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Translocator protein (TSPO) and stress cascades in mouse models of psychosis with inflammatory disturbances

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Abstract

Changes in inflammatory cascades have been implicated in the underlying pathophysiology of psychosis. Translocator protein 18 kDa (TSPO) has been used to assess neuroinflammatory processes in psychotic disorders. Nonetheless, it is unclear whether TSPO, a mitochondrial protein, can be interpreted as a general marker for inflammation in diseases involving psychosis. To address this question, we investigated TSPO signaling in representative mouse models for psychosis with inflammatory disturbances. The maternal immune activation and cuprizone short-term exposure models show different TSPO signaling. Furthermore, we observed similarities and differences in their respective stress pathways including stress hormone signaling and oxidative stress that are functionally interconnected with the inflammatory responses. We propose that more careful studies of TSPO distribution in neuroinflammation and other stress cascades associated with psychotic symptoms will allow us to understand the biological mechanisms underlying psychosis-related behaviors.

Keywords

cuprizone short-term exposure (CSE); maternal immune activation (MIA); inflammatory disturbances; Translocator protein 18 kDa (TSPO); stress cascades; psychosis

1. Introduction

Psychotic conditions, such as schizophrenia and major depressive disorder with psychotic features, are devastating medical conditions that affect certain percentages of the general

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population (Bogren et al., 2009; Kendler et al., 1996; Kessler et al., 2005; Perälä et al., 2007; van Os et al., 2001). Etiological factors and pathophysiological mediators for schizophrenia and related disorders have been studied using a variety of research approaches. Epidemiological studies consistently report that infection and other immune disturbances may underlie the etiology of the diseases (Brown and Derkits, 2010). Recent advances in human genetics implicated inflammatory molecules in psychotic disorders by both specific candidate molecular approaches as well as group functional pathway analyses (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Sekar et al., 2016). In addition, examinations of biospecimens from patients including blood and cerebrospinal fluid implicated dynamic changes in inflammatory cascades that are associated with symptoms (Coughlin et al., 2016; Hayes et al., 2014; Miller et al., 2011). Postmortem brain studies also show alterations in the inflammatory responses in these diseases (Fillman et al., 2013; Trepanier et al., 2016).

Given that inflammatory cascades may be changed in patients with psychotic features, both clinical and preclinical studies have addressed how inflammatory changes may affect behaviors relevant to psychosis. Several clinical studies explored the correlation between specific molecular changes in the inflammatory responses and symptoms or treatment responses (Fillman et al., 2016; Mondelli et al., 2015). In preclinical studies, animal models in which inflammatory mechanisms are primarily disturbed have been studied for behaviors relevant to psychosis. For example, administration of polyinosinic:polycytidylic acid [poly(I:C)], a synthetic analogue of double-stranded RNA, to pregnant dams disturbs the inflammatory responses of the offspring during early development and subsequently affects their behavior in adulthood (Meyer and Feldon, 2010). Note that, depending on the timing of poly(I:C) administration, the maternal immune activation (MIA) model may also emphasize behaviors relevant to autistic disorder spectrum (Careaga et al., 2017). Furthermore, a deficiency of schnurri-2, a nuclear factor-κB site-binding protein, induces neuroinflammation and leads to behavioral deficits relevant to schizophrenia (Takao et al., 2013). Our group recently reported that a new animal model exposed to cuprizone, a copper chelator, for one week only in young adulthood shows inflammatory disturbances and subsequent behavioral deficits relevant to psychosis (Kondo et al., 2016; Tezuka et al., 2013).

