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Abstract:

Background: Cognitive deficits and structural brain changes co-occur in patients with schizophrenia. Improving our understanding of the relationship between these is important to develop improved therapeutic strategies. Back-translation of these findings into rodent models for schizophrenia offers a potential means to achieve this goal. Aims: To determine the extent of structural brain changes and how these relate to cognitive behaviour in a sub-chronic phencyclidine (scPCP) rat model. Methods: Performance in the novel object recognition (NOR) task was examined in female Lister Hooded (LH) rats at 1 and 6 weeks after scPCP (2 mg/kg i.p. n=15) and saline controls (1 ml/kg i.p. n=15). Locomotor activity (LMA) following acute PCP challenge was also measured. Brain volume changes were assessed in the same animals using ex vivo structural magnetic resonance imaging (sMRI) and computational neuroanatomical analysis at 6 weeks. Results: Female scPCP treated LH rats spent significantly less time exploring novel objects (p<0.05) at both time points and had significantly greater LMA response to an acute PCP challenge (p<0.01) at 3-4 weeks of washout. At 6 weeks, scPCP treated LH rats displayed significant global brain volume reductions (p<0.05; q<0.05), without apparent regional specificity. Relative volumes of the perirhinal cortex were however positively correlated with novel object exploration time in scPCP rats at this time point. Conclusion: A sustained scPCP-induced cognitive deficit in NOR is accompanied by global brain volume reductions in female LH rats. The relative volumes of the perirhinal cortex however are positively correlated with novel object exploration time.
correlated with novel object exploration, indicating some functional relevance.
Global brain volume reductions in a sub-chronic phencyclidine animal model for schizophrenia and their relationship to recognition memory

Nazanin Doostdar1§, Eugene Kim2§, Ben Grayson1, Michael K. Harte1, Joanna C. Neill1*, Anthony C. Vernon3, 4*✪

1Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Medicine, Biology and Health, University of Manchester, Manchester, M13 9PT, UK
2King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Neuroimaging, Centre for Neuroimaging Sciences, De Crespigny Park, London, SE5 8AF, UK
3King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London, SE5 9RT, United Kingdom
4King’s College London, MRC Centre for Neurodevelopmental Disorders, New Hunt's House, Guy's Hospital Campus, London SE1 1UL, United Kingdom.

§These authors contributed equally to this publication

*Shared senior authorship

Corresponding author: Anthony C. Vernon (anthony.vernon@kcl.ac.uk); King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London, SE5 9RT, United Kingdom

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Abstract
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Aims: To determine the extent of structural brain changes and how these relate to cognitive behaviour in a sub-chronic phencyclidine (scPCP) rat model. Methods: Performance in the novel object recognition (NOR) task was examined in female Lister Hooded (LH) rats at 1 and 6 weeks after scPCP (2 mg/kg i.p. n=15) and saline controls (1 ml/kg i.p. n=15). Locomotor activity (LMA) following acute PCP challenge was also measured. Brain volume changes were assessed in the same animals using ex vivo structural magnetic resonance imaging (sMRI) and computational neuroanatomical analysis at 6 weeks. Results: Female scPCP treated LH rats spent significantly less time exploring novel objects (p<0.05) at both time points and had significantly greater LMA response to an acute PCP challenge (p<0.01) at 3-4 weeks of washout. At 6 weeks, scPCP treated LH rats displayed significant global brain volume reductions (p<0.05; q<0.05), without apparent regional specificity. Relative volumes of the perirhinal cortex however were positively correlated with novel object exploration time only in scPCP rats at this time point. Conclusion: A sustained scPCP-induced cognitive deficit in NOR is accompanied by global brain volume reductions in female LH rats. The relative volumes of the perirhinal cortex however are positively correlated with novel object exploration, indicating some functional relevance.

Introduction

Impairments in cognition are a core feature and clinical unmet need in the treatment of schizophrenia and related psychoses (Bortolato et al., 2015, Bora and Pantelis, 2015).
Cognitive deficits and negative symptoms have an adverse impact on quality of life (Green, 2006) bear a large socioeconomic cost and are refractory to current drugs for psychosis, including dopamine receptor (D2) antagonists such as haloperidol. Understanding the neural basis of impaired cognition in schizophrenia is therefore of significant importance (Cadinu et al., 2018). In parallel, structural brain abnormalities are also core features of schizophrenia and other serious neuropsychiatric disorders. In particular, reduced total brain and hippocampal volume, ventricular enlargement and thinning of the frontal and parietal-temporal cortical lobes, are the most commonly replicated findings in patients with schizophrenia, with robust effect sizes (Haijma et al., 2013, van Erp et al., 2016, van Erp et al., 2018). At least some of these anatomical abnormalities are present in non-medicated patients with schizophrenia, suggesting that abnormal brain structure is part of the disease pathophysiology and not solely the result of exposure to drugs for psychosis (Brugger and Howes, 2017). Whilst meta-analyses provide evidence for significant associations between frontal or temporal cortical thinning and positive and negative symptoms, respectively (Walton et al., 2017, Walton et al., 2018), there is evidence both for and against relationships between brain structure and cognitive performance in patients with schizophrenia. This most likely reflects heterogeneity in diagnosis, illness stage and the specific cognitive domains under investigation (Karnik-Henry et al., 2012, Massey et al., 2017, Heinrichs et al., 2017, Jirsaraie et al., 2018, Dempster et al., 2017). As such, efforts to improve our understanding of these associations, has the potential to help uncover the neuronal substrates of impaired cognition and so facilitate the discovery of novel therapeutic targets.
Disturbances in glutamatergic and GABAergic systems potentially induced by hypofunction of the N-methyl-d-aspartate receptor (NMDAR) remains a leading candidate mechanism for schizophrenia pathogenesis (Cadinu et al., 2018, Moghaddam and Javitt, 2012). This was derived partly from the early observation of reduced glutamate in the cerebrospinal fluid (CSF) of patients (Kim et al., 1980) and the finding that NMDAR antagonists, including ketamine and phencyclidine (PCP) robustly induce certain aspects of disease symptomatology in humans, including cognitive impairments (Krystal et al., 1994). Moreover, NMDAR antagonists exacerbate symptoms in schizophrenia patients that were otherwise stable on antipsychotic medication (Lahti et al., 1995b, Lahti et al., 1995a). Chronic intake of ketamine, at least in the context of addiction, is also reported to cause decreases in frontal lobe grey matter volume (Liao et al., 2010, Chesters et al., 2017) and abnormal white matter microstructure (Edward Roberts et al., 2014, Liao et al., 2011). NMDAR hypofunction therefore potentially contributes to both abnormal brain structure and function and cognitive dysfunction in patients with schizophrenia. Back-translating these findings into rodents through the development of NMDAR antagonist rodent models (Cadinu et al., 2018, Pratt et al., 2012, Moghaddam and Javitt, 2012) therefore offers a potentially fruitful approach to investigate the associations between cognitive behaviour and brain structure, with full control of genetic and environmental factors and in the absence of confounds such as antipsychotic drug treatment. Sub-chronic administration of PCP (scPCP) has been widely reported by ourselves and others to induce both aspects of negative symptoms and cognitive impairments of relevance for schizophrenia, as reviewed extensively elsewhere (Cadinu et al., 2018, Pratt et al., 2012, Neill et al., 2014, Neill et al., 2010). We have also shown several pathological changes of relevance to the disorder in this
model including reduced parvalbumin-positive interneurons and abnormal prefrontal
dopamine levels during performance of the novel object recognition (NOR) task

