Neonatal Lipopolysaccharide Treatment Has Long-Term Effects on Monoaminergic and Cannabinoid Receptors in the Rat

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KEY WORDS dopamine D2; serotonin 5HT1A; CB1; neonatal immune activation; LPS

ABSTRACT Brain inflammation in early life has been proposed to play important roles in the development of anxiety and psychosis-related behaviors in adulthood, behaviors that rely on the integrity of dopamine and/or serotonin systems. Moreover recent behavioral and anatomical evidence suggests involvement of CB1 receptors in the control of emotion and mood. In this study, we determined the effects of neonatal LPS treatment on dopamine, serotonin, and cannabinoid receptor binding in adulthood. Rats were treated with the bacterial endotoxin lipopolysaccharide (LPS) on postnatal day (PND) 3 and 5. Dopamine D1, D2, serotonin 5HT1A, 5HT2A, and serotonin transporter and cannabinoid CB1 receptor binding across several brain regions were measured autoradiographically in adulthood (PND 85). Neonatal LPS treatment caused a significant increase in dopamine D2 in the nucleus accumbens and olfactory tubercle, a decrease in 5HT1A receptor binding in the hippocampus CA1 and ventromedial hypothalamus. A decrease in CB1 receptor binding after neonatal LPS was observed in the amygdala. Neonatal LPS had no significant impact on dopamine D1, serotonin 5HT2A or serotonin transporter binding in any of the brain regions examined. Our results suggest long lasting, region specific effects and differential impact on dopamine, serotonin and cannabinoid receptor systems following neonatal inflammation, that may form the basis for compromised anxiety and psychosis related behaviors. Synapse 67:290–299, 2013. © 2013 Wiley Periodicals, Inc.

INTRODUCTION Early life events can have significant effects on an organism’s health and well-being during adulthood (Harvey and Boksa, 2012). Immune system activation during the neonatal period, such as exposure to the bacterial endotoxin lipopolysaccharide (LPS), has been demonstrated to result in lifelong changes to behavioral (Bilbo et al., 2005; Fan et al., 2011; Granger et al., 1996; Lucchina et al., 2010), neuroendocrine (Hodgson et al., 2001; Iwasa et al., 2010; Shanks et al., 1995; Wu et al., 2011) and neuroimmune (Kentner et al., 2010; Spencer et al., 2011; Walker et al., 2006, 2009b, 2010) responses in rodents.

Increased adulthood anxiety-related behaviors following neonatal LPS exposure on postnatal Days 3 and 5 have been reported across a range of behavioral tests (Sominsky et al., 2012b; Walker et al., 2006, 2009b, 2010) and are likely to be modulated by both the endocrine and immune systems changes. Corticosterone levels have been reported to increase in prepubertal, adolescent, and adult rats after neonatal LPS exposure, indicating a prolonged effect of neonatal immune challenge on hypothalamic-
pituitary-adrenal (HPA) axis functioning (Sominsky et al., 2012a). In addition, inhibition of IL-1β synthesis in neonatally infected rats prevented LPS-induced cognitive impairments in adulthood (Bilbo et al., 2005). Although some of the long-term consequences of early immune challenge have been identified, pathophysiological determinants have yet to be fully elucidated. In particular, the long-term neurobiological changes that are occurring in adulthood after neonatal immune challenge have not been well investigated.

Evidence exists that neonatal LPS may have long-term effects on the integrity of the dopamine system (Fan et al., 2008a,b,2011; Tenk et al., 2007). In animal studies, neonatal intracerebral LPS exposure resulted in significantly increased locomotor activity in the open field test in preadolescent male and female rats (Fan et al., 2008b), as well as enhanced drug-free and methamphetamine-induced locomotion and stereotypy in adult males (Fan et al., 2011). In addition, neonatal intracerebral LPS injection induced injury of the dopaminergic system including loss of tyrosine hydroxylase positive neurons in the substantia nigra and the ventral tegmental areas of the rat brain (Fan et al., 2008a,b). Neonatal LPS treatment also resulted in enhanced behavioral sensitisation to the dopamine D2/3 agonist quinpirole, in adult female rats (Tenk et al., 2007). Collectively, these studies suggest that neonatal LPS may have an impact on dopamine receptor density.