The peripheral benzodiazepine receptor/translocator protein 18 kDa (TSPO) has utility as a specific molecular target using positron emission tomography (PET) to probe possible neuroinflammation. TSPO protein is located in the mitochondrial membrane of several types of cells in the brain, in particular astrocytes and microglia (Gut et al., 2015; Rupprecht et al., 2010). Since TSPO is upregulated in some inflammatory conditions in the brain, TSPO was once expected to be a good marker for inflammation using PET-based neuroimaging (Rupprecht et al., 2010). Indeed, in brain inflammatory conditions that are acquired in adulthood, high TSPO PET signals have been reported (Gerhard et al., 2005; Gershen et al., 2015; Oh et al., 2011; Ramlackhansingh et al., 2011; Suridjan et al., 2015). Consistent with these reports, our group successfully visualized high TSPO PET signals in inflammatory conditions such as human immunodeficiency virus-associated dementia and history of sports-related, repeated traumatic brain injury found in National Football League players (Coughlin et al., 2014; Coughlin et al., 2017). Nevertheless, in subjects with psychotic

disorders (e.g., those with schizophrenia and first-episode psychosis), as well as subjects with an at-risk mental state for psychosis, the data TSPO imaging are inconsistent across reports (Bloomfield et al., 2016; Collste et al., 2017; Coughlin et al., 2016; Doorduin et al., 2009; Hafizi et al., 2017; Hannestad et al., 2013; Holmes et al., 2016; Kenk et al., 2015; Notter et al., 2017; Setiawan et al., 2015; Takano et al., 2010; van Berckel et al., 2008; van der Doef et al., 2016) (also see Table 1). Although some studies have reported higher TSPO PET signals in subjects in psychotic disorders and in subjects with an at-risk mental state for psychosis compared to controls (Bloomfield et al., 2016; Doorduin et al., 2009; Setiawan et al., 2015; van Berckel et al., 2008), several other groups have observed no change or even lower TSPO PET signals associated with psychosis (Collste et al., 2017; Coughlin et al., 2016; Hafizi et al., 2017; Hannestad et al., 2013; Holmes et al., 2016; Kenk et al., 2015; Notter et al., 2017; Takano et al., 2010; van der Doef et al., 2016). Our group reported that patients with recent onset of schizophrenia displayed an increase in the pro-inflammatory cytokine interleukin-6 (IL-6), whereas there was no difference in the binding of [^{11}C]DPA-713 to TSPO across the brains of these patients compared to matched healthy controls (Coughlin et al., 2016).

Inflammatory responses are functionally interconnected with other stress pathways, such as those involving stress hormones and those associated with oxidative stress (Landek-Salgado et al., 2016). Stress hormones, the hypothalamic-pituitary-adrenal (HPA) axis, and oxidative stress are implicated in the pathophysiology of schizophrenia and related disorders (Coughlin et al., in press; Emiliani et al., 2014; Garner et al., 2005; Hardingham and Do, 2016; Johnson et al., 2013; Niwa et al., 2013; Niwa et al., 2016; Tanaka et al., 2017). Therefore, evaluating the interconnection of multiple stress cascades in animals may provide insight into the biology of schizophrenia and related disorders.

In the present study, we examined two representative animal models of inflammatory disturbances in the context of psychosis: a MIA model and a cuprizone short-term exposure (CSE) model. The MIA model is associated with immune/inflammatory disturbance in early development, whereas in the CSE model the immune disturbance is initially triggered in young adulthood. A recent study using the MIA model reported lower TSPO signal in the prefrontal cortex (PFC) of affected animals compared to controls, which is similar to findings in patients (Notter et al., 2017). Building on this background we conducted TSPO autoradiography in the CSE model. Furthermore, we examined possible changes in cascades related to the HPA axis and oxidative stress in both MIA and CSE models, with the goal of drawing a comprehensive and comparative picture of how stress cascades affect psychosis-related behaviors in these preclinical animal models. To focus on the frontal lobe pathology of psychosis, we studied oxidative stress cascades in the PFC of the CSE and MIA models.

2. Experimental materials and methods

2.1. Animal models

In the CSE model, 8-week-old C57BL/6J male mice were fed either a diet containing 0.2% cuprizone (Sigma-Aldrich, St. Louis, MO), or a control diet consisting of standard chow (Harlan Teklad, Indianapolis, IN) for one week according to our published protocol (Kondo et al., 2016; Tezuka et al., 2013). Experiments were performed on the 7th day of diet

exposure. In the MIA model, pregnant C57BL/6J mice were administered poly(I:C) (20 mg/kg, *intraperitoneal*, Sigma-Aldrich, St. Louis, MO) on embryonic day 9.5 as described previously with minor modifications (Meyer et al., 2005). Experiments were conducted when the offspring reached 8 weeks of age. All experimental procedures followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and the Johns Hopkins University Animal Care and Use Guidelines.