In contrast, whilst several studies have elegantly demonstrated the impact of acute or
scPCP treatment on rodent brain function using either pharmacological MRI (phMRI)
or semi-quantitative 2-deoxyglucose (2-DG) mapping of local cerebral glucose
utilization, in combination with functional connectivity network analyses (Dawson et
al., 2015, Dawson et al., 2014, Dawson et al., 2010, Gozzi et al., 2008), we are the only
group to have confirmed that scPCP induces MRI-detectable structural changes in the
rodent brain with relevance for schizophrenia (Barnes et al., 2015). These included
ventricular enlargement, decreased hippocampal volume and thinning of the frontal and
parietal cortex (Barnes et al., 2015). In the same study, we reported that scPCP also
induced significant deficits in sustained attention; however, the behavioural and MRI
analyses were carried in separate cohorts of male rats. No studies have investigated
whether these scPCP-induced structural brain deficits are transient, static, or progress
further with a longer duration of follow-up. Furthermore, no studies have explicitly
tested for any relationship between abnormal brain anatomy and impaired cognition in
this translational animal model. Therefore, we currently lack any evidence for the
functional relevance of these apparent brain structural deficits as they relate to the
model we have thoroughly validated in female rats, we use females for a variety of
reasons explained elsewhere (see Cadinu et al. 2018 for a review of this topic). In the
current study, we begin to address these gaps in our knowledge. Specifically, we
examined the long-term effects of scPCP exposure (six weeks) on memory (using our
well established NOR paradigm) and brain anatomy using high-resolution, ex vivo
magnetic resonance imaging (MRI), coupled with computational neuroanatomical analysis methods, as we have reported for other rodent models of relevance for schizophrenia (Crum et al., 2017, Hamburg et al., 2016, Richetto et al., 2017). Finally, we also tested for any correlations between measures of performance in NOR and regional brain volumes at this time-point.
Experimental procedures

Animals

All in vivo work including injections and behavioural testing was undertaken at the University of Manchester (performed by ND). Female Lister Hooded rats (n=30; Charles River, UK; weighing 194.3±2.24 g (mean±SD) were housed in groups of 5 per cage in ventilated plastic cages (38 x 59 x 24 cm, GR1800 Double-Decker Cage, Tecniplast, UK) containing sawdust, paper sizzle nest and cardboard tunnels (Datesand group, UK) under a standard 12 hour light: dark cycle (lights on 07:00 h). The environment was maintained at 21±2 °C, 55±5 % humidity.

Behavioural testing took place during the light cycle, under normal lighting (100 lux). Animals undergoing behavioural experiments had access to standard rat chow (Special Diet Services) and water ad libitum in the home cage. All experimental procedures were performed in accordance with the relevant guidelines and regulations, specifically, the Home Office (Scientific Procedures) Act 1986, United Kingdom and European Union (EU) directive 2010/63/EU. Furthermore, all work was carried out with the approval of the local Animal Welfare and Ethical Review Body (AWERB) panels at both the University of Manchester and King’s College London (KCL).

Sub-chronic PCP Administration

Phencyclidine hydrochloride (PCP) was purchased from Sigma-Aldrich (P3029; Gillingham, Dorset, UK). Animals were administered saline (0.9% saline, n=15) or PCP (2 mg/kg, n=15) via the intra-peritoneal (i.p) route. The scPCP treatment regimen has been described many times and consisted of 7 twice-daily (at approx. 9 AM and 5 PM) injections followed by a 7-day washout period (Barnes et al., 2015, Neill et al., 2010,
Neill et al., 2014). During this total 14-day period, animals received no behavioural testing and were handled only while receiving injections.

**Experimental design**

A summary of the timeline of experimental procedures is shown in Figure 1. After the 7-day washout period, saline and PCP-treated rats underwent behavioural testing in the NOR paradigm twice, once at 1 week post-dosing and again at 6 weeks post-dosing. All animals also underwent a locomotor activity (LMA) test, 3-weeks post-dosing to confirm successful scPCP treatment. All animals were culled 24 hours after the final NOR test to enable ex-vivo magnetic resonance imaging to be carried out.

**Behavioural apparatus and testing**

NOR testing was conducted in open Plexiglas boxes (52 cm w, 52 cm L, 30 cm h). Each box consisted of black walls and a white floor divided into 9 square sectors. The objects used in the NOR consisted of Coca-Cola ® cans and brown medicine bottles, as previously described in detail by us (Grayson et al. 2007). Two days prior to the first NOR test, rats were placed in the testing box with their cage-mates for 15 minutes. On the following day, rats were allowed 10 minutes to explore the NOR test box individually. On the test day, rats were placed in the NOR box to explore two identical objects for 3 minutes (acquisition phase). Rats were then removed from the NOR box and placed into an unfamiliar Plexiglas box (24 cm w, 44 cm L, 19 cm h) for a 1-minute inter-trial-interval (ITI). Following this, rats were returned to the NOR box to explore a novel object and a replica of the familiar object for 3 minutes (retention phase). The NOR box and all objects were cleaned with 70% ethanol at the end of each NOR testing.
session to remove olfactory trails. All experiments were filmed and video recorded for subsequent analysis by an experienced blinded experimenter. Object exploration time in each phase of the task was scored using the “Jack Rivers-Auty” online stopwatch (http://jackrrivers.com/program/). Object exploration was defined as licking and sniffing of the object whilst leaning on or touching the object. Turning towards and sitting on or next to the object without sniffing was not considered exploration (Grayson et al., 2007, Grayson et al., 2015a). The discrimination index (DI) was also calculated, defined as \((\text{time exploring novel object [sec]} - \text{time exploring familiar object [sec]} / (\text{time exploring novel object [sec]} + \text{time exploring familiar object [sec]}))\). Locomotor activity (LMA) was also evaluated by counting the total number of lines crossed by the rat in both the acquisition and retention phase. The locomotor activity (LMA) response to acute PCP challenge was monitored in automated testing chambers (Photobeam Activity system, San Diego instruments). The testing chambers were translucent Plexiglas boxes (24 cm w, 44 cm L, 19 cm h) with a perforated translucent Plexiglas lid (to allow air flow). All chambers were controlled using Pas764 LMA software to record the number of interruptions to the photo beam within the chamber. Rats were habituated to the LMA chambers one day prior to testing, which involved leaving individual rats in the LMA chamber for one hour. On the day of testing, rats were placed in the LMA chambers and baseline activity levels were monitored every 5 minutes for 30 minutes. Rats were then treated with an acute dose of vehicle (0.9% saline; i.p) or PCP (2 mg/kg; i.p) and placed back into the testing chamber to be monitored every 5 minutes over 60 minutes. LMA testing was conducted over 2 weeks with a cross over in the treatment groups so that all rats received both vehicle and PCP.

