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Alterations of expression of inflammation/immune-related genes in the dorsal and ventral striatum of adult C57BL/6J mice following chronic oxycodone self-administration: A RNA sequencing study

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Abstract

Non-medical use of prescription opioids such as the mu opioid receptor (MOP-r) agonist oxycodone is a growing problem in the United States and elsewhere. There is limited information about oxycodone's impact on diverse gene systems in the brain.

Objectives—The current study was designed to examine how chronic oxycodone self administration (SA) affects gene expression in the terminal areas of the nigrostriatal and mesolimbic dopaminergic pathways in mice.

Method—Adult male C57BL/6J mice underwent a 14-day oxycodone self-administration procedure (4 hours/day, 0.25mg/kg/infusion, FR1), and were euthanized 1 hour after the last session. The dorsal and ventral striatum were dissected and total RNAs were extracted. Gene expressions were examined using RNA sequencing.

Result—We found that oxycodone self-administration exposure led to alterations of expression in numerous genes related to inflammation/immune functions: in the dorsal striatum (54 upregulated genes, 1 downregulated gene), ventral striatum (126 upregulated genes and 15 downregulated genes), with 38 upregulated genes identified in both brain regions.

Conclusion—This study reveals novel neurobiological mechanisms underlying some of the effects of a commonly abused prescription opioid. We propose that inflammation/immune gene systems may undergo a major change during chronic self-administration of oxycodone.

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Conflict of interest

The authors declare that, except for income received from our primary employer, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

Keywords

Oxycodone self-administration; RNA sequencing; Inflammation and immune related genes; C57BL/6J mice

Introduction

Over the past 15 years, there has been a dramatic increase in the medical and non-medical use of prescription opioids (short-acting MOP-r agonists). Oxycodone is one of the most commonly abused prescription opioids, which accounts for a substantial percentage of opioid-related deaths in the United States (Paulozzi et al. 2015). The effects of oxycodone are predominantly mediated initially through MOP-r (Beardsley et al. 2004). There is limited information about oxycodone's impact on the brain and the underlying neurobiological mechanisms involved in its abuse.

An early study (using a microarray technique) reported that repeated experimenter-administered oxycodone in rats (15 mg/kg, twice a day for 8 days) led to upregulation of 31 genes and downregulation of 25 genes, in the whole brain (Hassan et al. 2007). We extended this study by examining the effects of oxycodone in a mouse model of 14-day oxycodone self-administration on expression of genes in specific areas of the brain. We identified 61 genes that were downregulated and 18 genes that were upregulated in several brain regions in male adult and adolescent mice. Those studies focused on genes involved in neurotransmission or synaptic plasticity (Mayer-Blackwell et al. 2014; Zhang et al. 2015; Zhang et al. 2014).

Although microarrays have been widely used in quantitative transcriptomics, these techniques have several limitations: reliance on prior knowledge about genes for probes or PCR primer design, and monitoring only some portions of the known genes. RNA sequencing (RNA-seq) has been recently introduced for transcriptome profiling based on massively parallel, unbiased sequencing. RNA-seq offers several important advantages over other gene expression technologies: it provides more accurate, sensitive and reliable gene expression data, in addition to the detection of gene variant and alternative splicing (e.g., Goldman and Domschke 2014; Wang et al. 2014). However, RNA-seq technology is still in a developmental stage, and there is little consensus regarding optimal methods for quantification and downstream analysis, especially for brain tissues. In the current study, RNA-seq was used for the first time to identify changes in the genome-wide expression of transcripts in the mouse dorsal and ventral striatum following 14-day extended access (4-hour) oxycodone self-administration. The dorsal and ventral striatum (terminal regions of the nigrostriatal and mesolimbic dopaminergic pathways, respectively) are thought to mediate different aspects of reward and drug-seeking (e.g., Koob and Volkow 2010; Lobo and Nestler 2011). Numerous studies have shown that the dorsal striatum is gradually engaged in habitual and compulsive-like drug-seeking (e.g., Barker and Taylor 2014; Belin et al. 2009; Everitt 2014; Everitt and Robbins 2013; Porrino et al. 2007). The ventral striatum plays a crucial role in processing rewarding stimuli, as well as stimulations which are both rewarding and reinforcing (e.g., Olsen 2011).

Methods

Subjects

Male adult (11 weeks old on arrival) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were housed in groups of four, with free access to food and water in a light-(12:12 hour light/dark cycle, light on at 7:00 pm and off at 7:00 am) and temperature-(25 °C) controlled room. Animal care and experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources Commission on Life Sciences 2016). The experimental protocols used were approved by the Institutional Animal Care and Use Committee of The Rockefeller University. In the current study, we examined gene expression using RNA-seq in the male C57BL/6J mice because we had carried out numerous studies in this strain of male mice in the past (e.g., Mayer-Blackwell et al. 2014; Zhang et al. 2015; Zhang et al. 2014). Comparative studies of female mice would be of great interest for follow-up studies.

