Effect of chronic D-Amphetamine and Phencyclidine treatment on Parvalbumin expressing interneurons.

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INTRODUCTION

•The cognitive deficits in Schizophrenia (e.g. impaired attention and disrupted working memory) have been linked to dysfunction of the prefrontal cortex (PFC) and hippocampus.

•Changes in GABAergic system markers, particularly in a subset of inhibitory neurons, in these regions are a well documented characteristic of post mortem tissue from schizophrenic patients (Lewis et al. 2004 & Lewis et al. 2005). Thus, a reduction of the Ca^{2+} binding protein parvalbumin (PV) (a marker for chandelier neurons) mRNA expression levels has been observed in the dorso-lateral PFC, CA1 and Dentate gyrus (Hippocampus) regions (Lewis et al. 2004& Eyles et al. 2002).

•The ability of phencyclidine (PCP) and d-amphetamine (AMPH) to trigger schizophrenia-like symptoms in healthy subjects and potentiate existing symptoms in schizophrenic patients makes both drugs attractive tools to model schizophrenia-like symptomatology in animals.

•Treating rats repeatedly with PCP results in histological changes comparable to that seen in post mortem brains of schizophrenic patients (e.g. reduction of PV mRNA expression, Cochran et al. 2003 & Thomsen et al. 2009).

•Repeated administration of AMPH has well documented long term behavioural effects in rodents (Featherstone et al. 2007), although only few publications address the histological impact of chronic AMPH administration.

Objective:

•The goal of the present study was to compare the impact of a chronic AMPH dosing regimen known to result in cognitive impairments in rodents (Tenn et al. 2003), with a PCP dosing regimen demonstrated to decrease the expression of the Ca^{2+} binding protein parvalbumin (PV) mRNA in the PFC (Cochran et al. 2003), on: (I) the level of PV immunoreactive cells in the PFC and hippocampus, and, (II) in a preliminary study, prepulse inhibition (PPI) 2 weeks after treatment termination.

MATERIALS AND METHODS

Subjects

32 male Sprague dawley rats (Harlan, Netherlands), with a start weight of 200g. The animals were kept under normal 12:12h dark-light cycle (light on at 6:00AM) in groups of four per cage under controlled conditions (temperature 21C, humidity at 60%). No food or drink restrictions have been applied.). Subjects were divided into 4 treatment groups (n=8): AMPH & Vehicle (AMPH), PCP & Vehicle (PCP)

Drugs and administration

PCP was administered at 2.6 mg/kg (s.c.) for 4 weeks, 5-days in the first week and three days per week for the remaining period (see Cochran et al. 2003). AMPH was administered 3-days per week for 3-weeks using an escalating dosing regimen: 1-3mg/kg s.c. at a rate of 1mg/kg/week (see Tenn et al. 2003). The two sets of control (0.9%NaC, s.c.) animals were "treated" using the relevant dosing protocols described above.

Immunohistochemistry

Six rats from each treatment group were anaesthetized 72 h after last injection, followed by transcardial 4% paraformaldehyde-PBS fixation for 10min. Brains were removed, immersed in fixative at 4°C overnight and the left hemispheres submerged for 3 days in 30% sucrose in PBS at 4°C. The tissue was cut into coronal 40µm serial sections on a freezing microtome. Immunohistochemistry was performed on sections from PFC and hippocampus based on appropriate coordinates (Paxinos 2007), using monoclonal mouse anti-Parvalbumin (Sigma-Aldrich, P3088) The number of PV-immunoreactive neurons was counted manually (blinded) in the PFC (prelimbic & infralimbic cortex) and hippocampus (pyramidal cell layer of CA1 and CA2/3 and the granular cell layer of the dentate gyrus, DG) on digital images in a 10x magnification with ImageJ (NIH, USA).