Similarly, a change in serotonin receptors may underlie the neonatal LPS induced changes in anxiety. In animal studies, early deficits in serotonin signalling via the serotonin 5HT1A receptor led to heightened social anxiety in 5HT1A receptor knockout mice (Zanettini et al., 2010), and in humans, patients with social anxiety disorder have reduced 5HT1A receptor binding in the brain (Akimova et al., 2009; Lazenberger et al., 2007). Serotonin has been shown to play a key role in anxiety and depression through regulation of the HPA axis (Cryan and Leonard, 2000; Rittenhouse et al., 1994; Stockmeier, 2003; Van de Kar et al., 2001). Activation of subtype 5HT1A and 5HT2A serotonin receptors stimulates hypothalamic corticotrophin neurons (Contesse et al., 2000; Mikkelsen et al., 2004) increasing the release of adrenocorticotrophin and expression of corticotropin releasing hormone messenger RNA (Jørgensen et al., 2002). Additionally, enhanced proinflammatory actions in the brain (such as the proinflammatory cytokines tumour necrosis factor-α, interleukin 1β and interleukin-6) have been shown to lead to reduced bioavailability of tryptophan for serotonin synthesis, affecting serotonin levels (Müller and Schwarz, 2007).

In addition to serotonin, growing evidence suggests that the endocannabinoid system acting through CB1 cannabinoid receptors also represents an important substrate for the control of emotional behavior and mood. Mice lacking CB1 receptors display anxiety-like responses (Valverde and Torrens, 2012) and pharmacological CB1 receptor inactivation is anxiogenic (Marsicano et al., 2002). In contrast, cannabinoid receptor activation decreases anxiety in a variety of rodent tests such as the elevated plus maze and others (reviewed by Degroot, 2008). In humans, cannabis has been shown to have biphasic effects on anxiety with low doses being anxiolytic and high doses being anxiogenic (reviewed in Ashton and Moore, 2011). Furthermore, we have recently found that CB1 receptor binding is increased in paranoid schizophrenia, implicating the endocannabinoid system in psychotic disorders (Dalton et al., 2011).

The increased behavioral sensitisation to dopamine agonists in adult animals neonatally exposed to LPS compared to controls suggests long-term effects in the integrity of the dopamine receptor system. In addition, the demonstrated links between neonatal LPS treatment and anxiety-related behaviors in the adult offspring, together with preclinical and clinical evidence of involvement of serotonin and cannabinoid receptors in the control of emotion and mood make alterations in these receptor systems in the brain of adult animals prenatally exposed to LPS highly likely. However explicit evidence for these associations is still lacking. A direct examination of the efficacy of neonatal immune activation to affect neurotransmitter receptor levels is therefore warranted.

The aim of this study was to investigate the impact of neonatal LPS exposure on receptor levels involved in key neurotransmitter systems that have been implicated in anxiety/depression and/or psychotic behaviors. We examined dopamine D1 and D2, serotonin 5HT1A, 5HT2A and serotonin transporter and cannabinoid CB1 receptor binding in brain regions implicated in the pathophysiology of affective disorders of adult rats (postnatal age 85) exposed as neonates to LPS (on postnatal Days 3 and 5), using an identical treatment regimen with the one shown to affect behavioral, endocrine and neuroimmune functions in our previous studies (Sominsky et al., 2012a,b; Walker et al., 2009a,b,2010).

**MATERIALS AND METHODS**

**Animals and neonatal drug administration**

Animals were held in conventional housing conditions in a temperature (21 ± 1°C) and humidity (34 ± 2°C) controlled environment, under a 12 h light/dark schedule (lights on 06:00 h). Food (Rat and Mouse Pellets, Glen Forest, Western Australia) and water availability was ad libitum. Experimentally naïve female Wistar rats obtained from the University of Newcastle animal house were mated with sexually-experienced male studs in the University of Newcastle Psychology vivarium. Pregnant females were caged separately and litters were randomly allocated into either

*Synapse*
Autodigraphy

Coronal brain sections (16 μm) were cut with a cryostat and thaw mounted onto microscope slides. Three sections per brain region were collected non-consecutively so both the rostral and caudal parts of each brain structure were represented on each individual slide. Sections were stored at 2°C until individual slide. Sections were stored at -80°C until individual slide. Sections were stored at -80°C until.