Self-Administration Procedure

Catheter Implantation and Intravenous Self-administration Chambers—

Following acclimation for 7 days, the mice were anesthetized with a combination of xylazine (8.0 mg/kg, i.p.) and ketamine (80 mg/kg, i.p.). For details of surgical procedure and description of operant conditioning chambers, see Zhang et al. (Mayer-Blackwell et al. 2014; Zhang et al. 2014; Zhang et al. 2009). Immediately after surgery, the surgical sutures and incision areas of all the mice studied were covered with antibiotic ointment. No further antibiotic was used.

Oxycodone Self Administration

Study 1: A 4-hour self-administration session was conducted once a day for 14 consecutive days. Each day, mice were weighed and heparinized saline (0.01 ml of 30 IU/ml solution) was used to flush the catheter to maintain patency. During self-administration sessions, mice were placed in the self-administration chambers and a nose poke through the active hole led to an infusion of oxycodone (0.25 mg/kg/infusion; Sigma, St. Louis, MO) under a FR1 schedule. Drug dose was adjusted on a daily basis by a computer program which took into account the changes in an animal's body weight. Mice in the saline control groups received yoked saline infusions during all sessions (saline was infused in the control mouse whenever the oxycodone mouse self-administered oxycodone). At the end of the experiment, only data from mice that passed a catheter patency test, defined as loss of muscle tone within a few seconds after administration of 30 ul ketamine (5mg/ml) (Fort Dodge, IA), were included in the analysis of data.

Of a total 31 mice that started in this study, 22 mice finished the 14-day self-administration sessions and passed the catheter patency test. Of these 22 mice, the brain tissues of 12 mice (6 oxycodone and 6 yoked saline controls) were randomly chosen for RNA-seq study. These mice were studied as part of a recent publication and detailed behavioral data are available in that publication (Zhang et al., 2014).

Study 2: In order to have sufficient RNA for confirmation of RNA-seq data by qPCR, separate groups of mice underwent an identical 4-hour self-administration session once a day for 14 consecutive days. Of a total 26 mice that started in the second study, 16 mice completed the 14-day SA study and passed the catheter patency test. The brain tissues from these mice (9 oxycodone and 7 yoked saline controls) were used in the qPCR confirmation study.

RNA Extraction

Mice were sacrificed within 1 hr after the last self-administration session by decapitation after brief exposure to CO₂; the dorsal and ventral striatum from each mouse were dissected from the brain and homogenized in Qiazol (Qiagen, Valencia, CA). Total RNA was isolated from the dorsal and ventral striatum using the miRNeasy kit (Qiagen, Valencia, CA). The quality and quantity of RNA from each sample was determined using an Agilent 2100 Bioanalyzer.

RNA-seq library preparation and sequencing

The integrity of RNA samples was evaluated again by Agilent 6000 RNA Nano Chips. RNA-seq library preparation and sequencing of samples isolated from dorsal striatum was performed by LC Sciences (Houston, TX) whereas RNA-seq library preparation and sequencing of RNA isolated from ventral striatum was performed by the Genomic Resource Center at the Rockefeller University. Both RNA-seq libraries were prepared using Illumina's TruSeq® Stranded Total RNA LT kit (with Ribo-Zero™) following manufacturer's protocol. Briefly, starting with 100 ng total RNA, cytoplasmic ribosomal RNA was removed, and the remaining RNA was fragmented by incubating at 94°C for 8 minutes with divalent cations. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. This was followed by second strand cDNA synthesis using DNA Polymerase I and RNaseH. The double stranded cDNA fragments then had the addition of a single 'A' nucleotide to prevent self-ligation during the subsequent addition of the indexing adapters. PCR was then used to enrich only those DNA fragments that had adapter molecules on both ends to amplify the amount of DNA in the library. Libraries were validated using Agilent Tape Station High Sensitivity DNA kits and normalized. Libraries were multiplexed, four samples per lane and sequenced. Illumina HiSeq 2500 was used to obtain 100 bp single-end reads for samples from the ventral striatum. Illumina HiSeq 2000 was used to obtain 100 bp paired-end reads for samples from the dorsal striatum.

RNA-seq data quality assessment and differential gene expression (DE) Analysis

The fastq files were generated by configuring BclToFastq.pl from CASAVA v1.8.2 with the following parameters: --ignore-missing-stats, --ignore-missing-bcl, --ignore-missing-control, --positions-format, clocs, --fastq-cluster-count 350000000. They were then examined using FASTQC (Andrews, 2010). The reads were aligned to the mouse reference genome (version mm10) using STAR v2.3 (Dobin et al. 2013) aligner with default parameters. The alignment results were then evaluated through RNA-SeQC v1.17 (DeLuca et al. 2012) to ensure that all the samples had a consistent alignment rate, and no obvious 5' or 3' bias. Aligned reads were summarized through feature Counts (Liao et al. 2014) with the gene model from