Pilot Prepulse Inhibition experiment

Two animals from each of the four treatment groups were tested for PPI, with each animal tested on four occasions over a 2-week period beginning 2-weeks after the last dosing day. Each test session consisted of 82 trials following a 5-min acclimatization period. The background noise throughout the test was 65-dB. Each session consisted of three different trial types: PULSE-ALONE (P-alone), a 120-dB white noise burst for 40ms; PREPULSE+PULSE (pp+P), a 120-dB white noise burst for 40ms, this time following (100ms onset to onset) a 20ms white noise prepulse of either 4, 8, 16 or 24 dB above the 65-dB background noise; NOSTIM, a background noise only trial. The average of 32 2ms recording from the onset of the 120dB pulse in the P-alone or pp+P trials was used to determine the acoustic startle response (ASR)

Statistical analysis

PV-immunohistochemistry was analysed by 2-Way Anova (factors: treatment and area), for hippocampus and PFC, Fisher LSD post hoc analysis was used as appropriate. The preliminary PPI and startle data was likewise analysed by 2-way ANOVA.

RESULTS

Fig. 1: Prefrontal cortex analysis

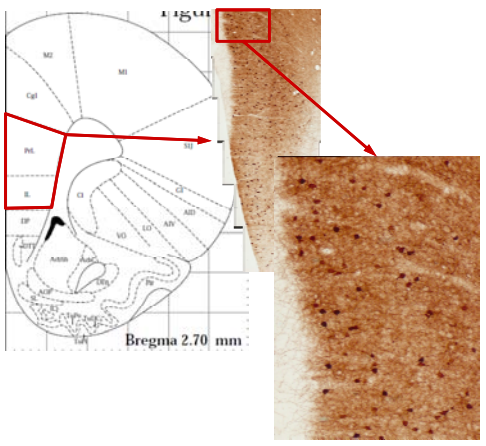


Fig. 2: Hippocampus analysis

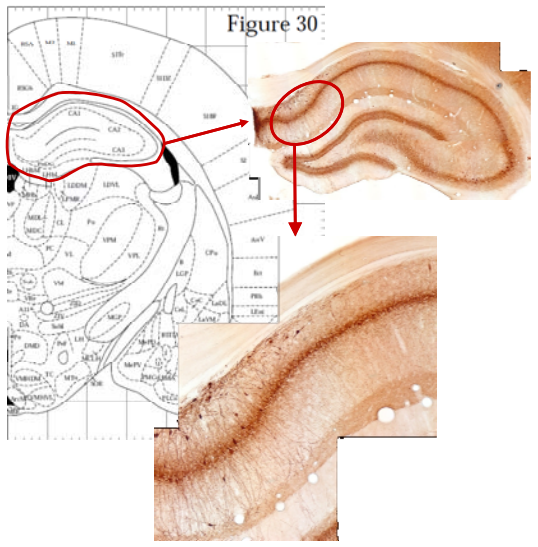
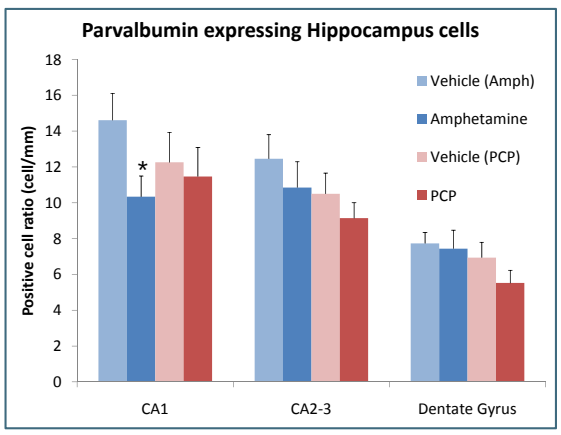


Fig. 3: Effect of chronic PCP and AMPH treatment on parvalbumin-positive interneurons