TABLE I. Summary of autoradiography protocols for dopamine D1 and D2 receptors, serotonin 5HT1A and 5HT2A receptors, serotonin transporter (SERT) and CB1 cannabinoid receptor

<table>
<thead>
<tr>
<th>Target</th>
<th>Preincubation</th>
<th>Incubation: Total binding</th>
<th>Incubation: Non-specific binding</th>
<th>Ligand concentration</th>
<th>Ligand specific activity</th>
<th>Post incubation washes at 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>10 min in D1 buffer: 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl, 1 mM MgCl, 50 mM Tris HCl, (pH 7.4)</td>
<td>1 hr in D1 buffer containing ligand and 5 μM ketanserin</td>
<td>1 hr in D1 buffer containing ligand and 10 μM SRF 38,939</td>
<td>4 nM [3H] SCH 23390</td>
<td>85.0 Ci/mm mol</td>
<td>2 × 1 min in D1 buffer</td>
</tr>
<tr>
<td>D2</td>
<td>20 min in D2 buffer: 120 mM NaCl, 2 mM CaCl, 1 mM MgCl2, 50 mM Tris HCl, (pH 7.4)</td>
<td>1 hr in D2 buffer containing ligand</td>
<td>1 hr in D1 buffer containing ligand and 10 μM (+)-butaclamol</td>
<td>5 nM [3H] raclopride</td>
<td>62.2 Ci/mm mol</td>
<td>2 × 5 min in D2 buffer</td>
</tr>
<tr>
<td>5HT1A</td>
<td>15 min in 5HT1A buffer: 50 mM Tris HCl, 120 mM NaCl and 4 mM CaCl2 (pH 7.4)</td>
<td>1 hr in 5HT1A buffer containing ligand</td>
<td>1 hr in 5HT1A buffer containing ligand and 10 μM MM 77 dihydrochloride</td>
<td>2 nM [3H] 8-OH-DPAT</td>
<td>170.2 Ci/mm mol</td>
<td>2 × 10 min in 5HT1A buffer</td>
</tr>
<tr>
<td>5HT2A</td>
<td>5 min in 5HT2A buffer: 170 mM Tris HCl, (pH 7.7)</td>
<td>1 hr in 5HT2A buffer containing ligand</td>
<td>1 hr in 5HT2A buffer containing ligand and 10 μM spiperone</td>
<td>7 nM [3H] ketanserin</td>
<td>67.0 Ci/mm mol</td>
<td>2 × 15 min in 5HT2A buffer</td>
</tr>
<tr>
<td>SERT</td>
<td>20 min in SERT buffer: 50 mM Tris HCl, 120 mM NaCl, 2 mM KCl, 1 mM MgCl2 and 1 mM CaCl2 (pH 7.5)</td>
<td>1 hr in SERT buffer containing ligand</td>
<td>1 hr in SERT buffer containing ligand and 10 μM fluoxetine</td>
<td>4 nM [3H] citalopram</td>
<td>83.0 Ci/mm mol</td>
<td>3 × 10 min in SERT buffer</td>
</tr>
<tr>
<td>CB1</td>
<td>30 min in CB1 buffer: 50 mM Tris HCl (pH 7.4)/5% BSA</td>
<td>2 hr in CB1 buffer containing ligand</td>
<td>2 hr in CB1 buffer containing ligand and 10 μM CP55,940</td>
<td>10 nM [3H] CP55,940</td>
<td>139.6 Ci/mm mol</td>
<td>1 × 1 min in 50 mM Tris HCl (pH 7.4)/1% BSA</td>
</tr>
</tbody>
</table>

Preincubation and incubation steps were carried out at room temperature. All post incubation washes were followed by dipping sections in ice cold distilled water. Sections were then dried. Abbreviations: BSA- bovine serum albumin.

Maternal behavior was not monitored in these animals. Differences in maternal care dependent on rodent strain have been reported in the neonatal LPS exposure model (Walker et al., 2004a; Hood et al., 2003) however poor maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care. On PND 3 and PND 5, animals were proved breeders and showed no history of maternal care.
raclopride, [3H] 8-OH-DPAT, [3H] ketanserin, [3H] citalopram and [3H] CP55,940 respectively. Films were then developed using Kodak GBX developer and fixed with Kodak GBX fixer.