Ensemble (Mus_musculus.GRCm38.75.gtf) at gene level: specifically, the uniquely mapped reads (NH 'tag' in bam file) that overlapped with an exon (feature) by at least 1bp were counted and then the counts of all exons annotated to an Ensemble gene (meta features) were summed into a single number. Only protein coding genes were used for the downstream analysis of this study. To eliminate the between-sample differences in read counts, the summarized counts matrix was then normalized through a set of housekeeping genes Ppia, Gusb and Gapdh (The sums of the counts are normalized to a fixed number for all samples). Principal Component Analysis (PCA) was then applied to the normalized count of all the samples from the two brain regions to detect outliers (see Figure 1). DESeq2 (Love et al. 2014) was applied to the normalized counts to estimate the fold change between the samples from mice that had self-administered oxycodone and those from yoked saline controls, using negative binomial distribution. An adjusted p-value of less than 0.05 (padj <0.05) was used to select genes that have a significant expression change.

Gene expression study with quantitative polymerase chain reaction (qPCR)

Samples from the replicate SA study (Study 2) were used to confirm expression differences between oxycodone and yoked saline control mice of a subset of randomly-selected genes with high and low expression level in the dorsal and ventral striatum, as identified by RNA-seq. We examined genes showing alteration in the expression levels in both the dorsal and ventral striatum, and also studied genes that were altered in response to oxycodone SA in only one of those brain regions. cDNA was synthesized from 1 µg of total RNA using the Super Script III first strand synthesis kit (Invitrogen, Carlsbad, CA). The reaction was performed in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA), applying the following program: 2 minutes at 50°C, 10 minutes at 95°C and 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Sequence Detection Software (Applied Biosystems, Foster City, CA) was used to calculate the cycle threshold (Ct) value for each well. Any sample with Ct greater than 35 was not included in the analysis according to manufacturer's recommendations. The relative expression of each gene was calculated by subtracting the Ct value of the housekeeping gene Gapdh from the Ct value of the target gene to determine the ΔCt . The final measure of relative expression level of a gene was calculated as $2^{-\Delta Ct}$ (details see Zhang et al. 2015; Zhang et al. 2014).

Statistical Analysis on qPCR data

In the qPCR study, the significance of the difference between oxycodone and yoked saline control mice in the expression levels of each gene was evaluated by one tailed t test. A p value of < 0.05 was accepted as indicating a significant difference. Adjustment for multiple comparisons (false discovery rate) was performed for the qPCR data.

Gene Ontology analysis

For functional enrichment analyses, DAVID (Ashburner et al., 2000) with default parameters and all mouse genes as background model, was used to test biological functional enrichment of genes with significant expression changes (padj <0.05). The analysis was done through Gene Ontology (GO) (Kanehisa and Goto 2000) and KEGG pathways (Ashburner et al. 2000). Briefly, DAVID tests the statistical significance of whether the identified genes are over-represented in a specific term (such as GO or KEGG).

Results

Extended access oxycodone self-administration

Self-administration behaviors of oxycodone and yoked saline control mice from Study 1 (for RNA-seq study, also see Zhang et al., 2014) and Study 2 (for qPCR study) were similar; we therefore combined the data from the two studies. The nose poking of mice from both SA studies is shown in Figure 2. A two-way ANOVA, for Drug Condition (oxycodone or saline) $\times$ Session (Days 1–14) showed a significant main effect of Drug Condition [$F(1,252)=672.6$, $p < 0.0001$], and a significant main effect of Session [$F(13,252)=2.359$, $p < 0.01$] with a significant interaction of Drug Condition $\times$ Session [$F(13,252)=21.12$, $p < 0.0001$]. Mice in the oxycodone group nose poked significantly more at the active hole than at the inactive hole. In contrast, the yoked saline control group did not differ in nose poking between the “active” and inactive hole. The daily oxycodone intake increased significantly across sessions.

Extended oxycodone self-administration led to transcriptional changes related to inflammation and immune function in the dorsal and ventral striatum

Dorsal striatum—Significant changes were observed in the expression of 55 genes related to inflammation/immune function in the dorsal striatum (Table 1), between mice that had self-administered oxycodone and the yoked saline controls. Fifty-four genes in the dorsal striatum showed significant upregulation, whereas one gene showed significant downregulation.

Ventral Striatum—In the ventral striatum, significant changes in the expression of 141 inflammation/immune-related genes were found between mice that had self-administered oxycodone and the yoked saline controls (Table 2). Two samples of ventral striatum were not included in the analysis due to their outlier status detected by PCA analysis (see Figure 1). Among the 141 inflammation/immune-related genes, 126 showed significant upregulation after oxycodone SA, whereas 15 genes showed significant downregulation.

Thirty-eight genes (labeled with *) in Tables 1 and 2 were found to be upregulated in both the dorsal and ventral striatum, following 14-day extended-access oxycodone SA, compared to yoked saline control. The heat map in Figure 3 shows normalized expression of all the differentially expressed inflammation and immune related genes ($p < 0.05$) from the dorsal and ventral striatum in the oxycodone and yoked saline control mice.

