

**Figure 2.** *Npas2* cKO mice have targeted deletion of *Npas2* in the liver only. (A) PCR amplification from *Npas2* exon 2 to exon 4 in cDNA synthesized from mRNA extracted from WT and cKO mouse liver, brain, heart and lung. The lower band of liver cKO samples indicated the absence of the 149bp exon 3 and this absence was not observed in brain, heart, and lung tissues from cKO mice. (B) qPCR analysis of *Npas2* circadian gene expression in *Npas2* cKO (red, solid line) and WT (grey, broken line) mouse livers. (C) RNAiish using a fluorescence probe covering a region of the *Npas2* mRNA from exon 2 to exon 4. Positive signals for *Npas2* mRNA are observed in both WT and cKO mouse livers and brain despite exon 3 being deleted in the cKO livers. NPY = neuropeptide Y.

### 36 Tadalafil treatment in mice for preeclampsia with fetal growth restriction has neurobenefic effects in offspring

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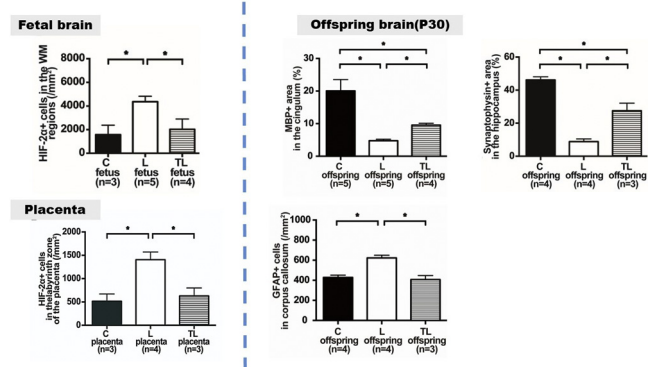
**OBJECTIVE:** We have demonstrated that tadalafil facilitates fetal growth in mice with L-NG-nitroarginine methyl ester (L-NAME)-induced preeclampsia (PE) with fetal growth restriction (FGR). Tadalafil is a selective phosphodiesterase 5 inhibitor that dilates the maternal blood sinuses in the placenta, thereby facilitating the growth of the fetus. The purpose of this study was to investigate the effects of tadalafil treatment for PE and FGR on the developing brain in FGR offspring using an L-NAME-induced mouse model of PE with FGR.

**STUDY DESIGN:** A control group of dams received carboxymethylcellulose (CMC). (C dams, n=5) L-NAME- administered groups received L-NAME dissolved in CMC from 11 d.p.c. The L-NAME-treated dams were divided into two subgroups 14 d.p.c. One subgroup continued to administer L-NAME. (L dams, n=8) The other subgroup administered L-NAME with tadalafil suspended in CMC. (TL dams, n=5) The dams were sacrificed 17 d.p.c. Placenta and fetal brain were collected. Another set of dams (C dams, n = 4; L dams, n = 5; TL dams, n = 6) was prepared as described above, and the dams were allowed to deliver spontaneously. After delivery, all dams were given normal drinking water during lactation. The pups were sacrificed on postnatal day 15(P15) or P30 and their brains were collected. The hypoxic conditions in the placenta and in the fetal brain were assessed by the expression of hypoxia- inducible factor (HIF)-2. The brains of pups on P15 or P30 were assessed by glial fibrillary acidic protein (GFAP) expression in the corpus callosum, myelin basic protein (MBP) expression in the cingulum, and synaptophysin expression in the hippocampus.

**RESULTS:** Tadalafil treatment for PE with FGR reduced the expression of HIF-2 in the placenta and in the brain of the FGR fetus. Moreover, tadalafil treatment *in utero* shows improved synaptogenesis and myelination in FGR offspring on P15 and P30. (The MBP positive area on P15 (C offspring,  $7.7 \pm 2.2\%$ ; L offspring,  $1.3 \pm 0.4\%$ ; TL offspring,  $5.0 \pm 0.4\%$ ) and P30 (C offspring,  $20.1 \pm 3.5\%$ ; L offspring,  $4.8 \pm 0.5\%$ ; TL offspring,  $9.6 \pm 0.5\%$ ), The synaptophysin positive

area on P15 (C offspring,  $13.8 \pm 3.3\%$ ; L offspring,  $5.1 \pm 1.6\%$ ; TL offspring,  $9.9 \pm 1.7\%$ ) and P30 (C offspring,  $46.2 \pm 1.9\%$ ; L offspring,  $8.8 \pm 1.7\%$ ; TL offspring,  $27.5 \pm 4.6\%$ )).

**CONCLUSION:** These results suggest that tadalafil treatment for PE with FGR not only facilitates fetal growth, but also has neuroprotective effects on the developing brain of FGR offspring through modulating prenatal hypoxic conditions.



### 37 Does *in utero* exposure to polybrominated diphenyl ethers affect neurodevelopment?

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**OBJECTIVE:** Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants and are ubiquitous environmental toxins, detectable at some level in  $\sim 100\%$  of women. Prior studies have suggested that PBDEs enhance placental inflammation, a known risk factor for neurodevelopmental disorders like autism. Therefore, our objective was to determine if *in utero* exposure to PBDE results in autism-like behaviors in offspring in an inflammation-dependent manner.

**STUDY DESIGN:** An IACAC-approved 2 x 2 factorial study design was implemented. Pregnant Wistar rats were assigned to one of 4 groups: control (VEH) without LPS, VEH with LPS, PBDE-209 without LPS, and PBDE-209 with LPS. Rats were received on gestational day (GD)3 and treated daily with cornflakes containing either PBDE-209 (1 mg/kg in corn oil) or VEH (corn oil alone) until delivery (GD23-24). On GD9, half of the dams of each group received LPS (100 mcg/kg intraperitoneally) or an equivalent volume of sterile saline (PBS). Pups from each litter underwent behavioral testing (open field and novel object) at postnatal day (PND)30 or PND60. Entries into center vs peripheral regions of the open field and interactions with or time spent with the novel object were recorded. Data was analyzed with generalized estimating equations.  $P < 0.05$  was considered statistically significant.

**RESULTS:** Of 24 dams, 17 of them delivered 162 pups. LPS-treated dams weighed less at GD16 than PBS-treated dams ( $P=0.009$ ). LPS and PBDE reduced PND9 pup weights ( $P=0.018$ ). For open field testing, there were no significant differences at PND30 for time spent in peripheral regions, entries into peripheral regions, time spent in center regions, or entries into center regions. At PND60, LPS increased entries into central regions but PBDE reduced the number of entries ( $P=0.03$ ) in LPS-treated rats. Between 78–100% of pups interacted with the novel object, more at PND60. Of the pups interacting with the novel object, PBDE exposure reduced the time to first encounter ( $P=0.03$ ).

**CONCLUSION:** *In utero* exposure to LPS, PBDE-209, or both did not cause the expected autism-like behaviors in the offspring. However, PBDE-treatment significantly reduced the weights of the pups of LPS-treated dams, suggesting some adverse effects on pregnancy outcome.