2.2. *Ex vivo* autoradiography

Ex vivo autoradiography was performed using the second-generation TSPO radiotracer of [^{125}I]iodo-DPA-713 (Wang et al., 2009), because it is regarded as one of the best TSPO ligands in regard to sensitivity and specificity. Although the disadvantage is that this is affected by a critical genetic polymorphism in humans (Kobayashi et al., 2017), the corresponding polymorphism does not exist in mice (Parente et al., 2016).

Mice were injected intravenously with an average of 9.25 ± 0.55 MBq of [^{125}I]iodo-DPA-713, specific activity 2,200 Ci/mmol, in 200 μl of 10% ethanol in phosphate buffered saline. All mice were sacrificed after 45 min of radiotracer uptake. The brains of the mice were cryosectioned into 20 μm thick horizontal sections according to the atlas of Franklin and Paxinos (Franklin and Paxinos, 2013), and were exposed to Kodak Biomax XR film along with serial dilutions of radiotracer standard. The film was developed using a Kodak X-O-Mat film processor and digitized using an MCID Core densitometry station and software. The regions of interest (ROIs) were set in one mm circles of the same size and shape for each subregion (> 20/subregion). The signals for the ROIs in sections were averaged across each subregion for each mouse section. Radiotracer uptake by region is expressed as fmol radiotracer/mm^3 of tissue section.

The specific binding signals of DPA-713 to TSPO were obtained by subtraction of the nonspecific binding from the total binding. The non-specific binding was estimated by evaluating the signals of [^{125}I]iodo-DPA-713 radiotracer when this was co-injected with 20 mg/kg of (R,S)-PK11195.

2.3. Levels of TSPO protein and 3-nitrotyrosine (3-NT)

The PFC (anteroposterior, +2.34 to +1.54 mm from Bregma; mediolateral, −0.50 to +0.50 mm from Bregma; dorsoventral, −0.75 to −3.00 mm from the dura) was collected according to the Mouse Brain Atlas (Franklin and Paxinos, 2013), and protein samples were prepared as previously described (Niwa et al., 2010). The levels of TSPO protein, 3-NT, and β-actin in the lysates were determined using western blotting as previously described (Niwa et al., 2010) with minor modifications. Briefly, TSPO, 3-NT, and β-actin were identified with primary antibodies against TSPO (1:10,000, #Ab109497, Abcam, Cambridge, UK), 3-NT (1:2,000, #06-284, Millipore, Billerica, MA), and β-actin (1:1,000, #4970, Cell Signaling Technology, Danvers, MA) respectively. Horseradish peroxidase-conjugated anti-rabbit IgG (1:2,000, #7074, Cell Signaling Technology, and 1:5,000, NA934, GE Healthcare Bio-Sciences, Piscataway, NJ) was used as the secondary antibody.

2.4. Levels of serum corticosterone

Blood was collected from the inferior vena cava under isoflurane anesthesia between 9 am and 11 am. Serum levels of corticosterone were determined using a commercially available enzyme immune assay kit (Cayman Chemical, Ann Arbor, MI) as described previously (Niwa et al., 2013).

2.5. Levels of protein carbonylation

Levels of protein carbonylation were measured according to our published protocols with minor modifications (Johnson et al., 2013). Quantitative analysis of protein carbonylation levels of the PFC lysates was performed by a dot blot procedure with the OxyBlot Protein Oxidation Detection Kit (Millipore, Billerica, MA).