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Tissue preparation for ex vivo MRI
Saline ($n=15$) and scPCP ($n=15$) treated-rats were culled by cardiac perfusion (0.9% saline followed by 4% paraformaldehyde) under terminal anaesthesia (sodium pentobarbital, 60 mg/kg i.p) and prepared for ex vivo MRI at the University of Manchester as described elsewhere (Vernon et al., 2011). In brief, fixed brain tissues were kept intact in the cranium and post-fixed for 12 hours in 4% PFA. Samples were then shipped to KCL and on arrival transferred into 0.01M phosphate buffer containing 0.05% w/v sodium azide and 2 mM Magnevist (Bayer Plc) for 4 weeks prior to MR imaging. We did not conduct a formal power analysis to establish sample sizes. However, our final group size ($n=15$) per group is larger than the only prior structural neuroimaging study in the scPCP rat model (Barnes et al., 2015).

**MRI acquisition**

A 9.4T horizontal small bore magnet (Bruker Biospec®; Bruker BioSpin GmbH, Germany) and a quadrature volume radiofrequency coil (39 mm internal diameter, RAPID Biomedical GmbH, Germany) were used for all MRI acquisitions. Fixed brain samples were placed securely one at a time in a custom-made MR-compatible holder and immersed in proton-free susceptibility matching fluid (FluorinertTM FC-70; Sigma-Aldrich). Samples were scanned in a random order, with the KCL operator (ACV) blinded to treatment group (saline or PCP) by numerical coding of samples undertaken at the University of Manchester (performed by ND). Scanning of samples was interspersed with phantoms to ensure consistent operation of the scanner. From each animal a single 3D Fast-Spin Echo (FSE) image was acquired for brain structural analyses, with the following parameters: TE/TR=30/300 ms, echo train length=4,
number of averages=2, matrix size=375×225×225 and field of view (FOV)=30×18×18 mm, yielding isotropic voxels of 80µm. Total scan-time was 2 hr, 6 min per brain.

**MR image analysis**

The raw MR images were downloaded from the scanner server and converted to the NIFTI image file format, inspected for artefacts and then pre-processed as described previously (Wood et al., 2016) (performed by EK). After a quality control inspection, one scan in the scPCP group was excluded due to mechanical injury to the brain sustained during sample preparation. The final n values per group for statistical comparisons of MRI data were therefore \( n = 15 \) saline and \( n = 14 \) scPCP. A study template image was constructed using the 3D FSE MR images from the whole dataset to avoid bias \( (n=29) \). The resulting 3D FSE template was then non-linearly registered to two open access rat MRI atlases, the Waxholm space Sprague-Dawley rat brain atlas (Papp et al., 2014) and the rat cortical in vivo MRI Template (Valdes-Hernandez et al., 2011). This approach combines the best features of these two atlases, with excellent sub-cortical and cortical parcellation, respectively. MR images from individual animals were then non-linearly registered to this study template using their T2-weighted FSE images. Logarithmic Jacobian determinants \( (J) \) were calculated from the inverse warp fields in standard space to estimate apparent volume change (Crum et al., 2017, Wood et al., 2016, Vernon et al., 2014).

**Statistical analysis**

**Behavioural data**
Statistical analyses on all behavioural datasets were performed using Prism software (v7.0; Graph Pad Software Inc., La Jolla, CA, USA). Data were first confirmed as normally distributed using a combination of the Kolgorov-Smirnov normality test and D’Agostino and Pearson omnibus normality test. For the NOR task, group-level differences between saline (n=15) and PCP-exposed rats (n=15) with object exploration time as the dependent variable were assessed using a 2-tailed t-test, with α=0.05 for each phase of the task separately. Group-level differences using the DI as the dependent variable were also assessed using a 2-tailed t-test, with α=0.05. Locomotor activity (LMA), defined as total number of lines crossed in both the acquisition and retention phases of the NOR tasks were also calculated and compared between saline and PCP-exposed animals using a 2-tailed t-test, with α=0.05. In the LMA test, a 2x2 repeated measures ANOVA with “time” as within subject factor (and repeated measure) and “treatment” as between subject factor were used to assess group-level differences between saline and PCP-exposed animals using total distance moved as the dependent variable. Post-hoc tests (Bonferroni, corrected for multiple comparisons) were performed for any significant time x treatment interactions arising from the 2x2 ANOVA model, with α=0.05. An area under the curve (AUC) analysis was also carried out for total distance moved in each group, analysed using 1-way ANOVA with post-hoc Bonferroni test (corrected for multiple comparisons, with α=0.05).

MR image analysis

We employed a combination of atlas-based segmentation (ABS) and voxel-wise tensor-based morphometry (TBM) analyses (Crum et al., 2017). In the ABS approach, we took advantage of two freely available rat MRI atlases. First, the Waxholm MRI atlas, which
is parcellated into $n=80$ regions of interest (ROI) that are predominantly sub-cortical, including hippocampal sub-fields as well as the olfactory system and cerebellum (Papp et al., 2014). Second, the rat cortical \textit{in vivo} MRI atlas, which is parcellated into $n=47$ exclusively cortical ROIs (Valdes-Hernandez et al., 2011). The ROIs of the Waxholm MRI atlas were transformed to the \textit{in vivo} MRI atlas space via non-linear registration of the respective template images. This combination therefore allows an in-depth exploration of both sub-cortical and cortical volumes in the rat brain that is not possible using either atlas alone. From this combined atlas, we successfully extracted values for volume ($\text{mm}^3$) from 72/80 ROIs in the Waxholm atlas, (missing ROIs: optic nerve, inner ear, commissural stria terminalis, central canal, spinal trigeminal tract, frontal association cortex, habenular commissure, nucleus of the stria medullaris and medial lemniscus decussation) and 45/47 ROI in the rat cortical atlas (missing ROIs: dorsal intermediate entorhinal cortex [DIEnt] and medial entorhinal cortex [MEnt]). Total brain volumes were also calculated. In total, we therefore obtained volume measurements from $n=117$ brain ROIs per animal, per treatment group. Group-level differences contrasting saline ($n=15$) vs. scPCP ($n=14$) using volume ($\text{mm}^3$) as the dependent variable were then performed using 2-tailed t-tests, corrected for multiple comparisons using the False Discovery Rate (FDR) procedure of Benjamini and Hochberg with a 0.05 (5%; $q<0.05$) threshold (Genovese et al., 2002). The same statistical analyses were run for both absolute volumes and relative volumes, the latter calculated by expressing individual absolute ROI volumes as a percentage of total brain volume (Crum et al., 2017). This approach also controls for potentially spurious volume differences emerging due to overall between group-differences in total brain volume, reflecting the tight correlation between volumes of a structure and total brain volumes.
To complement and extend the ABS approach, we also carried out a voxel-wise TBM analysis of apparent volume change from the log-scaled Jacobian determinant maps, using permutation tests and Threshold-Free Cluster Enhancement (TFCE) in FSL randomise corrected for multiple comparisons by controlling the family-wise error (FWE) rate as described elsewhere (Crum et al., 2017, Vernon et al., 2014, Wood et al., 2016). As for the ABS, we ran this analysis both with and without total brain volume as a regressor of no interest in the design matrix.

