Schizophrenia Research 212 (2019) 243–245

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Letter to the Editor

Increased translocator protein (TSPO) binding throughout neurodevelopment in the perinatal phencyclidine rodent model of schizophrenia

Radioligands for the 18 kDa mitochondrial translocator protein (TSPO) have been used in positron emission tomography (PET) and Single Photon Emission Tomography (SPECT) studies to investigate the potential neuroinflammatory status of patients with neuropsychiatric disorders. TSPO is primarily expressed on the mitochondrial membrane within microglia, astrocytes and vascular endothelial cells (Notter et al., 2018), whereby its expression is increased under certain neuroinflammatory states (Dupont et al., 2017). However, TSPO imaging studies in subjects diagnosed with schizophrenia have revealed inconsistent results (Fukudome et al., 2018), leading to some confusion as to the neuroinflammatory status. Such discrepancies may be due to medication history, polymorphisms of the TSPO gene, TSPO tracer utilized, stage of illness or the diverse heterogeneity of schizophrenia.

Animal models can aid in illustrating the development of and heterogeneity associated with schizophrenia. To examine the relationship between TSPO binding and schizophrenia pathology, Notter et al. (2018) investigated *ex vivo* TSPO expression and binding in adult mice from the maternal immune activation model of schizophrenia. Whilst they reported no change in TSPO ([^{3}H]PK11195) binding, a reduction in TSPO immunoreactivity was observed in the prefrontal cortex, concurrent with increased cytokine levels. Contrary, Fukudome et al. (2018) recently reported increased TSPO ([^{125}I]iodo-DPA-713) binding in adult mice following cuprizone short-term exposure (CSE), a model of psychosis, which has shown increased cytokine levels (Tezuka et al., 2013). Although limited in number, these animal studies appear to show conflicting results, similar to clinical studies. Disparities between animal models may highlight the differing mechanisms of altered neuroinflammatory processes or heterogeneity of symptom pathology, in addition to the type and timing of insult and examination. Therefore, the investigation of TSPO binding and neuroinflammation in a variety of animal models of schizophrenia may aid in dissecting the different mechanisms of schizophrenia pathology and neuroinflammation. The perinatal/neonatal phencyclidine (PCP) model provides an established neurodevelopmental model, to investigate these aspects of schizophrenia. Administration of PCP in neonatal rodents has shown to produce pre-pulse inhibition deficits, locomotor sensitization, working memory and executive function deficits at adulthood, in addition to neurochemical dysfunction to the glutamatergic, GABAergic and dopaminergic system (for review see Grayson et al., 2015). In an attempt to investigate the neuroinflammatory profile in the neonatal PCP rodent model of schizophrenia, we examined TSPO autoradiography in various brain regions, using the 2nd generation TSPO ligand, [^{125}I]CLINDE at critical neurodevelopmental time-points.

Male Sprague-Dawley rats were administered PCP (10 mg/kg, s.c.) or saline, as previously described (du Bois et al., 2012) and sacrificed at postnatal day (PN) 12, 5 weeks or 20 weeks of age (Animal Ethics Committee approval, AE09/03). Sections were incubated at 4 °C for 1 h in 50 mM Tris-HCl buffer, pH 7.4, containing 3 nM [^{125}I]CLINDE (specific activity 3.7 GBq/μmol). Non-specific binding was determined by incubating adjacent sections with [^{125}I]CLINDE in the presence of 10 μM PK11195 (Sigma). Two-way Analysis of Variance was used to analyze TSPO binding density in the prefrontal cortex (PFC), hippocampus, thalamus and caudate putamen (CPu). For significant interactions, post-hoc pairwise comparisons were used to compare binding density between treatment groups at individual time-points.