Quantitative analysis of autoradiographic images

All films were analyzed by using a computer-assisted image analysis system, Multi-Analyst, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). Brain regions for quantification were identified based on a standard rat brain atlas (Paxinos and Watson, 1998). [3H] SCH 23390 and [3H] raclopride binding were quantified in the lateral and medial caudate putamen, nucleus accumbens and olfactory tubercle. Brain regions quantified for [3H] 8-OH-DPAT and [3H] citalopram included the CA1 and dentate gyrus of the hippocampus, cingulate cortex, upper (Layers I–III) and lower layers (Layers IV–VI) of the somatosensory cortices, septum, ventromedial hypothalamus and amygdala. [3H] ketanserin and [3H] CP55,940 binding were assessed in the lateral and medial caudate putamen, nucleus accumbens, cingulate cortex, and ventromedial hypothalamus. [3H] CP55,940 binding was also quantified in the CA1 and dentate gyrus of the hippocampus, and the amygdala. Quantification of receptor binding in each brain region was performed by measuring the average optical density in three adjacent brain sections. Nonspecific binding from three adjacent brain sections was subtracted from the total binding to determine the specific binding. Optical density measurements for specific binding were then converted into fmoles [3H] SCH 23390, [3H] raclopride, [3H] 8-OH-DPAT, [3H] ketanserin, [3H] citalopram or [3H] CP55,940 per mg tissue equivalent (fmol/mg TE) according to the calibration curve obtained from the tritium standards.

Statistical analysis

The data are presented as ± SEM. Data were analyzed with two-way ANOVA (treatment X brain region) in order to identify statistically significant variation in [3H] SCH 23390, [3H] raclopride, [3H] 8-OH-DPAT, [3H] ketanserin, [3H] citalopram and [3H] CP55,940 binding across treatment groups in the various brain regions assessed. Post hoc LSD tests were performed where appropriate to determine variation between treatment groups within brain regions. Differences were regarded as statistically significant if P < 0.05. All data were analyzed using the SPSS statistical package.

RESULTS

Neonatal LPS treatment increases D2 receptor binding in striatal subregions of the nucleus accumbens and olfactory tubercle in adulthood

Two-way ANOVA analysis of the binding of D1 receptors in 4 striatal subregions (lateral and medial caudate putamen, nucleus accumbens, and olfactory tubercle) revealed a significant effect of brain region, F(3, 40)=47.992, P < 0.0001, no significant effect of treatment, F(1, 40)=0.550, P = 0.463 and no significant interaction between the two variables (P = 0.951; Table II). Nonspecific binding for D1 was less than 16%. Similar analysis on the binding of D2 receptors in the same brain regions revealed a significant effect of treatment, F(1,40)=11.129, P = 0.002, a significant effect of region, F(3,40)=181.574, P < 0.0001, but no significant interaction between the two variables (P = 0.712; Figs. 1A and 1B, Table II). Post hoc tests revealed that [3H]raclopride binding was higher in rats treated with LPS as neonates in comparison to vehicle-treated controls in the olfactory tubercle (P = 0.040), nucleus accumbens (P = 0.044) and increases approached significance in the medial part of caudate nucleus (P = 0.080). Nonspecific binding for D2 was less than 15% (Fig. 1A inset).

Neonatal LPS treatment increases 5HT1A receptor binding in CA1 and hypothalamus

Analysis of the effects of neonatal LPS treatment on the levels of 5HT1A receptor binding amongst 8 brain regions revealed a significant effect of treatment, F(1,80)=16.359, P < 0.0001, a significant effect of region, F(7,80)=307.046, P < 0.0001, but no significant interaction between the two variables (P = 0.266; Figs. 2A and 2B; Table III). Post hoc analysis revealed that rats treated with LPS as neonates had significantly lower levels of 5HT1A binding in the CA1 region of the hippocampus (P = 0.0001) and in the ventromedial nucleus of the hypothalamus (P = 0.019). Nonspecific binding for 5HT1A averaged less than 6% (Fig. 2A inset).

Analysis of 5HT2A receptor binding amongst five brain regions of LPS-treated animals and controls revealed a significant effect of region, F(4,50)=129.585, P < 0.0001, but not treatment, F(1,50)=0.410, P = 0.525, with no significant interaction between the two variables (P = 0.419; Fig. 3A; Table IV).