Correlations between brain volume and behaviour

To assess the potential functional relevance of brain volume changes in scPCP-treated rats, we examined the degree of correlation between parameters measured in the NOR task at 6 weeks and the volume of *a priori* selected brain regions, previously reported as neural correlates of performance in the NOR task as described below. Data were first confirmed as normally distributed using a combination of the Kolgorov-Smirnov normality test and D’Agostino and Pearson omnibus normality test. Pearson product moment (two-tailed) correlations were then run comparing exploration time (seconds) for novel and familiar objects and the discrimination index (DI), in the NOR task at week 6, against the volumes (both absolute and relative) of the cingulate cortex (Weible et al., 2009), perirhinal cortex, *split into areas 35 and 36* (Gilbert and Kesner, 2003, Brown and Aggleton, 2001, Kinnavane et al., 2016, Morillas et al., 2017, Peters et al., 2018, Winters et al., 2008), postrhinal cortex, *cornu ammonis (CA) 1, 2 and 3 and the* dentate gyrus (Chang and Huerta, 2012, Brown and Aggleton, 2001, Winters et al., 2008) for all animals in each group (scPCP or vehicle) separately. Correlations were only performed for animals where both behavioural and MR imaging data were...
available (saline, \(n=15\); scPCP, \(n=14\)). Significance was set at \(p<0.05\). As these are exploratory correlations we did not perform corrections for multiple comparisons.
Results

Sub-chronic PCP impairs performance in the NOR paradigm in female Lister Hooded rats after a 7-day washout

In the acquisition phase, both vehicle and PCP-exposed rats spent similar time exploring the identical objects (Figure 2a). In the retention phase, saline-treated animals spent significantly more time exploring the novel object ($p<0.05$), whilst scPCP-treated animals explored both objects equally (Figure 2b). The DI was clearly reduced in scPCP rats compared to vehicle controls, but this failed to reach statistical significance ($p=0.1$; Figure 2c). Total LMA did not differ significantly across both phases of testing between saline and scPCP-treated rats ($p=0.54$; Figure 2d).

Sub-chronic PCP leads to sustained deficits in the NOR paradigm in female Lister Hooded rats up to six weeks post-exposure.

To confirm if the observed deficits in the NOR paradigm at 1 week post-dosing are sustained, rats were re-tested in this paradigm after an additional five-weeks from the cessation of PCP or saline treatment (6 weeks in total from the end of PCP treatment). In the acquisition phase, there were no differences between vehicle and scPCP-treated rats, which spent similar time exploring the identical objects (Figure 2e). In the retention phase saline, but not scPCP-treated rats spent more time investigating the novel object ($p<0.05$; Figure 2f). The DI was clearly reduced in scPCP rats compared to vehicle controls, but this failed to reach statistical significance ($p=0.07$; Figure 2g).

No significant differences in LMA were detected across both phases of testing between saline and scPCP-treated rats, although this trended towards a reduction in the scPCP group ($p=0.052$; Figure 2h).
Increased total LMA following PCP-challenge in Female Lister Hooded rats previously exposed to sub-chronic PCP but not saline.

In a crossover design, all rats in either the saline or scPCP groups were tested for total LMA after a saline or PCP (2 mg/kg) challenge, 3-4 weeks after the initial scPCP or vehicle treatment. Two-way repeated measures ANOVA of total LMA confirmed significant main effects of time (F(17,952)=57.3; \(p<0.0001\)), treatment (F(3,56)=4.89; \(p<0.01\)) and time x treatment interaction (F(51,952)=8.44; \(p<0.0001\)). Post-hoc testing on the interaction term confirmed that total LMA was higher in the scPCP exposed animals, following acute PCP challenge in comparison to all other treatment groups between 25 and 55 minutes after the challenge (55 to 80 minutes from the beginning of the experiment; Figure 2i). A 1-way ANOVA analysis of the area under the curve (AUC) for total LMA also found a significant overall difference across treatment groups (F(3,56)=8.07; \(p<0.0001\)). Post-hoc testing confirmed that the AUC for total LMA was significantly higher for the scPCP group challenged with scPCP compared to all other groups (\(p<0.01\); Figure 2j).

Sub-chronic PCP results in sustained apparent global brain volume reductions without regional specificity

Six weeks after cessation of treatment, the total brain volumes of scPCP-treated rats were significantly reduced by -14.7% compared to saline controls (\(t=2.51;\ df=28;\ p<0.05;\ Cohen’s \(d=0.84\); Figure 3). Using our hybrid MRI atlas of the rat brain, ABS revealed that the absolute volumes of 115/117 (98%) atlas ROIs were significantly reduced in scPCP-treated rats compared to saline controls after FDR correction at \(q<0.05\) (Supplementary Table 1). Effect sizes ranged from \(d=1.06\) in the posterior parietal cortex (PtPC) to \(d=0.40\) (ascending fibres of the facial nerve). There were no
ROIs showing a positive volume increase in the scPCP group relative to saline controls.

The ROIs with the largest effect sizes were PtPC ($d=1.06$); commissure of the superior colliculus ($d=1.06$) and anterior commissure ($d=1.01$). Only 2/117 (2%) of atlas ROIs were not significantly different between the groups, these being the genu and ascending fibres of the facial nerve (Supplementary Table 1). To check if these effects are a function of the global brain volume reduction in scPCP-treated rats, we repeated the ABS analysis using data corrected for total brain volume (relative volumes). In this analysis, only the relative volumes of 7/117 (6%) of the atlas ROIs were different when comparing scPCP-treated rats to vehicle controls and only then at trend-level significance ($p<0.05$ uncorrected), with no results surviving correction for multiple comparisons at $q<0.05$ (Supplementary Table 2). Of these, the relative volume of the granule cell level of the cerebellum was modestly increased (+2.6%; Cohen’s $d=1.14$), whereas the relative volumes of the other 6 atlas ROIs were reduced in scPCP treated animals by 2-3% with effect sizes in the range of $d=0.77$ to 1.08 (Supplementary Table 2).

To confirm and extend these data, we next performed voxel-wise tensor based morphometry (TBM) analysis, since this may be more sensitive than ABS to subtle apparent volume differences between the treatment groups (Crum et al., 2017, Sawiak et al., 2009c, Lerch et al., 2008b). TBM analysis without whole brain volume as a covariate revealed widespread clusters of voxels that were significantly smaller (FWE-corrected $p<0.05$) in scPCP-treated rats as compared to vehicle controls (Figure 4). These were widely distributed across the rat brain affecting almost all major structures, including the cortex as a whole, the basal ganglia, hippocampal formation, hypothalamus, basal forebrain and ventral midbrain. In contrast, in the cerebellum, only
the upper layers appeared to be affected by scPCP-treatment (Figure 4). No voxels were larger in scPCP-treated rats as compared to vehicle controls (Figure 4). To confirm if these apparent volume changes reflect the global change in brain volume in scPCP rats, we repeated the TBM analysis using total brain volume as a covariate. In this analysis, no clusters of voxels were either significantly smaller or larger when comparing scPCP and saline groups after FWE correction, even at highly exploratory statistical thresholds ($p<0.2$; data not shown). Taken together these data suggest apparent global, rather than local or region-specific effects of scPCP on female Lister hooded rat brain volume 6 weeks post-exposure.