2.6. Statistical analysis

Data were assessed for normal distribution and homogeneity of variance using the Shapiro-Wilk and F-tests, respectively. Parametric statistical comparisons were performed using a two-tailed unpaired Student's t-test, except for the comparison of relative carbonylation levels of protein in MIA mice, which didn't show normal distribution. Thus, the data in Fig. 3B was analyzed with the Mann-Whitney U-test. For the comparison of relative levels of 3-NT protein, one-way analysis of variance was used. An α value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. TSPO distribution in CSE

We recently reported that TSPO binding was significantly lower in the MIA model that has inflammatory disturbances from early development, compared to controls (Notter et al., 2017). Here we examined radiotracer binding to the TSPO target in another inflammatory animal model relevant to psychosis, the CSE model, although the initial trigger for immune/inflammatory disturbance is in young adulthood, and not in early development. We observed augmentation of TSPO binding in broad areas of the brain, especially in the PFC, HP, and STR, of CSE mice compared with control (CON) animals (Fig. 1 and Supplemental Fig. 1). No difference of TSPO expression was observed in the PFC between the CSE model and CON animals at least through the biochemical methodology that we used (Supplemental Fig. 2).

3.2. HPA axis in CSE and MIA

Inflammatory responses are functionally interconnected with other stress pathways, including stress hormones and oxidative stress (Landek-Salgado et al., 2016). To address stress cascades other than inflammatory responses, we also examined changes in the HPA axis. We observed a significant increase in the level of serum corticosterone in both CSE and MIA models compared to baseline (Fig. 2).

3.3. Oxidative stress in CSE and MIA

We next addressed possible involvement of oxidative stress in the two models. We previously reported the preclinical evidence that oxidative stress is linked to neurocognitive deficits relevant to psychotic disease (Johnson et al., 2013). Using the protein carbonylation assay, we did not observe any increase of oxidative stress in the PFC of the CSE model (Fig. 3A). Interestingly, the level of protein carbonylation in the PFC of the MIA animals was significantly reduced compared with the CON animals (Fig. 3B). In contrast, we observed no significant difference in the 3-NT signals among the CSE, MIA, and CON mice (Supplemental Figure 3).

4. Discussion

One of the main goals in the present study was to examine TSPO binding in the CSE model and the connection with the HPA axis and oxidative stress in both the CSE and MIA models as compared to controls. Both CSE and MIA models displayed common signatures in stress cascades, such as an increase in the IL-6 signaling (a representative indicator for inflammation) and the HPA axis (Table 2). Nevertheless, a sharp contrast between these two models exists in the TSPO signals (Table 2).

As described above, TSPO findings in clinical studies in psychotic disorders are not consistent. These differences in data from TSPO brain imaging could be due to disease heterogeneity, analysis during different time points in pathological trajectory, the TSPO gene (rs6971) polymorphism, differences of medications, and differences between each tracer targeting the same molecule (DAA1106, DPA-713, FEPPA, PBR28, and PK11195). We propose that an overarching proposal of upregulated TSPO signaling in psychosis may be avoided. On the other hand, we propose that multiple animal models displaying abnormalities in the inflammatory responses and behavioral deficits relevant to psychosis should be utilized in parallel to decipher different aspects of inflammatory disturbances in patients with psychosis. PK11195 (1st generation TSPO ligand) may be inferior to other 2nd generation TSPO ligands such as DAA1106, DPA-713, FEPPA, and PBR28 in signal-noise ratio and brain penetration (Boutin et al., 2007; Brown et al., 2007; Endres et al., 2009; Rusjan et al., 2011; Wilson et al., 2008; Zhang et al., 2003). Thus, in such comparative studies, the data from 2nd generation TSPO ligands might be useful. A working hypothesis is that TSPO levels in the brain may be downregulated in psychotic conditions that are originally elicited in immune/inflammatory insults in early development (e.g., the MIA model or most of psychotic disorders), whereas the signals may be upregulated in neuroinflammatory conditions. More careful studies of TSPO in neuroinflammatory conditions that are associated with psychotic symptoms are needed to dissect the different mechanistic cascades.

In the long run, the mechanism of increased TSPO binding in the CSE model should be defined not merely by protein levels of TSPO protein but also by protein conformation and many other biological factors. The use of micro-PET, in which the Bmax/Kd values *in vivo* are to be available by reflecting pharmacokinetics, will provide us with more mechanistic insight in future studies. Furthermore, understanding the effects of antipsychotic drugs on TSPO binding and stress cascades would also be important.

