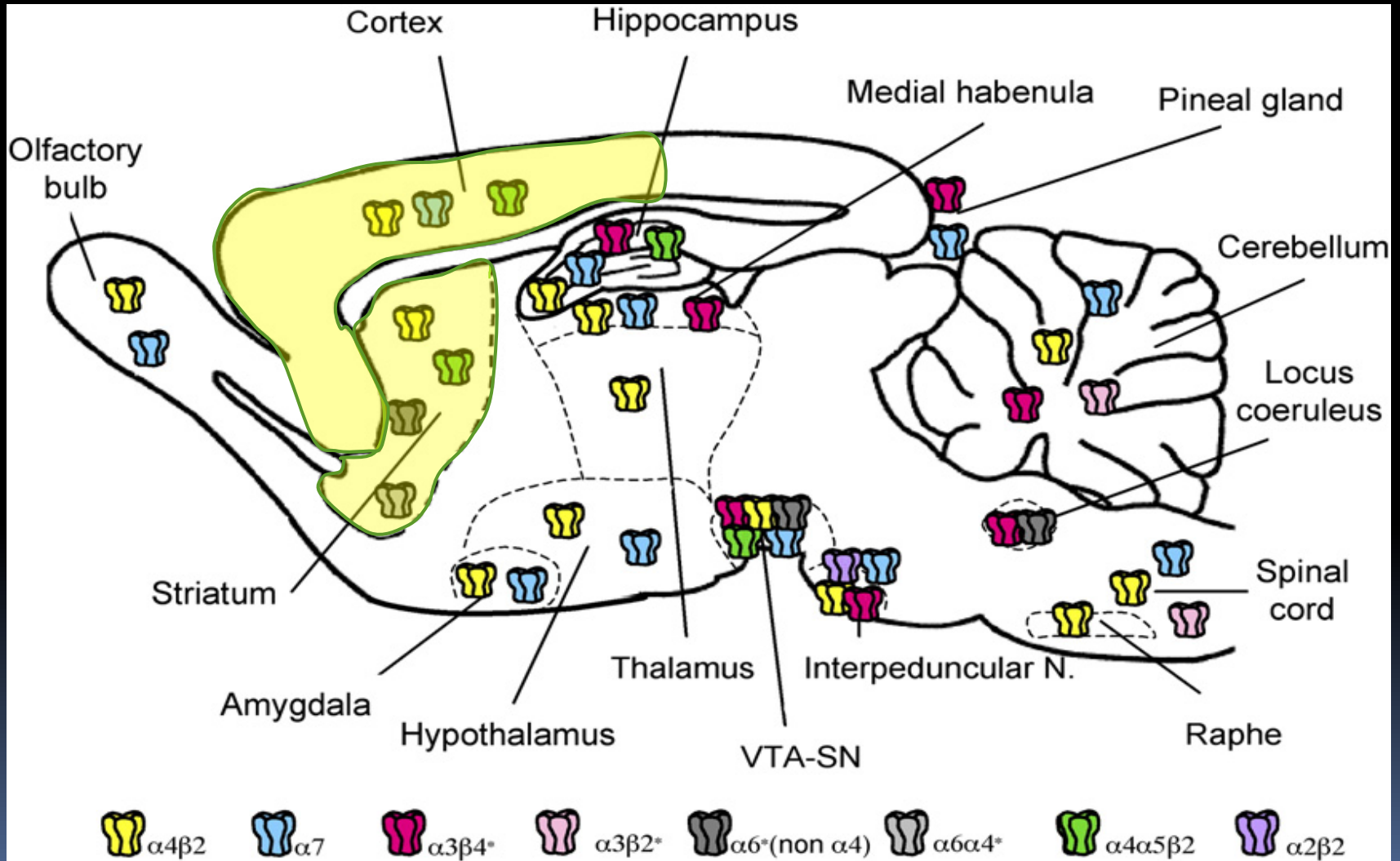
- Increased risk for ADD/ADHD
- Increased aggression
- More prone to addictive behaviors
- Some deficits are first recognized during puberty

Nicotinic acetylcholine receptors are key mediators in brain development

- Acetylcholine activates nicotinic acetylcholine receptors (nAChR)
- Nicotine is a nAChR agonist
- Neuronal nAChRs are composed of alpha (α_2 - α_{10}) and beta (β_2 - β_4) subunits
- Subunits assemble into heteromeric or homomeric pentamers



Differential expression of nAChR receptors in the brain



Adapted from Champtiaux, N., et al., 2003. *J. Neurosci.* 23, 7820–7829.