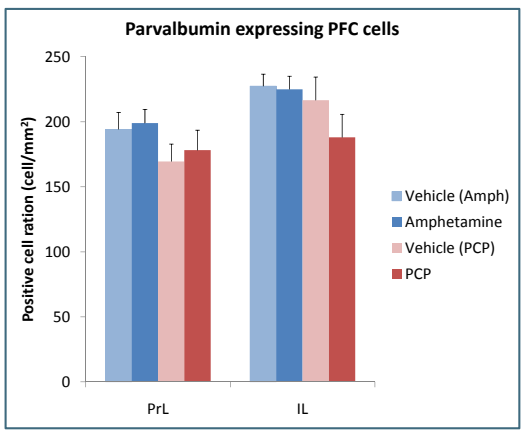
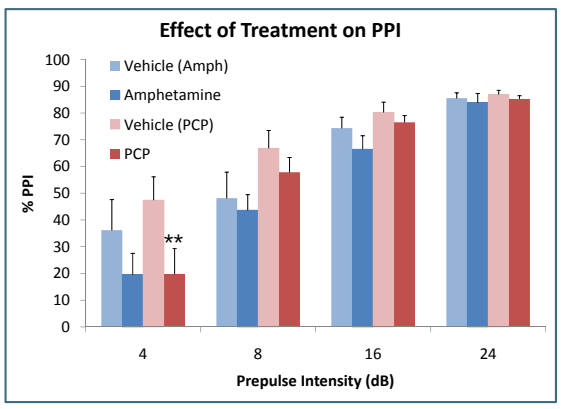
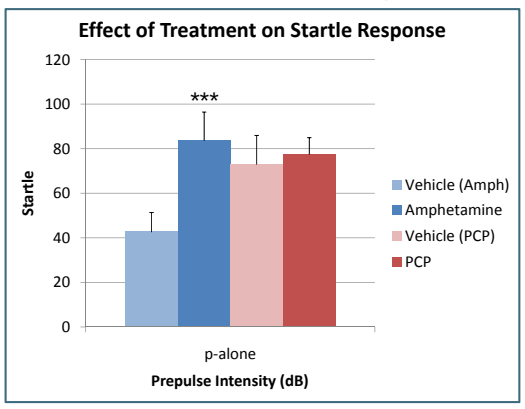


Fig. 4: Effect of chronic PCP and AMPH treatment on acoustic startle response and PPI





*/**/*** = p<0.05/ <0.01/ <0.001, compared to relevant control group. Data are presented as means + SEMs.

DISCUSSION

•Neither chronic PCP nor AMPH treatment altered the number of cells expressing PV in the mPFC (fig. 3), even at the level of single layer analysis (data not shown).

•In the hippocampus, chronic AMPH treatment significantly decreased PV expressing cells, specifically in the CA1 region of the molecular layer (fig. 3)

•These results are generally consistent with clinical data showing no change in the number of GABAergic interneurons in schizophrenic patients.

•Our methodology does not allow us to determine if the level of PV protein content per cell is reduced, a finding in schizophrenia.

•*Interestingly*, our preliminary behavioural data showed that chronic treatment with PCP significantly disrupted PPI at the 4 dB prepulse. This effect was apparent after a 2 weeks wash out period, and has not previously been reported.

•Likewise there was also a strong tendency towards disrupted PPI at 4dB (fig 4) in rats that had a 2-week washout after chronic AMPH treatment.

•The robustness of the PPI data in fig. 3 above is rather surprising given an N=2. However, it supports the power of the cross-over design when using the PPI procedure.

•Overall our conclusions can only be *preliminary* at this point since we are:

I. still determining immunostaining for GAD67 at the gross/fine resolution level, and
II. are repeating the PPI experiment with increased N.

References

Cochran, S.M. et al., 2003. *Neuropsychopharmacology*, 28(2), 265-275; **Eyles, D.W. et al., 2002**. *Schizophrenia Research*, 57(1), 27-34; **Featherstone, R.E., et al., 2007**. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 31(8), 1556-1571; **Lewis, D.A., et al., 2005**. *Nature Reviews. Neuroscience*, 6(4), 312-324; **Lewis, D.A., et al., 2004**. *Psychopharmacology*, 174(1), 143-150; **Paxinos, G., 2007**. *The rat brain in stereotaxic coordinates* 6th ed., Amsterdam; Boston: Elsevier; **Tenn, C.C., et al., 2003**. *Schizophrenia Research*, 64(2-3), 103-114; **Thomsen, M.S. et al., 2009**. Neuropharmacology, 56(6-7), 1001-1009.