The overlap of inflammation and immune-related genes from the dorsal and ventral striatum is shown in the union map (see Figure 4).

Examination of gene expression changes in the dorsal and ventral striatum with qPCR

qPCR was used to examine the relative mRNA levels of a subset of genes identified by RNA-seq in the dorsal striatum (Table 3A), and three genes were found to increase in expression levels in the oxycodone group compared with yoked saline group as identified by RNA-seq: cytochrome b-245, beta polypeptide (*Cybb*), proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2) (*Psmb9*) and solute carrier family 2

(facilitated glucose transporter), member 1 (*Slc2a1*). Figure 5 shows the expression levels of these three genes as measured by qPCR.

qPCR was used to examine the relative mRNA levels of a subset of genes identified by RNA-seq in the ventral striatum (Table 3B), and four genes were found to increase in expression levels in the oxycodone group compared with the yoked saline group as identified by RNA-seq: Cytochrome b-245, beta polypeptide (*Cybb*); Chemokine (C-C motif) receptor 5 (*Ccr5*); Intercellular adhesion molecule 1 (*Icam*) and Inositol 1,4,5-trisphosphate receptor 1 (*Itpr1*). Figure 6 shows the expression levels of these four genes measured by qPCR.

The expression levels of three genes in the dorsal striatum and seven genes in the ventral striatum were found to be not significantly different between the oxycodone and yoked saline groups, as measured by qPCR. Figure 7 shows the expression levels of these genes.

After adjustment for multiple comparisons of these qPCR data, none of the genes retained an experimental-wise significance.

Enriched function-related gene groups

Thirty Gene Ontology (GO) terms were found to be significantly enriched (Table 4). Among these processes are immune response (GO: 0006955), defense response (GO: 0006952), immune effector process (GO: 0002252) and inflammatory response (GO: 0006954), suggesting that the expression of inflammatory/immune-related genes found in this study was altered as a result of chronic oxycodone self-administration. We also found that the genes listed in Tables 1 and 2 were enriched in several immune functions including response to bacterium (GO: 0009617) and defense response to bacterium (GO:0042742).

Discussion

RNA-seq was used to examine differential gene expression in the dorsal and ventral striatum of adult male mice that had undergone 14-day extended-access self-administration of oxycodone, compared with yoked saline controls. This regimen resulted in substantial daily intake of oxycodone and escalation across sessions. We found here that the mRNA levels of numerous genes related to the inflammation and immune functions changed as a result of oxycodone self-administration, in the dorsal and ventral striatum.

Early studies revealed that chronic morphine and oxycodone can modulate both adaptive and innate immune systems, as well as activate neuroinflammation (e.g., Cui et al. 2014; Wang et al. 2012). Acute and chronic morphine administrations showed inhibitory effects on humoral and cellular immune responses (Fecho et al. 1996; Manfredi et al. 1993; Novick et al. 1989; West et al. 1998). In contrast, one study found that hydromorphone and oxycodone were not immunosuppressive, and may act as immune stimulants (e.g., Sacerdote et al. 1997). In our prior hypothesis-based qPCR studies, we did not examine whether oxycodone SA affected the expression of genes related to inflammation and immune functions (Mayer-Blackwell et al. 2014; Zhang et al. 2015; Zhang et al. 2014). One study had examined the effect of chronic high-dose experimenter-administered oxycodone on gene expression in

whole rat brain, and found that this led to changes in the expression of the following immune function-related genes: Hla-dra, CD163, Lims2, Dsipi, Cklfsf6, Tnfrsf11b (Hassan et al. 2007). However, it is unknown which brain region(s) were involved in these effects, and whether these or other changes would be observed in animals self-administering oxycodone.

In the current study, we observed changes in the expression levels of numerous genes related to inflammation and immune functions following oxycodone SA. For example, a significant increase in mRNA for C-C chemokine receptor type 5 (Ccr5) was found in this study. Ccr5 are seven-transmembrane G-protein-coupled receptors that associate with Gi/Go (Raport et al. 1996). Ccr5 regulates chemotaxis and cell activation via interactions with several chemokine ligands (Allen et al. 2007). Ccr5 is expressed by several cell types including lymphocytes, microglia, and astrocytes, as well as neurons in the brain (Sorce et al. 2011; Westmoreland et al. 2002). Several in vitro experiments have shown an increase in Ccr5 expression following 24–48 hours of morphine exposure in primary normal human astrocytes (Mahajan et al. 2005; Mahajan et al. 2002) and human lymphocytic CEMx174 cells (Miyagi et al. 2000; Suzuki et al. 2002a; Suzuki et al. 2002b). Co-immunoprecipitation studies have shown that Ccr5 heterodimerizes with MOP-r opioid receptors (Chen et al. 2004). Further, heterologous cross-deactivation of the MOP-r and Ccr5 has been shown in both in vivo and in vitro experiments (Szabo et al. 2003).