The differences in the stress signals may originate from the timing of the original immune/inflammatory insults. The acute nature of the CSE model induces the increase in TSPO signals by massive activation of glial cells. In contrast, in the MIA model, the immune/inflammatory response occurs in early development, which is likely to alter the homeostatic set point inside the brain as a compensatory mechanism (McEwen et al., 2015). This may lead to reduction in protein carbonylation (oxidative stress) and suppression of glial activation associated with a decrease in TSPO binding. In contrast, it is interesting that such a compensatory mechanism did not seem to affect the levels of corticosterone that were commonly upregulated in both CSE and MIA models.

Interconnection of stress cascades among inflammation, HPA axis, and oxidative stress are important. For each paradigm, corresponding peripheral biomarkers may be utilized in clinical settings, including blood levels of C-reactive protein, cytokines, cortisol, and glutathione. We believe that this is a study with two animal models that aims to contribute to this long-term goal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CBL	cerebellum
CON	control
CSE	cuprizone short-term exposure
HP	hippocampus
HPA	hypothalamic-pituitary-adrenal
MIA	maternal immune activation
PET	positron emission tomography
PFC	prefrontal cortex
poly(I	C), polyinosinic:polycytidylic acid
ROIs	regions of interest
STR	striatum

TSPO	Translocator protein 18 kDa
3-NT	3-nitrotyrosine

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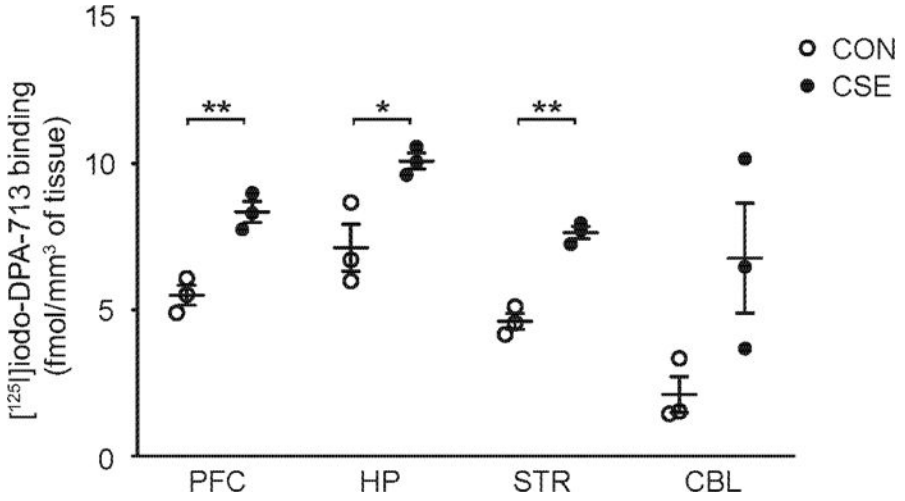

Fig. 1. Translocator protein 18kDa (TSPO) disposition in cuprizone short-term exposure (CSE)

Quantitative analyses of regional [^{125}I]iodo-DPA-713 binding to TSPO: There was a significant increase of [^{125}I]iodo-DPA-713 binding in the prefrontal cortex (PFC), hippocampus (HP) and striatum (STR), but not in the cerebellum (CBL) in CSE mice compared with CON mice. N=3 for each group. *p<0.05, **p<0.01, as analyzed by Student's t-test (p=0.0045 for PFC, p=0.0250 for HP, p=0.0009 for STR, p=0.0775 for CBL). Data are presented as mean ± S.E.M.