[^{125}I]CLINDE binding increased in an age-dependent manner in all brain regions ($p < 0.01$, see Fig. 1), similar to previous observations in humans (Kumar et al., 2012). Furthermore, [^{125}I]CLINDE binding was upregulated in perinatal PCP-treated rats in an age and brain region-specific manner. [^{125}I]CLINDE binding was significantly increased in the hippocampus of perinatal PCP-treated rodents compared to their saline-treated counterparts at PN12 ($p = 0.040$), 5 weeks ($p < 0.001$) and 20 weeks ($p < 0.001$). Similarly, PCP-induced increases were observed in the thalamus at 5 ($p < 0.001$) and 20 weeks ($p = 0.003$). In the PFC, a significant increase in [^{125}I]CLINDE binding was observed in PCP-treated rats at 20 weeks ($p = 0.001$), indicating perinatal PCP treatment induces delayed effects on [^{125}I]CLINDE binding in the PFC. No changes in [^{125}I]CLINDE binding were observed in the CPu at any time-points between saline- and PCP-treated rats.

There is conflicting clinical evidence regarding TSPO binding in schizophrenia patients. Collectively, these varying findings are also reflected in schizophrenia-relevant animal models, including the present results, and may represent the pathogenic heterogeneity of schizophrenia. Fukudome et al. (2018) hypothesized the discrepancies in TSPO binding between the MIA model and their CSE model may be due to the timing of immune/inflammatory activation, whereby early insults may lead to a reduction in TSPO levels. However, the present results challenge this notion, whereby we provide evidence of increased TSPO binding in the hippocampus and thalamus following an early (excitotoxic) insult, which continues into adulthood. Furthermore, following perinatal PCP treatment, we show a delayed increase of TSPO binding in the PFC, with changes not detected until adulthood. Despite animal models being able to represent an entire pathology, the PCP model permits investigation of a potential inflammatory aetiology of schizophrenia throughout neurodevelopment. The clinical relevance of this model is supported by a recent meta-analysis investigating *in vivo* TSPO PET binding in individuals with schizophrenia, similarly finding a significant increase in binding when binding potential was used as the outcome measure (Marques et al., 2018).

As far as we are aware, this is the first study to examine TSPO binding during several critical periods of neurodevelopment. These results suggest brain region-specific responses that may reflect the