SYNAPSE
Nonspecific binding for 5HT2A averaged 23%. Similarly, analysis of binding to serotonin transporter across 8 brain regions, revealed a significant effect of region, $F(7,80)=39.402$, $P<0.0001$, no significant interaction between the two variables ($P=0.860$; Fig. 3B; Table III). Nonspecific binding for the serotonin transporter averaged 29%.

**Fig. 1.** $[^3H]$ raclopride binding (fmoles/mg tissue equivalent (TE)) to dopamine D2 receptors, in brain regions of adult rats treated as neonates with saline or the bacterial endotoxin lipopolysaccharide (LPS). A: Typical autoradiographs showing $[^3H]$ raclopride binding in saline and LPS-treated animals. Boxed insets show nonspecific binding. Line diagrams showing location of brain regions are adapted from Paxinos and Watson, 1998. B: Comparison of D2 receptor levels in saline (open bars) and LPS (filled bars) treated animals. Two-way ANOVA revealed a significant effect of treatment $F(1,40)=11.129$, $^*P=0.002$ ($n=6$ per group). $^*P=0.040$ and $^SP=0.044$ saline vs. LPS, post hoc LSD tests. ACB, nucleus accumens; CPUL, lateral caudate nucleus; CPUM, medial caudate nucleus; TU, olfactory tubercle; CING, cingulate cortex; CTXDO, layers IV-VI of somatosensory cortex; CTX UP, layers I-III of somatosensory cortex; SEPT, septum. Scale bar = 2 mm.

**Fig. 2.** $[^3H]$ 8-OH-DPAT binding (fmoles/mg tissue equivalent (TE)) to serotonin 5HT1A receptors. A: Typical autoradiographs showing $[^3H]$ 8-OH-DPAT binding in brain regions of adult rats treated as neonates with saline or lipopolysaccharide (LPS). Boxed insets show nonspecific binding. Line diagrams showing brain regions are adapted from Paxinos and Watson, 1998. B: Comparison of 5HT1A receptor binding in saline and LPS-treated animals. Two-way ANOVA revealed a significant effect of treatment $F(1,80)=16.359$, $P<0.0001$ ($n=6$ per group). $^*P<0.0001$, $^SP=0.019$, saline vs. LPS, post hoc LSD tests. AMY, amygdala; CA1, CA1 region of hippocampus; CING, cingulate cortex; CTXDO, Layers IV-VI of somatosensory cortex; CTX UP, Layers I-III of somatosensory cortex; DG, dentate gyrus of hippocampus; SEPT, septum; VMH, ventromedial nucleus of hypothalamus. Scale bar = 2 mm.

Nonspecific binding for 5HT2A averaged 23%. Similarly, analysis of binding to serotonin transporter across 8 brain regions, revealed a significant effect of region, $F(7,80)=39.402$, $P<0.0001$, no significant effect of treatment, $F(1, 80)=0.157$, $P=0.693$, and no significant interaction between the two variables ($P=0.860$; Fig. 3B; Table III). Nonspecific binding for the serotonin transporter averaged 29%.
Neonatal LPS treatment induces a decrease in CB1 binding in the amygdala

Analysis of the effects of neonatal LPS treatment in rats on CB1 receptor binding amongst 8 brain regions revealed a significant overall effect of treatment, (ANOVA, $F(1, 80)=15.020, P<0.0001$). A significant effect of region was also observed, $F(7,80)=5846.222, P<0.0001$, with a significant interaction between the two variables $F(7,80)=3.649, P=0.002$ (Figs. 4A–4C; Table IV). Post hoc tests revealed a significant decrease in CB1 receptor binding in the amygdala ($P<0.0001$). Nonspecific binding for CB1 averaged 0.4% (Figs. 4A and 4B inset).

DISCUSSION

This study characterized the effects of an early immune challenge in the long term, by measuring binding densities of the monoaminergic and cannabinoid receptor systems in the adult rat. Our results suggest that administration of the bacterial endotoxin, LPS, on PNDs 3 and 5, leads to increased dopamine D2 receptor binding in the nucleus accumbens and olfactory tubercle, a decrease of serotonin 5HT1A receptor binding in the CA1 subregion of the hippocampus and ventromedial hypothalamus, and a decrease in cannabinoid CB1 receptor binding in the amygdala.