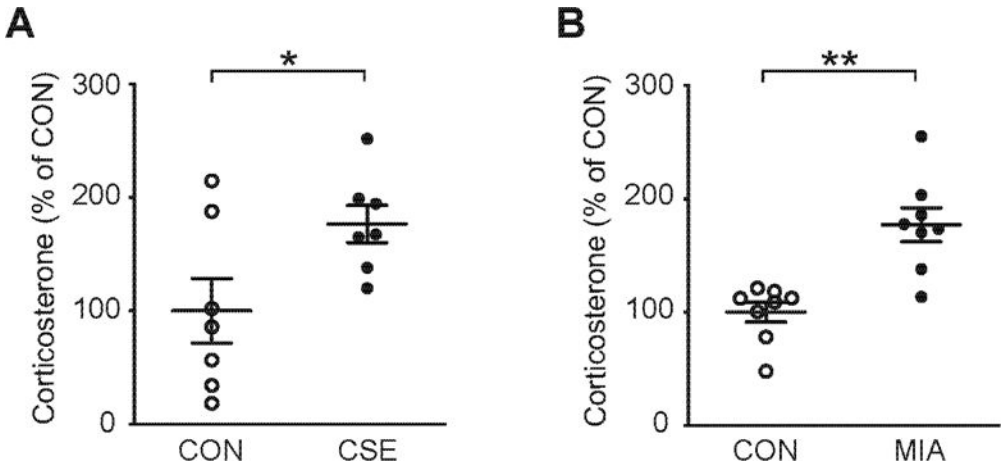

Fig. 2. Hypothalamic-pituitary-adrenal (HPA) axis in CSE and maternal immune activation (MIA)

(A) Serum levels of corticosterone in CSE mice. CSE mice showed a significant elevation in levels of serum corticosterone. The serum levels of corticosterone in CON mice were 63.10 ± 17.92 ng/mL. N=7. *p<0.05, as analyzed by Student's t-test (p=0.0380). **(B)** Serum corticosterone levels in MIA mice. MIA mice showed a significant elevation in levels of serum corticosterone. The serum levels of corticosterone in CON mice were 128.01 ± 11.29 ng/mL. N=8. **p<0.01, as analyzed by Student's t-test (p=0.005). Data are presented as mean ± S.E.M.

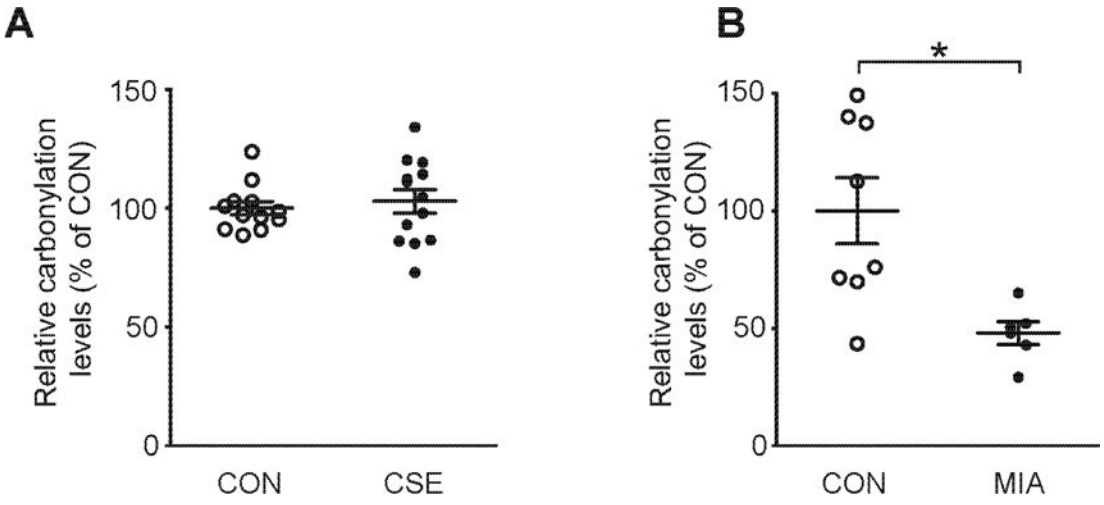

Fig. 3. Oxidative stress in CSE and MIA

(A) Relative carbonylation levels of protein in the PFC of CSE mice. No significant differences between CON and CSE mice were detected. N=12-13. p=0.6228, as analyzed by Student's t-test. **(B)** Relative carbonylation levels of protein in the PFC of MIA mice. MIA mice showed significantly reduction in relative carbonylation levels of protein. N=6-8. *p<0.05, as analyzed by Mann–Whitney U-test (p=0.0118). Data are presented as mean ± S.E.M.