Rodent models are used to investigate effects of maternal smoking

Prenatal nicotine exposure results in:

- Perturbation of differentiation leading to changes in the number of neurons during development
- Altered synaptic activity
- Changes in arousal and sensory gating mechanisms

Nicotine exposure models:

- Acute systemic exposure (injection)
- Chronic systemic exposure (osmotic pump)
- Oral consumption
- **Mainstream smoke inhalation**



Potential Mechanisms for the Effects of Maternal Smoking: “Direct Effects”

- **Direct effects** of NIC result from the interactions of nicotine with nicotinic acetylcholine receptors (nAChRs) to produce changes in ion fluxes. nAChRs are present very early in the fetal brain (Hagino and Lee, 1985).
- Activation of ionotropic nicotinic receptors (nAChRs) is recognized as highly relevant to cognition, locomotion and analgesia (Champtiaux and Changeux, 2002; Drago et al 2003; Picciotto et al 2000, 2001).
- Chronic nicotine exposure has been shown to up-regulate functional α_4/β_2 nAChRs (Flores et al 1991)

Potential Mechanisms for the Effects of Maternal Smoking: “Indirect effects”

- NIC exposure-induced changes in Ca^{+2} ion permeability and can activate or inhibit other pathways (serotonergic, GABAergic, dopaminergic) that interact with cholinergic pathways.
- This could result in long-term changes in ion permeabilities, might regulate the activity of some genes or alter release of other neurotransmitters

Experimental Design



Smoke Exposure

- Begins on gestational day 4 and continues thru parturition
- Dams are exposed 3 times a day for 15 minutes



Offspring are dissected

- PND 20 (beginning puberty)
- PND 35 (mid puberty)
- PND 60 (end of puberty)



Expression assessed

- RNA extracted and converted into cDNA
- Rt -rt-Pcr

nAchr subunit primers and reference genes

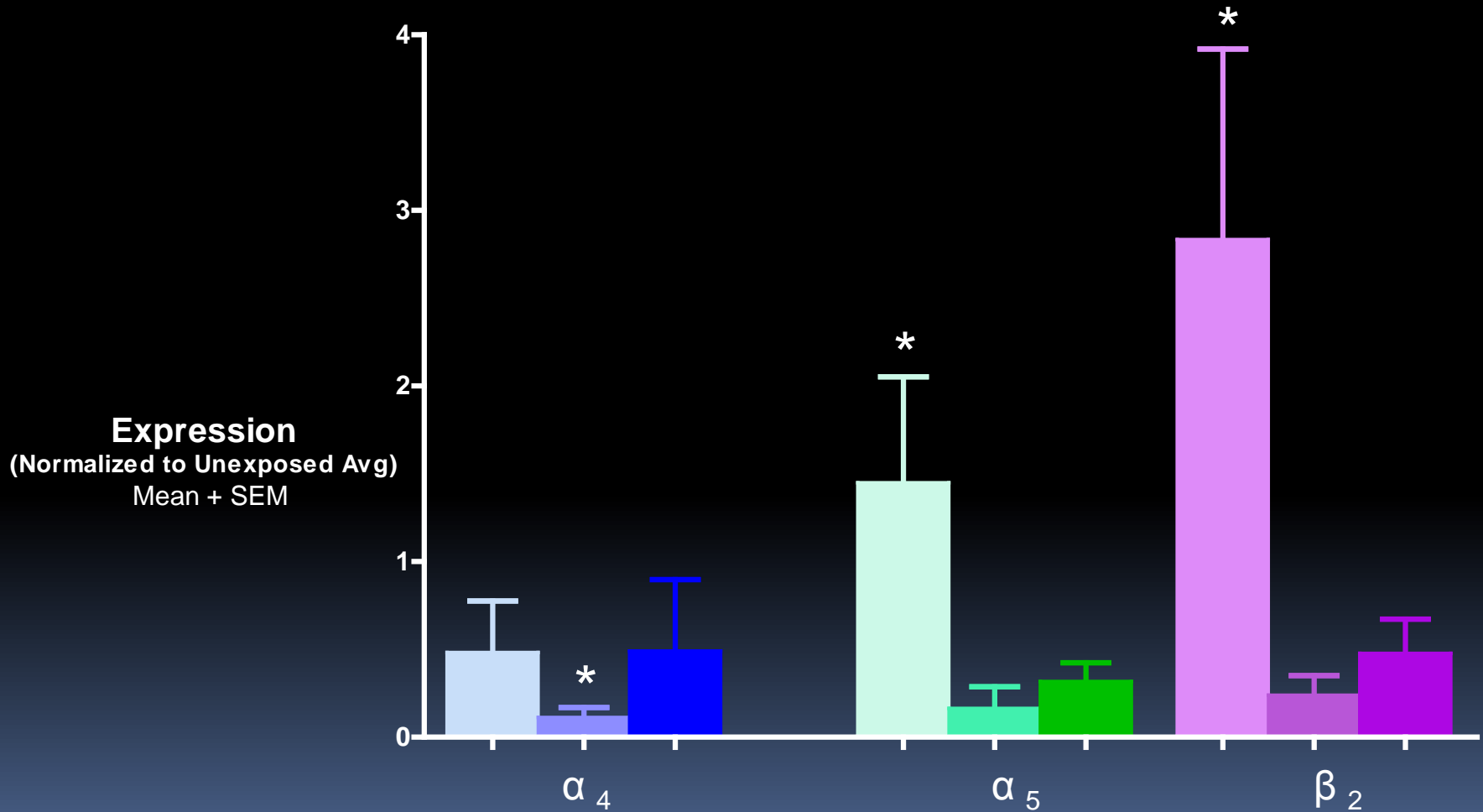
Striatum

- Target genes Chrna₄, Chrna₅, and Chrnb₂
- Reference genes assessed : Atp5b, Hprt, Gapdh, Pgk-1, Gusb, and Rpl13a