The increased D2 receptor binding in the nucleus accumbens and olfactory tubercle of adult rats following neonatal LPS suggests a link between neonatal immune activation and long-term effects on dopaminergic brain function. Although the nucleus accumbens is not generally viewed as a critical nucleus in mediating anxiety, it is possible that altered levels of D2 receptors in this brain region may contribute to anxiety-related behaviors that have been previously reported in the model (Sominsky et al., 2012b; Walker et al., 2006, 2009b, 2010). In support of this, Chen et al. (2012) have reported that corticotropin-releasing factor induces depressive and anxiety-related behaviors by altering dopamine levels in this nucleus.

Recent studies have provided evidence for a link between neonatal immune activation induced by LPS and sensitivity to dopamine-based drugs of abuse. Neonatal intracerebral LPS exposure results in significantly increased locomotion and stereotypy compared to controls following a one-off dose of methamphetamine in adult male rats, suggesting that the dopamine system is more sensitive to methamphetamine stimulation in the LPS exposed brain (Fan et al., 2011). In addition, neonatal LPS enhances behavioral sensitization to the dopamine D2/3 agonist quinpirole in adult female but not male rats (Tenk et al., 2007). Our findings of increased D2 receptor binding in striatal subregions are in line with the above studies suggesting alterations in the dopamine system after neonatal LPS exposure although in contrast to Tenk et al. (2007), our cohort was made up of

![Fig. 3. $[^{3}H]$ketanserin (A) and $[^{3}H]$citalopram (B) binding (fmol/mg tissue equivalent (TE)) to serotonin 5HT2A receptors and serotonin transporter (SERT) respectively, in brain regions of adult rats treated as neonates with saline or lipopolysaccharide (LPS). See Figures 1 and 2 for abbreviations.](image-url)
males only and included no female animals. It is noteworthy however that sensitisation to quinpirole was enhanced in females naïve to neonatal LPS treatment (Tenk et al., 2007) and D2 receptor levels can fluctuate during the oestrous cycle (Di Paolo et al., 1988), which was not controlled for by Tenk et al. (2007). It is possible that females will show even greater D2 receptor binding in response to neonatal LPS treatment and this warrants further investigation.

We have previously shown using the same treatment regimen as in the present study, that in adulthood, LPS challenged neonates exhibited increased anxiety-like behavior on the elevated plus maze and the holeboard apparatus, both of which are widely used to assess anxiety-like behaviors in rodents (Sominsky et al., 2012b; Walker et al., 2009a, 2011). Abnormalities in 5-hydroxytryptamine (5-HT; serotonin) neurotransmission are known to be associated with symptoms of depression and anxiety (Cowen, 2008). Selective serotonin reuptake inhibitors (SSRIs), commonly used to treat depression and anxiety (Vaswani et al., 2003), are thought to act by blocking serotonin reuptake at the transporter and increasing serotonin availability at the synapse in patients suggesting that abnormalities in the serotonin transporter (SERT) may be involved in the etiology of mood disorders and anxiety (Cowen, 2008; Schloss and Henm, 2004). In the current study, we did not observe any alterations in SERT levels following neonatal LPS exposure. Investigations of SERT levels in patients with depression have provided equivocal results (Meyer, 2012; Cowen, 2008). In vitro research has shown that SSRIs do not alter SERT mRNA but increase SERT internalisation thus lowering receptor availability (Lau et al., 2008). Furthermore, altered functionality of the SERT in human depression and anxiety appears to be linked to single nucleotide polymorphisms in the SERT gene (Nugent et al., 2011; Petersen et al., 2012). Taken together, these studies suggest that dysfunction of the SERT in mood disorders and anxiety may be linked to alterations in receptor functionality rather than a change in SERT levels, in line with our finding that SERT levels were unchanged despite the previously described anxious phenotype in the neonatal LPS model (Sominsky et al., 2012b; Walker et al., 2009a, 2011).