Ccr5 plays an important role in HIV infection in humans. Individuals with the Ccr5 variant, 32 bp deletion, thus without functional Ccr5 protein, are highly resistant to HIV infection and show delayed progression to AIDS (Alkhatib and Berger 2007; Kaslow et al. 2005). Genetic modification in Ccr5 gene results in HIV resistance in T cells (Romano Ibarra et al. 2016). Pharmacological blockade of Ccr5 inhibits the entry of HIV viruses into target cells (e.g., Romano Ibarra et al. 2016). The Ccr5 antagonists maraviroc and cenicriviroc are used for the treatment of HIV infection, but also for improving impairment in HIV- related cognitive function (e.g., Gates et al. 2016; Kim et al. 2016; Kramer et al. 2014; Ndhlovu et al. 2014).

The observed increase in Ccr5 mRNA following 14-day oxycodone self-administration extends the in vitro findings of protracted exposure to MOP-r agonists resulting in increased Ccr5 expression. Further experiments are needed to examine the mechanism by which oxycodone self-administration results in increased Ccr5 mRNA expression, and whether activity at Ccr5 influences escalation of oxycodone self-administration.

It is postulated that the gene expression changes in the brains of mice that had self-administered oxycodone were initiated by microglia. Microglia represent 10–15% of all cells found within the brain (Lawson et al. 1992). Microglia are extremely sensitive to small changes in the micro-environment, and are activated by various factors such as proinflammatory cytokines, necrosis factors, and changes in extracellular potassium (e.g., Aloisi 2001; Brown et al. 1998; Kust et al. 1997; Tooyama et al. 2002). Recent neuroimaging studies have detected microglial activation in the brains of rats exposed to repeated morphine administration (Auvity et al. 2016). We therefore hypothesize that in response to persistent MOP-r mediated activity caused by oxycodone, microglia may

proliferate and become activated. The involvement of microglia in the activation of immune-related genes in the brain following chronic oxycodone self-exposure could be of interest in future studies.

Like macrophages in the periphery, microglia secrete inflammatory mediators to orchestrate the immune response in the brain. Using RNA-seq, we have found upregulation of the expression of genes encoding for interleukin 1 beta (Il1b), monocyte chemotactic protein-5 (Ccl12), tumor necrosis factors (Tnfaip2) and interferon gamma-induced GTPase (Igtf), which may play important roles in proinflammatory functions.

The immune systems modulate a variety of brain functions and affect behaviors (Boulanger 2009; Miller et al. 2013).

Prescription opioids such as oxycodone are thought to initiate their reward-related effects by acting as MOP-r agonists, and also by subsequently increasing dopamine in the mesolimbic and nigrostriatal dopaminergic pathways (Di Chiara and Imperato 1988a; Di Chiara and Imperato 1988b). However, recent studies suggest that innate immune signaling in the brain also plays an important part in the rewarding properties of opioids. For example, suppression of glial proinflammatory responses by AV411, a glia inhibitor, significantly reduced morphine or oxycodone withdrawal, while improving analgesia (Hutchinson et al. 2009) and blocking morphine-induced increases of dopamine in the nucleus accumbens (Bland et al. 2009). Thus, whether inflammation and immune responses are involved in escalation of oxycodone self-administration and development of vulnerability to oxycodone addiction will be an important area of future study.

We identified expression changes in a large number of inflammation/immune-related genes in this study after chronic oxycodone SA, in the absence of provoked bacterial and viral infections. These altered inflammation/immune systems were found in the terminal regions of the nigrostriatal and mesolimbic dopaminergic pathways (e.g., Lindvall et al. 1984; Skagerberg et al. 1984). Future studies could determine whether alterations in inflammation/immune genes and gene products are involved in the rewarding properties of oxycodone, or escalation of oxycodone self-administration. The potential impact of prescription opioid exposure on these inflammation/immune gene targets in the context of clinical analgesia is also a potential area for further study.

Since gene expression changes were examined immediately after 14 consecutive days of chronic oxycodone self-administration, it is unknown whether such alterations occurred immediately after one session of oxycodone self-administration, or resulted from chronic oxycodone self-administration exposure, or from escalation of intake. It is also not clear whether the changes in inflammation/immune gene expression are long-lasting, which may play an important role in the development of sensitivity to oxycodone upon re-exposure ("relapse"), or during withdrawal. Further studies could also determine whether similar changes also occur in other brain regions, or in the peripheral nervous system.

Conclusion

We found here, using an unbiased RNA-seq approach, that chronic oxycodone SA resulted in alterations in multiple inflammatory and immune genes in the dorsal and ventral striatum. Some human studies also report changes in various immune and inflammatory mediators, in brain of persons with chronic heroin exposure (Neri et al. 2013).

The role of neuroinflammatory processes and immune response in the effects of opioids (either to heroin or prescription opioids such as oxycodone) is not well understood. Overall, the present study on the transcriptome provides an initial set of targets and gene systems to examine how the brain inflammatory and immune gene systems are affected by chronic self-administration of the widely abused prescription opioid oxycodone.