Correlation between the volumes of a priori brain ROIs and NOR performance at 6 weeks post-scPCP

In the vehicle control group, we found no significant correlations between the volumes of our a priori selected brain ROIs (using either absolute or relative volumes) and the behavioural measures from the NOR task performed 6 weeks post-treatment (Table 1). In contrast, in the scPCP group, we found a positive correlation at trend-level significant ($p<0.05$ uncorrected for multiple comparisons) between the relative volumes of the perirhinal cortex areas 35 and 36 and the time spent exploring the novel object (Table 2). Plotting the data revealed that scPCP rats with larger volumes of these regions spent more time exploring the novel object, with no such relationship observed in the control group (Figure 5). We did not run correlations to the NOR task at week 1 or LMA at week 3 since these were temporally separated from the MR imaging time-point.

Table 1 about here. Correlations between behavioural data from the novel object recognition (NOR) task and MRI-derived volumes of a priori selected brain regions suggested to reflect the neural correlates of performance in this task in vehicle-treated rats (n=15), 6 weeks post-treatment. Data shown are correlation coefficients (Pearson’s $r$) and p-values (2-tailed t-test) for both absolute and relative volumes (mm$^3$).
Table 2 about here. Correlations between behavioural data from the novel object recognition (NOR) task and MRI-derived volumes of a priori selected brain regions suggested to reflect the neural correlates of performance in this task in scPCP-treated rats (n=14), 6 weeks post-treatment. Data shown are correlation coefficients (Pearson’s r) and p-values (2-tailed t-test) for both absolute and relative volumes (mm$^3$). Values in bold indicate a trend-level statistical significance ($p<0.05$ uncorrected for multiple comparisons).

Discussion

The main findings of the current study are that in female Lister Hooded (LH) rats, scPCP exposure induced the expected deficit in object exploration time at 1 week after drug exposure, which was sustained out to 6 weeks. Although DI was clearly reduced in the scPCP rats, this however failed to reach statistical significance. Exposure to scPCP also resulted in an elevated LMA response to an acute PCP challenge. At 6 weeks after cessation of treatment, scPCP rats had significantly reduced global brain volume, which lacked any apparent regional specificity, since all significant differences in volume were lost after a correction for global brain volumes, strongly suggesting an allometric effect (smaller brain, smaller regional volumes). At 6 weeks post-treatment, there were no significant correlations between the volumes of a priori selected brain ROIs and behavioural measures recorded in the NOR task in vehicle-controls. In contrast, the relative volumes of the perirhinal cortex areas 35 and 36 were positively correlated with time spent exploring the novel object in the scPCP group, such that scPCP rats with smaller relative volumes of these structures spent less time exploring the novel object.

These data replicate previous reports of sustained impairment in a simple cognitive task based on novel object recognition (NOR) using this scPCP-dosing regimen (Leger et al., 2015, McLean et al., 2011). It is important to note that scPCP rats failed to discriminate the novel from familiar object in retention at both time points, shown as no significant
difference between novel and familiar object exploration times, unlike in the vehicle group. The DI, which represents a ratio of this discrimination, however failed to achieve statistical significance, when comparing scPCP to vehicle controls. This most likely reflects a relatively low value in the vehicle animals, rather than the absence of reduced DI in the scPCP rats (for reference please refer to values for vehicle-treated animals in (McLean et al., 2011)). Although the validity of this task for assessing cognitive deficits in the context of schizophrenia has been challenged (Pratt et al., 2012), we suggest it still represents a useful, non-food rewarded task to assess recognition memory deficits that are broadly applicable to a range of CNS disorders (Grayson et al., 2015).

Importantly, we and others have previously shown that this scPCP dosing regimen also induces long-lasting impairments in cognitive domains with greater relevance for schizophrenia, including executive function, problem solving and reasoning, attention, vigilance and aspects of negative symptoms including social behaviour and affect (Cadinu et al., 2018, Neill et al., 2010, Neill et al., 2014).

In contrast to this extensive behavioural validation, only a single published study to date has examined the impact of scPCP on brain volumes (Barnes et al., 2015). Furthermore, this was not done in the same animals that also underwent behavioural testing, thus any functional relevance of the structural MRI changes may only be inferred and not be directly tested. Nonetheless, Barnes and colleagues (Barnes et al., 2015) reported significant bilateral reductions in grey matter density in the anterior cingulate cortex (ACC), ventral striatum (VS), amygdaloid nucleus and hippocampal formation accompanied by lateralised reductions in the thickness of the somatosensory and insula cortices. These findings have clear face validity for structural brain alterations
commonly reported in patients with schizophrenia (Barnes et al., 2015). These data are also consistent with MRI findings in mice exposed sub-chronically to other NMDA receptor antagonists such as MK-801, in which grey matter volume reductions and microstructural alterations of cerebral white matter measured using diffusion tensor imaging (DTI) have also been previously reported (Xiu et al., 2015, Xiu et al., 2014, Wu et al., 2016). Our data extend these findings to suggest that after a 6-week washout period the female LH rat brain volume is globally reduced in the scPCP group compared to saline controls. The combined ABS and TBM analysis approach revealed that this reflects widespread significant regional differences in the apparent absolute volume of several cortical and sub-cortical brain regions in scPCP-exposed rats. Notably, these include the same regions that are affected at 7 days post-scPCP in males (Barnes et al., 2015). However, our analysis reveals that when relative volumes (corrected for global brain volume changes) are compared there were no longer any statistically significant differences in either cortical or sub-cortical regional brain volumes between scPCP and saline-exposed rats, after correcting for multiple comparisons. These observations suggest a lack of regional specificity in brain volume changes and that the widespread absolute volume changes most likely reflect an allometric effect due to the overall global brain volume reduction following scPCP exposure. These data would however be consistent with a recent report suggesting that brain-volume changes in a large clinical MR imaging data set (n=2,668 individuals with a diagnosis of schizophrenia, bipolar disorder or attention-deficit/hyperactivity disorder as compared to healthy controls) are strongly associated with global brain and intracranial volumes (Schwarz et al., 2019). Nonetheless, our data also show that the relative volumes of a small number of brain ROIs remained differentially affected by scPCP-exposure, albeit only at trend-level
significance ($p<0.05$ uncorrected for multiple comparisons). These included decreases in the apparent relative volumes of the lateral somatosensory and posterior parietal cortex. Whilst we cannot exclude the possibility that these results are Type-I errors, these ROIs are consistent with the anatomical location of cortical thickness deficits reported in the previous structural MRI analysis of male scPCP exposed rats (Barnes et al., 2015). The magnitude of these relative volume reductions is also small, (range: 2-3%), which in silico experiments on MR image registration sensitivity suggest would be unlikely to survive a conservative multiple comparisons correction, as is the case here (van Eede et al., 2013). Measures of cortical thickness may also reflect a more sensitive and topographically relevant index of subtle anatomical brain remodelling in both humans and preclinical models, respectively (van Erp et al., 2018, Vernon et al., 2014, Lerch et al., 2008a).

