Sub-chronic PCP administration alters GABAergic gene expression in the rodent frontal cortex – focus on Tonic Inhibition

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Introduction

• Schizophrenia is a complex, chronic neuropsychiatric disorder characterised by 3 symptom domains: positive symptoms, negative symptoms and cognitive deficits.

•GABAergic dysfunction has been strongly implicated in the symptomatology of schizophrenia (Harrison and Weinberger 2005, Beneyto et al 2011).

•Tonic GABAergic dysfunction is thought to contribute to cognitive deficits with a5-containing receptors being the focus for recent research (Atack 2011).

•Our sub-chronic PCP rodent model has been consistently shown to induce long-lasting cognitive deficits in rats, similar to those shown in schizophrenia patients (Neill et al 2010).

• PCP administration has been shown to alter expression of a variety of genes in humans (Liu et al 2010) and in rats (Kaiser et al 2004).

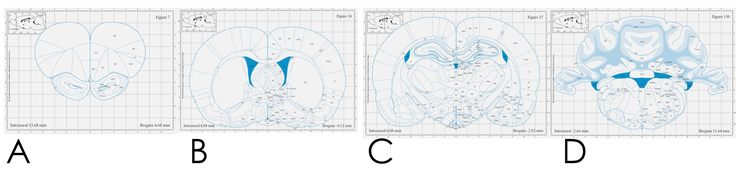
• **AIM: To investigate GABAergic gene expression in cortical, striatal, hippocampal and cerebellar brain regions in the sub-chronic PCP rodent model of schizophrenia symptomatology, with a focus on tonic GABAergic inhibition .**

Materials & Methods

•Adult female Hooded-Lister rats received bi-daily injections of PCP (2mg/kg i.p) or vehicle (0.9% saline i.p) for 7 days, (n=5 per group) followed by a 7 day washout period before killing by CO_2 overdose and cervical dislocation.

• Brains were removed on ice and dissected into the following regions of interest:

- Frontal Cortex (A)
  (Bregma = +4.7mm - +3.2mm)
- Striatum (B)
  (Bregma = +1.6mm - -0.3mm)
- Hippocampus (C)
  (Bregma = -2.5mm - -3.8mm)
- Cerebellum (D)
  (Bregma = -10.3mm - -12.7mm)



A B C D

Examples of brain structures sampled. Images taken from "The Rat Brain in stereotaxic coordinates" by Wilson and Paxinos (2005)

•RNA was extracted using Trizol® reagent and Invitrogen miniprep columns and converted to cDNA using a Qiagen reverse transcription kit.

•Gene expression was measured with SYBR green qRT- PCR.

•Data are shown as mRNA molecules relative to GAPDH ±S.E.M and analysed using REST software.

Results

• These data show sub-chronic PCP causes significant down-regulation of a4 and a5 ($p<0.001$) and gamma-2 ($p<0.01$) $GABA_A$ subunit genes in rat frontal cortex
• Significant down-regulation of other GABA-related genes including GAD1(GAD_{67})($p<0.01$), GAT-1 ($p<0.05$) and GAT-3 ($p<0.01$) and calcium-binding proteins parvalbumin ($p<0.01$) and calretinin ($p<0.001$) was also observed in PCP-treated frontal cortex
• A reduction in GFAP transcription was also observed in PCP-treated frontal cortex ($p<0.05$)
• A reduction in GFAP transcription was observed in the striatum ($p<0.05$)
• An increase was observed in delta subunit transcription in the cerebellum ($p<0.05$)
• Frontal Cortex data are also shown as $2^{-\Delta\Delta Ct}$ expression ratios relative to vehicle transcription