Table 1

Summary of findings from TSPO PET studies in patients with major mental illnesses

Diseases	Ligands	TSPO levels	References
Psychosis (ultra high risk for psychosis)	[^{11}C]PBR28	↑	[1]
Psychosis (first-episode psychosis)	[^{11}C]PBR28	↓	[2]
Psychosis (first-episode psychosis)	[^{18}F]FEPPA	→	[3]
Schizophrenia	[^{18}F]FEPPA	→	[4]
Schizophrenia	[^{11}C]DPA-713	→	[5], [6]
Schizophrenia	[^{11}C]DAA1106	→	[7]
Major depressive disorder	[^{11}C]PBR28	→	[8]
Major depressive disorder	[^{18}F]FEPPA	↑	[9]

[1] Bloomfield et al., 2016, [2] Collste et al., 2017, [3] Hafizi et al., 2017, [4] Kenk et al., 2015, [5] Coughlin et al., 2016, [6] Notter et al., 2017, [7] Takano et al., 2010, [8] Hannestad et al., 2013, [9] Setiawan et al., 2015

Table 2

Comprehensive characterization of cuprizone short-term exposure (CSE) mice and maternal immune activation (MIA) mice

Stress signals/Behavioral dimensions		Index	Tests	Results CSE		MIA (GD9, 5 mg/kg *i.v.* or GD9.5, 20 mg/kg *i.p.*)	
Stress signals	Inflammation	TSPO levels		↑	Fig.1	↓	[12]
		IL-6 levels		↑	[10]	↑	[12]
	HPA axis	Serum corticosterone levels		↑	Fig. 2A	↑	Fig. 2B
	Oxidative stress	Protein carbonylation levels		→	Fig. 3A	↓	Fig. 3B
Behavioral dimensions	Psychosis	Sensitivity to psychostimulants	OF	↑	[10] [11]	↑	[13]
	Memory	Spatial learning and memory	MWM	ND	[10]	Normal	[14]
		Recognition memory	NORT	↓	[10]	ND	[15]
		Spontaneous alternation	Y-maze	↓	[11]	↓	[13]
		Context/cue-associated fear learning and memory	FC	Normal		ND	
		Avoidance learning	AAL	ND		↓	
	Executive function	Behavioral flexibility	RL	Normal	[11]	Normal	[16]
	Social cognition	Sociability/social novelty	SIT	Normal	[11]	↓	[12]
	Anxiety	Neophobia	LDT	↑	[11]	ND	[16]
		Hyponeophagia	NSFT	Normal	[11]	ND	
		Exploration based conflict	OF	ND		↑	
	Depression	Behavioral despair	FST/TST	Normal	[11]	ND	
	Information processing	Sensorimotor gating	PPI	Normal	[10]	↓	[12]
	Attention	Latent inhibition	LI	ND		↓	[13]
	Motor systems	Motor coordination/learning	Rotarod	Normal	[11]	ND	

[10] Tezuka et al. 2013, [11] Kondo et al. 2016, [12] Notter et al. 2017, [13] Meyer et al., 2005, [14] Meyer et al., 2008, [15] Willi et al., 2013, [16] Meyer et al., 2006

GD, gestation day; TSPO, Translocator protein 18kDa; IL-6, Interleukin-6; HPA, Hypothalamic-pituitary-adrenal; OF, Open field; MWM, Morris water maze; ND, not determined; NORT, Novel object recognition test; FC, Fear conditioning; AAL, Active avoidance test; RL, Reversal learning; SIT, Social interaction test; LDT, Light/dark box test; NSFT, Novelty suppressed feeding test; FST, Forced swim test; TST, Tail suspension test; PPI, Prepulse inhibition; LI, Latent inhibition