On the other hand, our results suggest that early LPS exposure leads to neurochemical changes in the serotonin receptor system in adulthood that specifically affect the 5HT1A receptor subtype in the CA1 region of the hippocampus and in the ventromedial nucleus of the hypothalamus. Altered 5-HT function and impaired negative feedback control of the HPA axis have been described in depression for several

![Fig. 4.](image) | TABLE IV. | [3H] ketanserin and [3H] CP55,940 binding to serotonin 5HT2A and cannabinoid CB1 receptors respectively |
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<tr>
<td></td>
<td>[3H]ketanserin</td>
<td>[3H] CP55,940</td>
<td>    </td>
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<tr>
<td></td>
<td>Saline</td>
<td>LPS</td>
<td>Saline</td>
<td>LPS</td>
</tr>
<tr>
<td>CA1</td>
<td>124.56 ± 3.0</td>
<td>125.0 ± 5.3</td>
<td>100.6 ± 3.2</td>
<td>97.8 ± 1.8</td>
</tr>
<tr>
<td>DG</td>
<td>116.8 ± 3.7</td>
<td>108.1 ± 2.9</td>
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<td>108.1 ± 2.9</td>
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<td>CPUL</td>
<td>97.8 ± 2.9</td>
<td>103.5 ± 3.0</td>
<td>62.5 ± 3.4</td>
<td>57.1 ± 2.6</td>
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<td>CPUM</td>
<td>97.6 ± 2.7</td>
<td>106.2 ± 4.8</td>
<td>67.1 ± 3.7</td>
<td>59.7 ± 2.8</td>
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<tr>
<td>CING</td>
<td>129.8 ± 4.2</td>
<td>126.1 ± 5.6</td>
<td>65.6 ± 4.2</td>
<td>61.8 ± 3.9</td>
</tr>
<tr>
<td>ACB</td>
<td>52.3 ± 1.7</td>
<td>49.1 ± 3.3</td>
<td>85.9 ± 2.6</td>
<td>83.0 ± 3.3</td>
</tr>
<tr>
<td>AMY</td>
<td>62.6 ± 2.1</td>
<td>55.5 ± 1.9*</td>
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Data are represented as mean ± SEM of fmoles/mg tissue equivalent (n = 6 per group).

*P < 0.0001 saline vs. LPS, post hoc LSD tests. Abbreviations-See Table II and III.
decades (Belmaker and Agam, 2008). In view of the suggestion that cyclooxygenase-2 (COX-2) inhibitors might exert antidepressant effects by acting on serotonergic deficiency (Müller and Schwarz, 2007), it is of interest that elevated basal COX-2 expression in the hypothalamus (Kentner et al., 2010) has been shown in adult male rats treated neonatally with LPS, which was suggested to be a phenotype for disrupted hedonic behavior, a symptom of depression.

While our current findings do not directly implicate reduced 5HT1A receptor binding in the enhanced anxiety observed in our previous study, three independently introduced 5HT1A knockout mice (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) consistently showed elevated anxiety-related behaviors and antidepressant-like responses. In contrast, mice overexpressing the 5HT1A receptor displayed a behavioral phenotype where anxiety-related behaviors were considerably reduced (Kusserow et al., 2004). In human studies, patients with social anxiety disorder have been shown to have reduced 5HT1A receptor binding in the brain (Akimova et al., 2009; Lanzenberger et al., 2007).

Neonatal LPS administration may be considered a systemic stressor and many of the behavioral and physiological alterations reported in adulthood in rats treated with LPS as neonates are similar to those induced by repeated maternal separation early in life. Franklin et al. (2011), using a model based on unpredictable maternal separation combined with maternal stress from PNDs 1–14, reported decreased [3H] 8-OH-DPAT receptor binding to 5HT1A receptors in several brain regions important for anxiety and stress including the CA1 region of the hippocampus, whereas another study reported reduced 5HT1A mRNA in the adult hippocampus following early maternal separation (Maniam and Morris, 2010). However, in contrast to the HPA axis activation seen following maternal separation (Kuhn et al., 1990), neonatal LPS treatment activates both endocrine and immune systems (Hodgson et al., 2001; Shanks et al., 1995; Walker et al., 2006). The immune system activation leads to the release of proinflammatory cytokines such as tumour necrosis factor-α, interleukin 1β and interleukin-6 (Harvey and Boksa, 2012) and within the brain one of the likely mediators of inflammatory response is microglia (Sominsky et al., 2012b). Interestingly, using the same treatment regimen as in the present study, we have recently shown increased microglial activation in the hippocampus of adult rats prenatally exposed to LPS that corresponded with induced anxiety and stress responses (Sominsky et al., 2012b). Enhanced proinflammatory actions in the brain have also been shown to lead to reduced bioavailability of tryptophan for serotonin synthesis, affecting serotonin levels (Müller and Schwarz, 2007). Therefore, it is likely that mechanisms involving both the endocrine and/or immune systems may contribute to the 5HT1A changes found in this study, a clearly interesting and intriguing topic for future research.