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A. Dorsal Striatum

B. Ventral Striatum

Figure 1. Principal Component Analysis on normalized count in dorsal striatum (A) and ventral striatum (B).

Figure 2.

Oxycodone self-administration in 4 hour sessions across the 14 days is shown. Data represent mean daily number of nose pokes ($\pm$ SEM). Mice in the oxycodone group nose poked significantly more at the active hole than at the inactive hole. Mice in the yoked saline group did not differ in nose poking between the "active" and inactive hole. See also Zhang et al., 2014.

Figure 3.

A heat map shows normalized expression of all differentially expressed inflammation and immune related genes ($p < 0.05$) of the dorsal and ventral striatum samples in the oxycodone and yoked saline control groups.

Figure 4.

A union map shows the overlap between differentially expressed genes from the dorsal and ventral striatum. The genes expressed identically in the two brain regions are indicated by the overlap between the two circles.

Figure 5.

Confirmation of mRNA expression changes in the dorsal striatum. qPCR confirmed three upregulated transcripts. The value for each individual sample was determined and from these individual values, the means and SEMs were calculated. The mean (+SEM) levels of *Cybb* (A), *Psmb9* (B) and *Slc2a1* (C) mRNA levels in the dorsal striatum in mice that had self-administered oxycodone was higher than that of yoked saline controls (n=7–9/group).

Figure 6.

Confirmation of mRNA expression changes in the ventral striatum. qPCR confirmed upregulated transcripts of *Ccr5* (A), *Cybb* (B), *Icam* (C) and *Itpr1* (D) in mice that had self-administered oxycodone compared to yoked saline controls (n=7–9/group).

Figure 7.

The expression levels of genes, that were found to be not significantly different between oxycodone and yoked saline control groups as measured by qPCR, were shown in A for the dorsal striatum and B for the ventral striatum.

Table 1
Altered inflammation /immune related genes following oxycodone SA in the dorsal striatum

Genes that were upregulated following 14-day oxycodone self-administration in the dorsal striatum. Genes labeled with * were upregulated in both the dorsal and ventral striatum.

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Fcgr4*</i>	Fc receptor, IgG, low affinity IV	↑	3.02	2.26E-08	6.76E-05
<i>Trem14*</i>	triggering receptor expressed on myeloid cells-like 4	↑	3.01	1.27E-07	1.90E-04
<i>Plac8*</i>	placenta-specific 8	↑	2.89	6.10E-08	1.33E-04
<i>Lilrb4*</i>	leukocyte immunoglobulin-like receptor, subfamily B, member 4B	↑	2.85	8.04E-08	1.33E-04
<i>Tgtp1*</i>	T cell specific GTPase 1	↑	2.72	4.84E-09	3.61E-05
<i>Fpr2*</i>	formyl peptide receptor 2	↑	2.65	2.54E-06	1.65E-03
<i>Il1b*</i>	interleukin 1 beta	↑	2.65	1.19E-05	5.21E-03
<i>Spn*</i>	sialophorin	↑	2.64	1.14E-06	1.00E-03
<i>Cybb*</i>	cytochrome b-245, beta polypeptide	↑	2.59	4.24E-07	4.22E-04
<i>Gbp4</i>	guanylate binding protein 4	↑	2.55	1.32E-06	1.03E-03
<i>Iigp1</i>	interferon inducible GTPase 1	↑	2.53	1.33E-05	5.68E-03
<i>Itgal*</i>	integrin alpha L	↑	2.53	3.95E-07	4.21E-04
<i>C3*</i>	complement component 3	↑	2.52	4.08E-06	2.48E-03
<i>Ly6c2</i>	lymphocyte antigen 6 complex, locus C2	↑	2.51	3.51E-05	1.12E-02
<i>Casp4*</i>	caspase 4, apoptosis-related cysteine peptidase	↑	2.42	4.09E-05	1.22E-02
<i>Gbp5*</i>	guanylate binding protein 5	↑	2.39	2.23E-05	8.31E-03
<i>Tgtp2*</i>	T cell specific GTPase 2	↑	2.39	1.83E-05	7.01E-03
<i>Lcn2*</i>	lipocalin 2	↑	2.35	1.38E-05	5.70E-03
<i>Gvin1</i>	GTPase, very large interferon inducible 1	↑	2.34	2.41E-05	8.66E-03
<i>Six11</i>	syntaxin 11	↑	2.34	1.33E-04	2.96E-02
<i>Irf1*</i>	interferon regulatory factor 1	↑	2.33	9.61E-06	4.78E-03
<i>Nlr3*</i>	NLR family, CARD domain containing 5	↑	2.30	5.04E-06	2.79E-03
<i>Socs3*</i>	suppressor of cytokine signaling 3	↑	2.29	1.13E-05	5.10E-03
<i>Lyz2*</i>	lysozyme 2	↑	2.29	4.16E-06	2.48E-03
<i>Hck*</i>	hemopoietic cell kinase	↑	2.27	7.61E-08	1.33E-04
<i>Cx2tp*</i>	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	↑	2.27	1.01E-04	2.44E-02