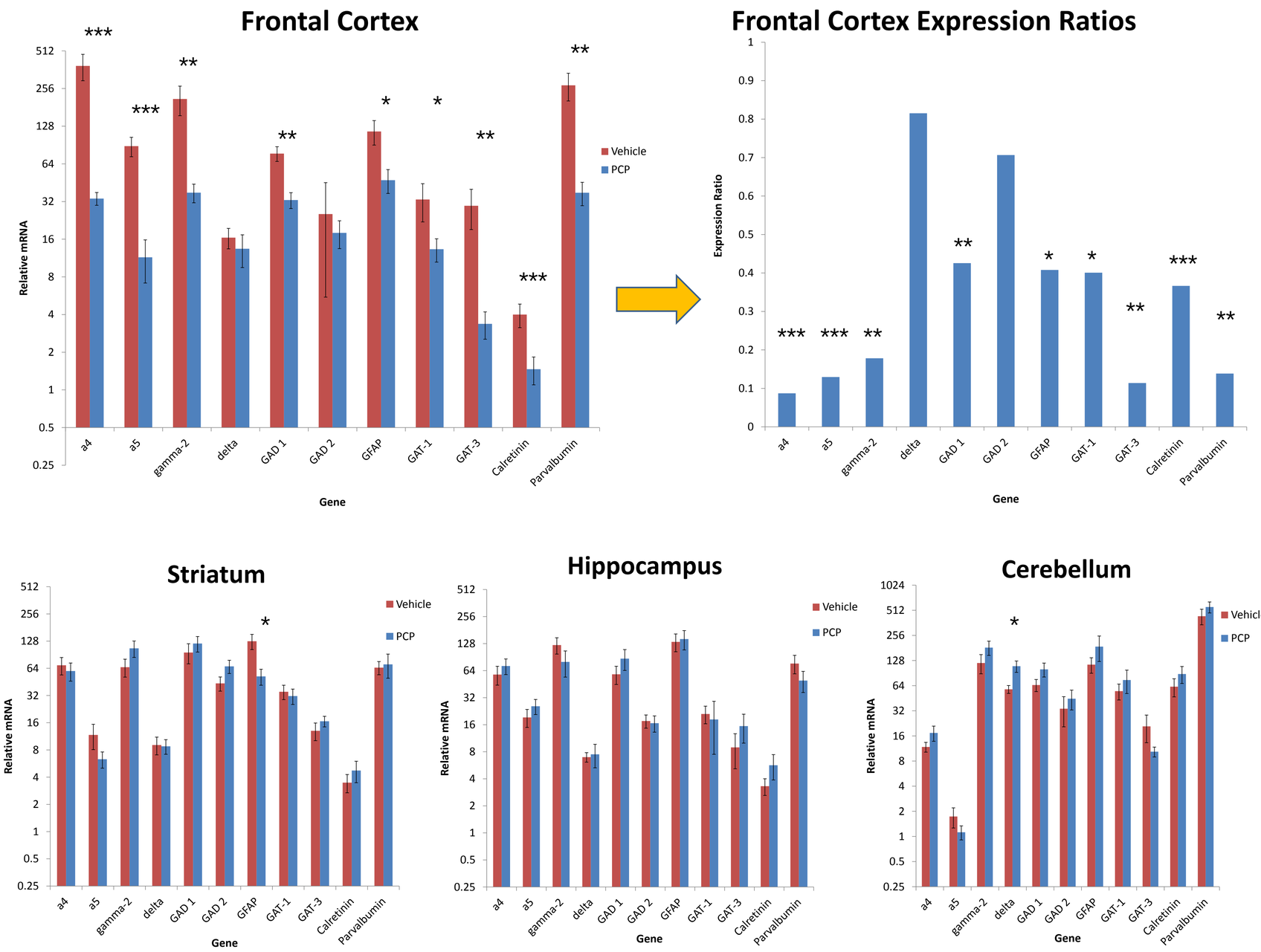


Figure 1: The effect of sub-chronic PCP(2mg/kg i.p) vs vehicle (0.9% NaCl i.p) on tonic GABAergic gene expression in Frontal Cortex, Striatum, Hippocampus and Cerebellum. Data are expressed as relative number of mRNA molecules per 1000 molecules of GAPDH ± S.E.M or expressed as $2^{-\Delta\Delta Ct}$ ratios between PCP and vehicle treated brain regions. N=5 per group. Data were analysed using REST software. *P<0.05, **P<0.01, ***P<0.001; significant difference between PCP treated and vehicle brain gene expression.

Conclusions

• These data show sub-chronic PCP causes significant down-regulation of multiple GABAergic genes in the frontal cortex with no significant change in transcription in other regions.
• These data suggest that dysfunction of tonic $GABA_A$ receptor –mediated signalling in the frontal cortex could play a role in the behavioural and cognitive deficits shown in the sub-chronic PCP rat model summarised in Neill et al (2010).
• These data also show close similarity between the GABAergic deficits in the sub-chronic rodent model and human post-mortem studies into GABAergic deficits in schizophrenia. (Lewis et al 2008, Guidotti et al 2000, Hashimoto et al 2008)

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