Taken together, whilst there are commonalities in the two MR imaging data sets, there are also clear differences. In this respect, key methodological differences between the two studies should be noted. First, Barnes and colleagues (2015) used male Lister Hooded rats, whereas we have used females. The female rat brain may be more sensitive to PCP; hence we observe proportionally greater neuroanatomical effects. Supporting this hypothesis, female rats are more sensitive to scPCP, with differential pharmacokinetic effects (Shelnutt et al., 1999), greater impairments in performance in an attention set shifting task and increased PCP-associated neurotoxicity, including widespread reductions in brain derived neurotrophic factor (BDNF) in several brain regions post-scPCP (Snigdha et al., 2011, Fix et al., 1995). Second, a higher dose of 5 mg/kg was used, as compared to the 2 mg/kg dose in our study due to pharmacokinetic
1 differences between male and female rats (Shelnutt et al., 1999). Third, MR images
2 were acquired after only 1 week of drug washout (Barnes et al., 2015) as compared to 6
3 weeks in the current study. It may be hypothesised that scPCP exposure initially leads
4 to a region-specific anatomical remodelling of the rat brain, which may proceed to a
5 more progressive course, resulting in global deficits with increasing time post-scPCP
6 treatment. In support of this, we observe robust reductions in parvalbumin (PV)
7 expression at 6 weeks post scPCP treatment, but not earlier (Leger et al., 2015). Fourth,
8 the MR images were analysed differently. Barnes and colleagues used voxel-based
9 morphometry (VBM) (Sawiak et al., 2009b, Barnes et al., 2015), whilst we used tensor-
10 based morphometry (TBM) (Vernon et al., 2014, Lau et al., 2008). A key difference in
11 these two methods is that VBM contains an additional series of steps to segment MR
12 images into tissue classes (grey, white matter and CSF), whereas TBM does not
13 (Sawiak et al., 2009b). It may be argued then, that the use of two different analysis
14 methods could lead to differential results between our study and that of Barnes and
15 colleagues (Barnes et al., 2015). To address this question, we refer to a direct
16 comparison of these methods performed using high resolution ex vivo MR images
17 acquired from a murine model of Huntington’s disease (HD; R6/2 mutant) and their
18 wild-type littermates (Sawiak et al., 2009a). This study demonstrates first, that a TBM
19 analysis finds all of the results that a VBM analysis would. Second, TBM analysis
20 identifies many additional regions that are significantly affected in terms of their
21 volume, when comparing R6/2 to WT mice as compared to VBM (Sawiak et al., 2009a).
22 This is not surprising since VBM uses the probability of grey matter as its statistical
23 measure, whereas TBM uses the local volume change independent of tissue type
24 (Sawiak et al., 2009a). Therefore using TBM and avoiding assumptions about tissue
classes that can be challenging to separate in the rodent brain due to the relative paucity of white matter (Lau et al., 2008) may reveal more information. Put simply, whilst VBM and TBM find common changes, it may be argued that TBM is more sensitive, hence the greater number of significant voxels found in our study, at least using absolute volumes. Future longitudinal MRI studies in scPCP and saline-exposed rats of both sexes are now required to establish the sex-dependence, timing, nature (progressive or static) and any regional specificity of brain volume changes following scPCP exposure. A direct head-to-head comparison of VBM and TBM methodology in this study may also prove useful for the field.

A goal of this study was to explore whether there is any potential functional relevance of the observed brain volume reductions in scPCP rats. Hence, we tested for associations between MRI-derived brain volumes and behavioural measures extracted from the NOR task 6 weeks post-treatment. We focussed this analysis on 9 a priori-selected brain ROIs from our MRI atlas, based on literature confirming a key role for these structures in object recognition memory (see materials and methods). We also included whole brain volume since this was globally reduced in the scPCP rats. This analysis revealed a trend-level correlation (p<0.05 uncorrected for multiple comparisons) between the relative volumes of the perirhinal cortex areas 35 and 36 and the time spent exploring the novel object in scPCP, but not vehicle-treated rats. These data are consistent with the established role of the perirhinal cortex in object recognition memory and novelty detection, based on lesioning and chemogenetic studies (Brown and Aggleton, 2001, Winters et al., 2008, Morillas et al., 2017, Kinnavane et al., 2016, Peters et al., 2018). Taken together, these data suggest there may well be functionally
relevant links between the volumes of individual brain regions and behavioural performance in this scPCP model. Changes in connectivity between brain regions are also clearly important in this context. Indeed, brain network changes beyond the perirhinal cortex are clearly implicated in object recognition memory formation (Tanimizu et al., 2018). Furthermore, the importance of looking at networks is evidenced by elegant studies combining LCGU and functional connectivity analysis, which have linked abnormalities in functional brain networks post-scPCP to impaired cognitive performance (Dawson et al., 2010, Dawson et al., 2015, Dawson et al., 2014). To the best of our knowledge however, the perirhinal area was not included in these studies. Future studies are now needed to replicate and extend these findings including more relevant behavioural tests, such as those of passive and active attention, previously reported to be deficient in male rats exposed to scPCP (Barnes et al., 2015).

We did not assess any correlation to LMA as this was temporally separated from the MR image acquisition. In the context of NOR, both male and female rats show robust deficits in this cognitive task, provided the dosing schedule is adjusted to account for these sex specific pharmacokinetic effects of PCP (see Janhunen et al. 2015 for an extensive review).

Mechanistic studies are also required to understand the cellular basis of brain volume changes in this scPCP model and how this might influence the relationship to behavioural data. Cognitive performance and memory processes have been suggested to be associated with remodelling of dendritic spine density on pyramidal neurons, including in the prefrontal cortex (PFC). Specifically, a reduction in dendritic spine density on these primary neurons may contribute to cognitive dysfunction (Kasai et al.,
In support of this, there are correlations between the loss of asymmetric spine synapses in the primate PFC and the emergence of cognitive dysfunction during ageing (Dumitriu et al., 2010, Peters et al., 2008). Decreased density of PFC pyramidal neuron dendritic spines, suggestive of spine loss, is also reported from *post-mortem* studies of PFC brain tissue from schizophrenia patients (Glantz and Lewis, 2001). Furthermore, two prior studies have found evidence for decreased dendritic spine density in the PFC following scPCP treatment in rats, which persists for at least 4 weeks (Elsworth et al., 2011, Hajszan et al., 2006). *No such data are however available in the perirhinal cortex following scPCP administration.* At least in the rodent brain, changes in neuronal dendritic spine density are suggested to correlate with apparent brain volume changes detected using voxel-wise morphometry analysis of MR images (Keifer et al., 2015).