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Irgm2*</i>	immunity-related GTPase family M member 2	↑	2.21	7.61E-08	1.33E-04
<i>Gbp9*</i>	guanylate-binding protein 9	↑	2.21	6.82E-07	6.37E-04
<i>Pirb*</i>	paired Ig-like receptor B	↑	2.14	1.51E-04	3.14E-02
<i>Psmb9</i>	proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2)	↑	2.14	1.45E-05	5.70E-03
<i>C1ra</i>	complement component 1, r subcomponent A	↑	2.11	1.09E-04	2.58E-02
<i>Bstl*</i>	bone marrow stromal cell antigen 1	↑	2.10	2.18E-04	4.23E-02
<i>Irgm1*</i>	immunity-related GTPase family M member 1	↑	2.09	7.16E-05	1.91E-02
<i>Tap1</i>	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	↑	2.08	5.75E-06	3.07E-03
<i>Il4ra*</i>	interleukin 4 receptor, alpha	↑	2.08	2.45E-07	2.82E-04
<i>Hp*</i>	haptoglobin	↑	2.08	1.50E-04	3.14E-02
<i>Ctla2a*</i>	cytotoxic T lymphocyte-associated protein 2 alpha	↑	1.95	3.11E-05	1.03E-02
<i>Ly6a</i>	lymphocyte antigen 6 complex, locus A	↑	1.91	1.80E-08	6.70E-05
<i>Ptpnc*</i>	protein tyrosine phosphatase, receptor type, C	↑	1.91	1.28E-06	1.03E-03
<i>Myo1g*</i>	myosin IG	↑	1.89	1.38E-04	2.98E-02
<i>Ifitm3</i>	interferon induced transmembrane protein 3	↑	1.88	8.07E-05	2.08E-02
<i>Itgb2*</i>	integrin beta 2	↑	1.88	2.75E-05	9.33E-03
<i>Pglyrp1*</i>	peptidoglycan recognition protein 1	↑	1.87	1.83E-04	3.64E-02
<i>Anxa2</i>	annexin A2	↑	1.81	2.20E-06	1.50E-03
<i>Sla</i>	src-like adaptor	↑	1.80	1.81E-04	3.64E-02
<i>Tgm2</i>	transglutaminase 2, C polypeptide	↑	1.80	1.44E-07	1.95E-04
<i>Slc11a1*</i>	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	↑	1.72		
<i>Zbtb16*</i>	zinc finger and BTB domain containing 16	↑	1.69	1.04E-05	5.01E-03
<i>Osmr*</i>	oncostatin M receptor	↑	1.65	1.30E-04	2.95E-02
<i>Abcc9*</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	↑	1.55	1.44E-05	5.70E-03
<i>Samhd1</i>	SAM domain and HD domain, 1	↑	1.52	2.37E-04	4.47E-02
<i>Ly6c1</i>	lymphocyte antigen 6 complex, locus C1	↑	1.50	1.13E-05	5.10E-03
<i>Slc2a1*</i>	solute carrier family 2 (facilitated glucose transporter), member 1	↑	1.44	1.67E-07	2.08E-04
<i>Pdzd2</i>	PDZ domain containing 2	↑	1.28	1.52E-04	3.14E-02
<i>Iiga9</i>	integrin alpha 9	↓	0.72	3.82E-05	1.17E-02

Table 2
Altered inflammation /immune related genes following oxycodone SA in the ventral striatum

Genes that were up- or down-regulated following 14-day oxycodone self-administration in the ventral striatum. Genes labeled with * were upregulated in both the dorsal and ventral striatum.