https://doi.org/10.1016/j.schres.2019.07.041

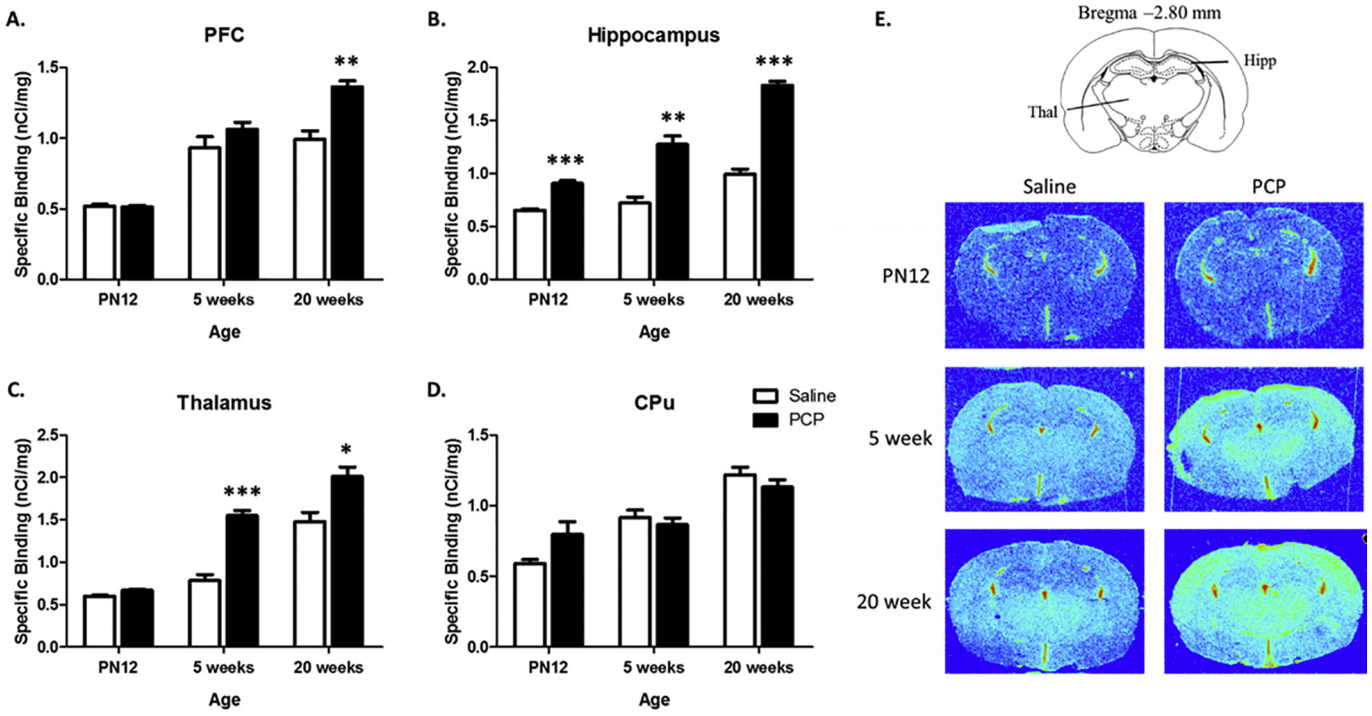

Fig. 1. Effects of perinatal PCP treatment on TSPO binding density. A. Perinatal PCP treatment increased [^{125}I]CLINDE in the prefrontal cortex (PFC) at 20 weeks of age (38%, $p = 0.001$). B. A significant increase of [^{125}I]CLINDE density was observed in the hippocampus of perinatal PCP-treated rats at PN12 (39%, $p = 0.040$), 5 weeks (77%, $p < 0.001$) and 20 weeks (84%, $p < 0.001$) compared to saline-treated rats. C. Furthermore, [^{125}I]CLINDE binding within the thalamus was significantly increased at 5 (97%, $p < 0.001$) and 20 weeks (36%, $p = 0.003$). D. The caudate putamen (CPu) showed no difference in [^{125}I]CLINDE binding between saline- and PCP-treated rats at PN12, 5 weeks or 20 weeks. E. Representative autoradiographs showing TSPO binding density in the thalamus and hippocampus at PN12, 5 weeks and 20 weeks of saline- and PCP-treated rats. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ *vs.* age matched controls.

neuronal damage previously observed following perinatal NMDA receptor antagonism (Jevtić et al., 2016; Wang et al., 2007). However, further studies are required to establish the source of the observed increase of TSPO binding and concurrent neuroinflammatory state. Furthermore, investigation is required as to the extent that microglia and astrocytes may contribute to the disease-relevant behavioural and neurochemical abnormalities observed in this model. However, taken together the TSPO response in the different animal models may provide avenues for investigating the different neurodevelopmental sequela in schizophrenia. This study suggests perinatal disruption to the NMDA receptor, which can arise *via* various avenues, can cause long-term region-specific increases in TSPO binding and may provide a useful platform for interrogating the underlying pathophysiology/mechanism of the increases in TSPO observed in a subset of schizophrenia subjects.

Contributors

T.M. Du Bois, K.A. Newell, and X.F. Huang designed the study. F. Mattner and A. Katsifis designed and synthesized the radioligand, [^{125}I]CLINDE. T.M. Du Bois and K.A. Newell acquired the data, which all authors analyzed. J.S. Lum and S.J. Brown wrote the original manuscript, which all authors reviewed and approved for submission.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

This research has been conducted with the support of the Australian Government Research Training Program Scholarship awarded to S.J.B. Funding for this study was provided by National Health and Medical Research Council (NHMRC) Project Grant ID573426, as well as the Schizophrenia Research Institute (SRI) Australia, utilising infrastructure from New South Wales Health.

References

du Bois, T.M., Newell, K.A., Huang, X.-F., 2012. Perinatal phencyclidine treatment alters neuregulin 1/erbB4 expression and activation in later life. Eur. Neuropsychopharmacol. 22, 356–363.

Dupont, A.-C., Largeau, B., Santiago Ribeiro, M.J., Guilloteau, D., Tronel, C., Arlicot, N., 2017. Translocator protein-18 kDa (TSPO) positron emission tomography (PET) imaging and its clinical impact in neurodegenerative diseases. Int. J. Mol. Sci. 18, 785.