Whilst a change in spine density would be predicted to contribute, at least in part, to the apparent volume changes detectable by MR imaging, *it should be remembered that the latter could also simply be an epiphenomena of this spine loss and thus not related to the behavioural changes.* Moreover, *apparent changes in brain volume as measured using MRI* (which, it should be remembered does not measure brain structure directly) may also reflect changes in several other cellular compartments, including glia, blood vessels and myelin content (Zatorre et al., 2012, Stolp et al., 2018), which have yet to be investigated in this scPCP model. Longitudinal MRI and behavioural studies in larger sample sizes, of both sexes, with detailed *post-mortem* follow up including assessments of spine density are therefore required to *map the cellular correlates of brain volume changes and identify which of these mediates the link to behavioural deficits in specific brain regions such as the perirhinal cortex.* In this context, the work of Wu and colleagues (2016) may suggest some important leads. Specifically, following chronic
exposure of mice to the NMDA-receptor antagonist MK-801, they report findings of increased numbers of necrotic cells in brain regions with reduced grey matter volume detectable by MRI, at least at trend level (p<0.01 uncorrected) (Wu et al., 2016). Furthermore, in specific sub-fields of the hippocampus, which also demonstrated apparent reduced grey matter volume (as detected by MRI), there was evidence for a reduction in the number of PV immunopositive interneurons and decreased dendritic spine complexity and density on pyramidal neurons (Wu et al., 2016). In the white matter tracts, of these animals, in which both reduced volume and microstructural changes (increased fractional anisotropy) are detectable by MRI, they provide evidence for decreased myelin basic protein immunoreactivity suggestive of demyelination (Wu et al., 2016), as reported previously in this model (Xiu et al., 2014, Xiu et al., 2015). As already indicated such future studies should also include testing for relationships between brain structure, but also function, (Dawson et al., 2010) and other cognitive domains that are impaired in the scPCP model, particularly given that human studies suggest complex relationships between brain anatomy and cognitive performance across different tasks and domains, including studies in patients with schizophrenia (Heinrichs et al., 2017, Jirsaraie et al., 2018, Karnik-Henry et al., 2012, Massey et al., 2017, Brandt et al., 2015).

Conclusions

In summary, our data confirm our previous work showing that scPCP exposure leads to long-lasting behavioural deficits in the NOR task when comparing object exploration time directly. We show for the first time that in the female LH rat brain these are accompanied by global brain volume reductions that are without apparent regional specificity. The latter may reflect either enhanced sensitivity to scPCP in female rats
compared to males, which show more regionally selective grey matter volume changes, or that progressive brain volume loss occurs at a later time point following scPCP exposure. Our analyses also suggest that some of these phenomena are related, specifically, the relative volumes of the perirhinal cortex are positively correlated with novel object exploration time at 6 weeks only in scPCP rats. These data provide a clear rationale for future in vivo longitudinal, multi-modal MRI studies; incorporating cognitive testing and detailed post-mortem analysis following scPCP exposure. Such studies have the potential to establish a set of non-invasive, cross-species biomarkers to increase the predictive validity of testing novel therapeutics with pro-cognitive actions in the scPCP model for cognitive impairments in schizophrenia and other serious mental illnesses.

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no role in the decision to publish this work. The remaining authors declare no conflicts of interest.

Supplementary data

Supplementary data associated with this article can be found in the online version.

Data availability

MR images used in this study are freely available upon reasonable request to the corresponding authors.

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therapy of cognitive dysfunction in schizophrenia. *Eur Neuropsychopharmacol*, 21, 333-43.


Figure legends

Figure 1. Experimental timeline. All in vivo animal work including drug treatment and behavioural analysis was carried out at the University of Manchester. Female Lister Hooded (LH) rats (n=30) were sub-chronically exposed to either PCP 2 mg/kg, i.p. (n=15) or 0.9% saline as a control (n=15), twice daily for 7 days, followed by 7 days washout. Animals were then tested in the NOR paradigm after 1 and 6 weeks of drug-washout. Locomotor activity (LMA) in response to acute saline or PCP challenge was examined in all animals in each group using a crossover design after 3 weeks of drug washout. Animals were culled after the final behavioural session and brain tissue prepared and shipped to King’s College London (KCL) for ex vivo MR imaging after 4 weeks hydration in 0.01M phosphate buffer containing 0.05% w/v sodium azide and 2% v/v Magnevist Gadolinium-based contrast agent.

Figure 2. The effect of sub-chronic PCP (2 mg/kg, i.p. twice daily for seven days, followed by a 7-day washout period) on the exploration time (seconds) of two identical objects, A and B, at 1 and 6 weeks after cessation of PCP treatment in the 3 min acquisition trial (Figure 2a, e) and the familiar object and a novel object in the 3 min retention trial (Figure 2b, f). Data for the discrimination index (DI) are also shown (Figure 2c, g). Data are expressed as mean ± s.e.m (n=15 per group) and were analysed by Student’s t-test. *p<0.05; significant difference between time spent exploring the familiar and novel object. There were no significant differences in the total number of line crossings in the acquisition plus retention trial as a measure of locomotor activity (LMA) at either timepoint (Figure 2d and 2h). Data shown are scatter plot plus mean.
where each data point represents the LMA for an individual animal. Acute PCP challenge elicits significantly greater locomotor activity (LMA) as shown by the time course (Figure 2i) and area under the curve analysis (Figure 2j) following sub-chronic exposure to PCP as compared to saline-treated rats. In Figure 2g, **p<0.01; PCP-PCP (n=15) vs. all other treatment groups (all n=15; Bonferroni post-hoc test corrected for multiple comparisons on significant time x treatment interaction from 2x2 RM ANOVA). In Figure 2h, **p<0.01; ***p<0.001 PCP-PCP (n=15) vs. all other treatment groups (all n=15; Bonferroni post-hoc test corrected for multiple comparisons following overall significant 1-way ANOVA model).

**Figure 3.** Total brain volume is significantly reduced in female LH rats sub-chronically exposed to PCP (n=15) as compared to saline-treated rats (n=15), 6 weeks post-exposure. *p<0.05 saline vs. scPCP, 2-tailed t-t-test. Data shown are volume (mm$^3$).

**Figure 4.** Voxel-wise tensor based morphometry (TBM) analysis (not covarying for whole brain volume) reveals widespread apparent volume changes in the female rat brain 6 weeks after sub-chronic exposure to PCP as compared to saline controls. Data shown are voxel-wise $t$-statistics of group-levels differences (contrast: saline [n=15] vs. PCP [n=15]) in the log scaled Jacobian determinant ($J$), corrected for multiple comparisons using Family-Wise Error (FWE) with a 5% threshold ($p<0.05$), overlaid on the minimum deformation template image. Cold colours apparent volume contraction (PCP< Saline). Note that co-varying for total brain volume results in no significantly different voxels between saline and scPCP-treated rats even at highly liberal and
exploratory FWE thresholds (20%; \( p<0.2 \); data not shown) suggesting a lack of regional specificity in scPCP-induced volume changes in female LH rats.