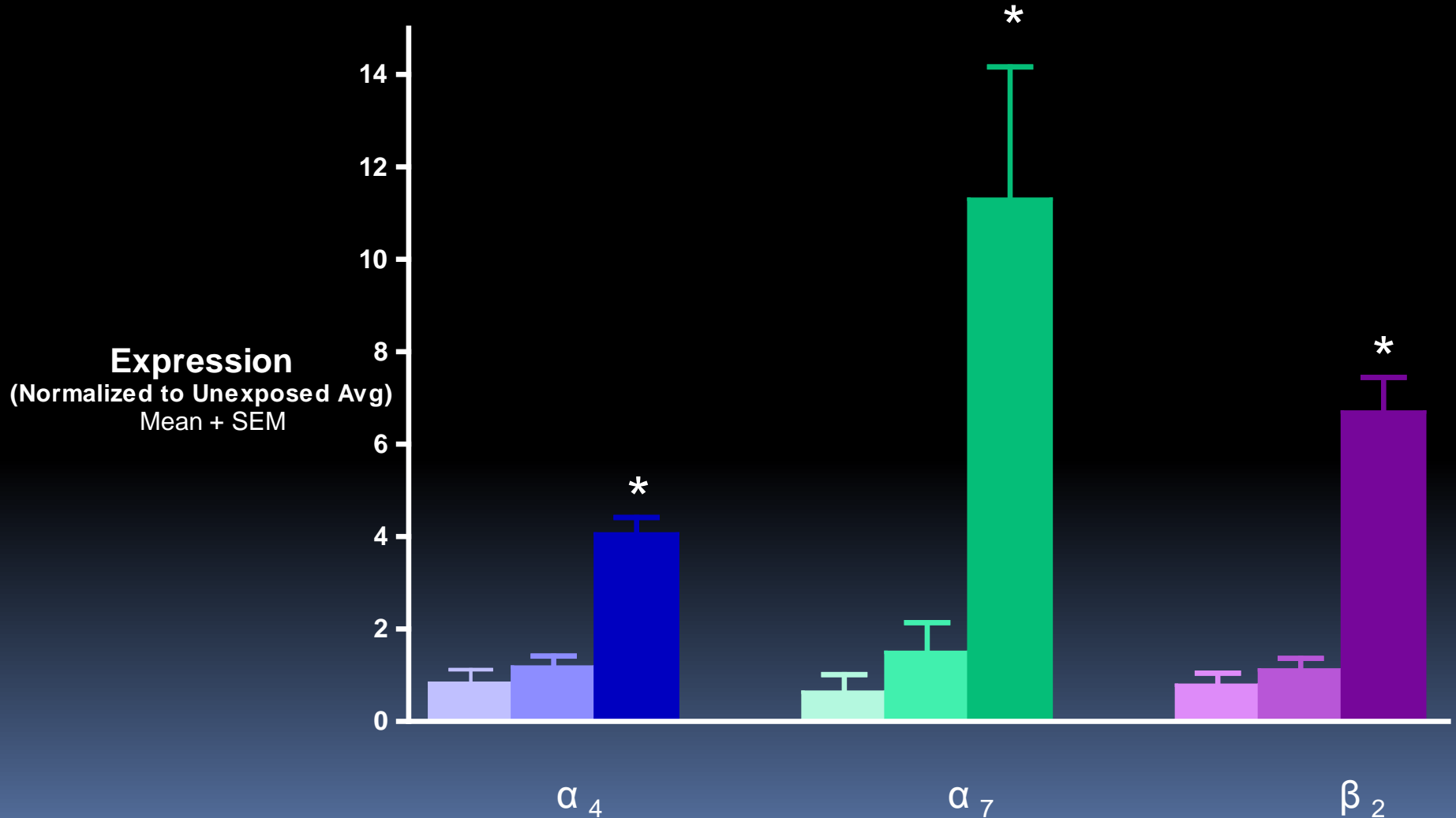
Frontal Cortex

- Target genes Chrna₄, Chrna₇, and Chrnb₂
- Reference genes assessed : Gapdh, 18s, β -Actin, Pgk-1, Gusb, and Rpl13a

Normalized Fold Expression of nAChR Receptor Subunit mRNAs in the **Striatum** of Pups Born to Exposed Dams



Normalized Fold Expression of nAChR Receptor Subunit mRNAs in the **Frontal Cortex** of Pups Born to Exposed Dams



Future Work

- Additional Brain regions: We have harvested hippocampus, hypothalamus, and cerebellum
 - Western blots to verify protein expression
 - Use GC-MS to determine serum concentration of nicotine so we can estimate absorbed dose.
 - Expose dams to smoke from nicotine-free cigarettes to examine impact of CO and exposure-induced stress.

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