Fukudome, D., Hayes, L.N., Faust, T.E., Foss, C.A., Kondo, M.A., Lee, B.J., Saito, A., Kano, S.-I., Coughlin, J.M., Kamiya, A., Pomper, M.G., Sawa, A., Niwa, M., 2018. Translocator protein (TSPO) and stress cascades in mouse models of psychosis with inflammatory disturbances. Schizophr. Res. 197, 492–497.

Grayson, B., Barnes, S.A., Markou, A., Piercy, C., Podda, G., Neill, J.C., 2015. Postnatal phencyclidine (PCP) as a neurodevelopmental animal model of schizophrenia pathophysiology and symptomatology: a review. In: Kostrzewa, R.M., Archer, T. (Eds.), Neurotoxin Modeling of Brain Disorders — Life-Long Outcomes in Behavioral Teratology, Current Topics in Behavioral Neurosciences. Springer International Publishing, pp. 403–428.

Jevtić, G., Nikolić, T., Mirčić, A., Stojković, T., Velimirović, M., Trajković, V., Marković, I., Trbovich, A.M., Radonjić, N.V., Petronijević, N.D., 2016. Mitochondrial impairment, apoptosis and autophagy in a rat brain as immediate and long-term effects of perinatal phencyclidine treatment — influence of restraint stress. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 66, 87–96.

Kumar, A., Muzik, O., Shandal, V., Chugani, D., Chakraborty, P., Chugani, H.T., 2012. Evaluation of age-related changes in translocator protein (TSPO) in human brain using ^{11}C-[R]-PK11195 PET. J. Neuroinflammation 9, 232.

Marques, T.R., Ashok, A.H., Pillinger, T., Veronese, M., Turkheimer, F.E., Dazzan, P., Sommer, I.E.C., Howes, O.D., 2018. Neuroinflammation in schizophrenia: meta-analysis of in vivo microglial imaging studies. Psychol. Med. 1–11.

Notter, T., Coughlin, J.M., Gschwind, T., Weber-Stadlbauer, U., Wang, Y., Kassiou, M., Vernon, A.C., Benke, D., Pomper, M.G., Sawa, A., Meyer, U., 2018. Translational evaluation of translocator protein as a marker of neuroinflammation in schizophrenia. Mol. Psychiatry 23, 323–334.

Tezuka, T., Tamura, M., Kondo, M.A., Sakaue, M., Okada, K., Takemoto, K., Fukunari, A., Miwa, K., Ohzeki, H., Kano, S., Yasumatsu, H., Sawa, A., Kajii, Y., 2013. Cuprizone short-term exposure: astrocytic IL-6 activation and behavioral changes relevant to psychosis. Neurobiol. Dis. 59, 63–68.

Wang, C.Z., Yang, S.F., Xia, Y., Johnson, K.M., 2007. Postnatal phencyclidine administration selectively reduces adult cortical parvalbumin-containing interneurons. Neuropsychopharmacology 33, 2442–2455.\

Jeremy S. Lum*,
Teresa M. du Bois
Illawarra Health and Medical Research Institute, Wollongong, Australia
Molecular Horizons and School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, New South Wales 2522, Australia

*Corresponding author at: Molecular Horizons and School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, New South Wales 2522, Australia.
E-mail address: jlum@uow.edu.au.

Filomena Mattner,
Andrew Katsifis
Department of Molecular Imaging, Royal Prince Alfred Hospital, Missenden Road, Camperdown, 2050, Australia

Samara J. Brown,
Xu-Feng Huang
Illawarra Health and Medical Research Institute, Wollongong, Australia
Molecular Horizons and School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, New South Wales 2522, Australia

Kelly A. Newell
Illawarra Health and Medical Research Institute, Wollongong, Australia
Molecular Horizons and School of Medicine, Faculty of Science, Medicine and Health, University of Wollongong, Wollongong, New South Wales 2522, Australia

12 July 2019
Available online 13 August 2019