In contrast to our current findings, we have previously shown that prenatal infection with the viral mimic polyIC at gestational day 15 leads to increased 5HT1A receptor binding in the adult offspring (Dalton et al., 2012). Both polyIC and LPS induce immune system activation through the production of cytokines (Harvey and Boksa, 2012), however there can be significant differences in the magnitude of cytokine responses induced by these two types of immunogens as well as qualitative differences in the cell types that respond to the profile of cytokine induced and activation of downstream signalling cascades (Harvey and Boksa, 2012). Also, maternal immune activation is somewhat protective in that the pathogen might not act directly on the fetus and thus changes could be a by-product of the maternal HPA axis activation, which we found was increased in the pregnant mothers after polyIC (Dalton et al., 2012). Although increased markers of anxiety have been reported in studies using maternal influenza or polyIC administration, collectively, our studies suggest that the nature of the immunogen and/or the timing of the infection can differentially affect serotonergic neurotransmission in the long term, resulting in reductions or increases in 5HT1A receptor expression. Additionally, numerous studies (reviewed in Harvey and Boksa, 2012) suggest that prenatal immune activation is more likely to result in “schizophrenia-like phenotype” whereas neonatal immune activation has been linked to anxiety and depression-like behaviors.

Cannabis and Δ-9-tetrahydrocannabinol (THC) have been shown to have biphasic effects on anxiety depending on dose, environment, personality and other factors, and can have anxiolytic or anxiogenic actions (Ashton and Moore, 2011). In view of the evidence that endocannabinoids exert regulatory control over different physiological mechanisms that are impaired in mood disorders, it is not surprising that neonatal LPS treatment had an impact on the expression of CB1 receptors in our study, as indicated by a significant overall treatment effect and a decrease on CB1 receptor binding in the amygdala, a brain region that has been implicated in the cannabinoid-related effects on anxiety (Marsicano et al., 2002; Viveros et al., 2005). Reduced CB1 receptor availability in CB1 knockout mice results in increased anxiety-like responses in several behavioral models, including the elevated plus maze (reviewed by Valverde and Torrens, 2012). In addition, pharmacological blockade of CB1 receptors produces similar anxiety-related phenotypes (Marsicano et al., 2002). Mice lacking the CB1 receptor for example exhibited an
improvement in the extinction of aversive memories and increased levels of endocannabinoids in the basolateral amygdala complex (but not in other brain structures) after the presentation of the conditioned stimulus (Marsicano et al., 2002).

There appear to have been no formal clinical trials of cannabinoids in anxiety/depression (Ashton and Moore, 2011) however in humans, blocking of CB1 receptors with the cannabinoid receptor antagonist, rimonabant, has been shown to precipitate depressive illness (Christensen et al., 2007). On the other hand, stimulation of the cannabinoid system appears to reverse depressive and anxious symptoms in some instances (Bergamaschi et al., 2011; Robson, 2001; Wilson and Cadet, 2009). For example, although studies were subjective and relied on self-reports, cannabis self-medication to improve mood has been reported in individuals with depression and schizophrenia (Wilson and Cadet, 2009). Treatment with THC in cancer patients improved mood and the synthetic cannabinoid, nabilone, has been shown to reduce anxiety in anxious patients (Robson, 2001). In addition, cannabidiol, a major non-psychotomimetic compound found in cannabis, has been shown to reduce anxiety associated with simulated public speaking in subjects with treatment-naïve social phobia (Bergamaschi et al., 2011). Taken together, the studies discussed above indicate that the decrease in CB1 receptors that we observed in the amygdala may play a role in the anxiety-like behavior we have previously reported in the neonatal LPS model (Somin sky et al., 2012b; Walker et al., 2009a, 2011).

In summary, our results provide evidence that early LPS exposure, determines the trajectory of a range of neurochemical changes including alterations in monoaminergic and cannabinoid receptor systems. These changes may represent a potential mechanism for the well documented increases in anxiety-like behaviors observed in the model in adulthood and add to the relevance of neonatal infection models in understanding the neural mechanisms underlying human conditions linked to mental illness.

REFERENCES