**Figure 5.** No relationship between the relative volumes of the Perirhinal cortex areas 35 and 36 and time spent exploring the novel object in vehicle controls (a, b). In contrast, a positive correlation is observed in scPCP rats (c, d). Data shown are exploration time for the novel object (seconds) and relative volume (as % of total brain) for each animal in each group. The \( r \) and \( p \)-values indicated reflect both the data from a Pearson’s correlation.
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389x78mm (300 x 300 DPI)
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<table>
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<th>Exploration time (sec)</th>
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<td>Familiar object</td>
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<td>Cornu ammonis 3 (CA1)</td>
<td>Pearson’s r .059, p-value .420</td>
<td>Pearson’s r .237, p-value .207</td>
<td>Pearson’s r -.155, p-value .299</td>
</tr>
<tr>
<td>CA3 (%)</td>
<td>Pearson’s r -.299, p-value .150</td>
<td>Pearson’s r .123, p-value .337</td>
<td>Pearson’s r -.226, p-value .219</td>
</tr>
<tr>
<td>Postrhinal cortex (PRC)</td>
<td>Pearson’s r .034, p-value .454</td>
<td>Pearson’s r .277, p-value .169</td>
<td>Pearson’s r -.201, p-value .246</td>
</tr>
<tr>
<td>PRC (%)</td>
<td>Pearson’s r -.356, p-value .106</td>
<td>Pearson’s r .264, p-value .181</td>
<td>Pearson’s r -.378, p-value .091</td>
</tr>
<tr>
<td>Perirhinal area (PA) 35</td>
<td>Pearson’s r .167, p-value .284</td>
<td>Pearson’s r .242, p-value .202</td>
<td>Pearson’s r -.111, p-value .352</td>
</tr>
<tr>
<td>PA35 (%)</td>
<td>Pearson’s r .631, p-value .008</td>
<td>Pearson’s r .220, p-value .225</td>
<td>Pearson’s r .114, p-value .349</td>
</tr>
<tr>
<td>Perirhinal area (PA) 36</td>
<td>Pearson’s r .142, p-value .314</td>
<td>Pearson’s r .258, p-value .187</td>
<td>Pearson’s r -.137, p-value .320</td>
</tr>
<tr>
<td>PA36 (%)</td>
<td>Pearson’s r .488, p-value .038</td>
<td>Pearson’s r .291, p-value .156</td>
<td>Pearson’s r -.038, p-value .448</td>
</tr>
<tr>
<td>Entorhinal cortex (EC)</td>
<td>Pearson’s r .050, p-value .432</td>
<td>Pearson’s r .282, p-value .165</td>
<td>Pearson’s r -.198, p-value .249</td>
</tr>
<tr>
<td>EC (%)</td>
<td>Pearson’s r -.190, p-value .257</td>
<td>Pearson’s r .297, p-value .151</td>
<td>Pearson’s r -.338, p-value .119</td>
</tr>
<tr>
<td>Cingulate cortex (Cg)</td>
<td>Pearson’s r .063, p-value .416</td>
<td>Pearson’s r .176, p-value .273</td>
<td>Pearson’s r -.116, p-value .347</td>
</tr>
<tr>
<td>Cg (%)</td>
<td>Pearson’s r -.088, p-value .382</td>
<td>Pearson’s r -.154, p-value .300</td>
<td>Pearson’s r .044, p-value .441</td>
</tr>
</tbody>
</table>
Global brain volume reductions in a sub-chronic phencyclidine animal model for schizophrenia and their relationship to recognition memory

Nazanin Doostdar1§, Eugene Kim2§, Ben Grayson1, Michael K. Harte1, Joanna C. Neill1*, Anthony C. Vernon3, 4*✪

1Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Medicine, Biology and Health, University of Manchester, Manchester, M13 9PT, UK
2King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Neuroimaging, Centre for Neuroimaging Sciences, De Crespigny Park, London, SE5 8AF, UK
3King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London, SE5 9RT, United Kingdom
4King’s College London, MRC Centre for Neurodevelopmental Disorders, New Hunt's House, Guy's Hospital Campus, London SE1 1UL, United Kingdom.

§These authors contributed equally to this publication

*Shared senior authorship

✪ Corresponding author: Anthony C. Vernon (anthony.vernon@kcl.ac.uk); King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London, SE5 9RT, United Kingdom

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SUPPLEMENTARY INFORMATION

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1Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Medicine, Biology and Health, University of Manchester, Manchester, M13 9PT, UK

2King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Neuroimaging, Centre for Neuroimaging Sciences, De Crespigny Park, London, SE5 8AF, UK

3King’s College London, Institute of Psychiatry, Psychology and Neuroscience, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London, SE5 9RT, United Kingdom

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SUPPLEMENTARY RESULTS
Supplementary Table 1. Absolute (mm$^3$) volumes extracted from n=117 regions of interest for all saline (n=15) and PCP-exposed (n=14) rats, derived using a custom-made hybrid of two publicly available rat brain MRI atlases combined with a computational atlas based segmentation (ABS) pipeline. Data shown are mean and 95% confidence intervals (95% CI).

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>saline (n=15)</th>
<th>PCP (n=14)</th>
<th>Mean Diff</th>
<th>95% CI Diff</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>dentate gyrus</td>
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<tr>
<td>hippocampus</td>
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<tr>
<td>parietal cortex</td>
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<tr>
<td>temporal cortex</td>
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<tr>
<td>Insula</td>
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<tr>
<td>sensory cortex</td>
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<tr>
<td>motor cortex</td>
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<tr>
<td>frontal cortex</td>
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<tr>
<td>cingulate cortex</td>
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<tr>
<td>prefrontal cortex</td>
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<tr>
<td>orbitofrontal cortex</td>
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<tr>
<td>accumbus</td>
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<tr>
<td>amygdalae</td>
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<tr>
<td>hypothalamus</td>
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<tr>
<td>thalamus</td>
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<tr>
<td>caudate nucleus</td>
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<td>pallidum</td>
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<tr>
<td>subthalamic nucleus</td>
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<tr>
<td>basal ganglia</td>
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<tr>
<td>substantia nigra</td>
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<tr>
<td>midbrain</td>
<td></td>
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<tr>
<td>diencephalon</td>
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</tbody>
</table>

Note: The table includes volumes for various brain regions, with columns for mean and 95% CI differences, and p-values for statistical significance. The data is presented in a tabular format with headers for each region of interest, followed by columns for saline and PCP groups, mean differences, 95% CI differences, and p-values. The p-values are used to determine the significance of the differences between the two groups.
standard deviation (SD) for each atlas ROI for each treatment group, as well as the results of statistical comparisons between groups using 2-tailed t-test (contrast: saline vs. PCP) at p<0.05 uncorrected (“TTEST”, trend-level significance) and after correction for multiple comparisons using the False Discovery Rate (FDR) with a 5% threshold (“FDR-p”; q<0.05). The percentage change and effect size are also shown.
Supplementary Table 2. Relative (percentage of whole brain) volumes extracted from n=117 regions of interest for all saline (n=15) and PCP-exposed (n=14) rats, derived using a custom-made hybrid of two publicly available rat brain MRI atlases combined with a computational atlas based segmentation (ABS) pipeline. Data shown are mean and standard deviation (SD) for each atlas ROI for each treatment group, as well as the results of statistical comparisons between groups using 2-tailed t-test (contrast: saline vs. PCP) at p<0.05 uncorrected (“TTEST”, trend-level significance) and after correction for multiple comparisons using the False Discovery Rate (FDR) with a 5% threshold (“FDR-p”; q<0.05). The percentage change and effect size are also shown.