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Sele</i>	selectin, endothelial cell	↑	3.84	3.10E-15	4.59E-11
<i>Lcn2*</i>	lipocalin 2	↑	3.26	2.95E-14	2.19E-10
<i>C3*</i>	complement component 3	↑	3.19	6.42E-12	2.38E-08
<i>Spn*</i>	sialophorin	↑	3.17	1.12E-10	2.37E-07
<i>Plac8*</i>	placenta-specific 8	↑	3.05	7.98E-10	1.18E-06
<i>Fpr2*</i>	formyl peptide receptor 2	↑	2.88	7.69E-09	9.50E-06
<i>Hp*</i>	haptoglobin	↑	2.87	4.56E-10	7.51E-07
<i>Selp</i>	selectin, platelet	↑	2.81	1.79E-08	1.91E-05
<i>Fgr</i>	FGR proto-oncogene, Src family tyrosine kinase	↑	2.79	1.80E-08	1.91E-05
<i>Ncf4</i>	neutrophil cytosolic factor 4	↑	2.66	2.14E-08	2.09E-05
<i>Il1b*</i>	interleukin 1 beta	↑	2.65	1.09E-07	8.48E-05
<i>Itgal*</i>	integrin alpha L	↑	2.54	3.43E-08	2.99E-05
<i>Trem14*</i>	triggering receptor expressed on myeloid cells-like 4	↑	2.53	4.15E-07	0.000228194
<i>Prl</i>	prolactin	↑	2.45	4.04E-07	0.000228194
<i>Cybb*</i>	cytochrome b-245, beta polypeptide	↑	2.42	8.88E-07	0.000356587
<i>Myo1g*</i>	myosin IG	↑	2.41	2.26E-08	2.09E-05
<i>Icam1</i>	intercellular adhesion molecule 1	↑	2.37	1.07E-06	0.000416759
<i>Cd14</i>	CD14 antigen	↑	2.35	1.20E-07	8.94E-05
<i>Hspa1b</i>	heat shock protein 1B	↑	2.32	8.90E-07	0.000356587
<i>Ly6i</i>	lymphocyte antigen 6 complex, locus I	↑	2.29	6.04E-06	0.00149208
<i>Bst1*</i>	bone marrow stromal cell antigen 1	↑	2.26	6.60E-06	0.001604811
<i>Pltra</i>	paired immunoglobulin-like type 2 receptor alpha	↑	2.25	5.78E-06	0.00149208
<i>Socs3*</i>	suppressor of cytokine signaling 3	↑	2.22	2.96E-06	0.000944922
<i>Mefv</i>	Mediterranean fever	↑	2.19	1.17E-05	0.0024599
<i>Cst2rb*</i>	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	↑	2.14	1.32E-05	0.002573582

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Pirb*</i>	paired Ig-like receptor B	↑	2.13	1.29E-05	0.002559713
<i>Runx1</i>	runt related transcription factor 1	↑	2.12	3.35E-06	0.001034761
<i>Casp4*</i>	caspase 4, apoptosis-related cysteine peptidase	↑	2.11	2.66E-05	0.004083134
<i>Cytip</i>	cytohesin 1 interacting protein	↑	2.08	4.26E-05	0.005519554
<i>Sfin2</i>	schlafen 2	↑	2.08	4.23E-05	0.005519554
<i>Fcgr4*</i>	Fc receptor, IgG, low affinity IV	↑	2.07	7.40E-05	0.008131867
<i>Tnfrsf2</i>	tumor necrosis factor, alpha-induced protein 2	↑	2.03	1.16E-05	0.0024599
<i>Irf1*</i>	interferon regulatory factor 1	↑	2.03	2.14E-05	0.003533595
<i>Ptprc*</i>	protein tyrosine phosphatase, receptor type, C	↑	2.02	8.71E-07	0.000356587
<i>Hck*</i>	hemopoietic cell kinase	↑	2.01	7.52E-07	0.000338049
<i>C5ar1</i>	complement component 5a receptor 1	↑	2.00	0.000108393	0.01086204
<i>Gbp5*</i>	guanylate binding protein 5	↑	1.98	0.000176751	0.015696982
<i>Il4ra*</i>	interleukin 4 receptor, alpha	↑	1.98	6.97E-07	0.000333552
<i>Ifi47</i>	interferon gamma inducible protein 47	↑	1.97	0.000200308	0.0171499
<i>Rnf125</i>	ring finger protein 125	↑	1.97	5.62E-05	0.006671
<i>Ccl12</i>	chemokine (C-C motif) ligand 12	↑	1.97	0.000213169	0.0171499
<i>Lilrb4*</i> (replaced <i>Lilrb4</i>)	leukocyte immunoglobulin-like receptor, subfamily B, member 4A	↑	1.94	0.000273844	0.019157455
<i>Irgm2*</i>	immunity-related GTPase family M member 2	↑	1.93	6.03E-05	0.006982474
<i>Lyz2*</i>	lysozyme 2	↑	1.91	0.000341027	0.022680563
<i>Fas</i>	Fas (TNF receptor superfamily member 6)	↑	1.90	2.36E-05	0.003801418
<i>Fcgr2b</i>	Fc receptor, IgG, low affinity IIb	↑	1.90	5.22E-05	0.00634768
<i>Batf2</i>	basic leucine zipper transcription factor, ATF-like 2	↑	1.89	0.000391349	0.025125934
<i>Tnfrsf10</i>	tumor necrosis factor (ligand) superfamily, member 10	↑	1.89	0.000246682	0.0180525
<i>Adams1</i>	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	↑	1.89	8.15E-06	0.00191844
<i>Robo3</i>	roundabout homolog 3 (Drosophila)	↑	1.87	0.000548487	0.030104231
<i>S100a9</i>	S100 calcium binding protein A9 (calgranulin B)	↑	1.87	0.000663427	0.033596569
<i>Fyb</i>	FYN binding protein	↑	1.86	1.81E-07	0.000127701
<i>Il2rg</i>	interleukin 2 receptor, gamma chain	↑	1.86	0.000603152	0.031721065
<i>Isg20</i>	interferon-stimulated protein	↑	1.85	0.000428365	0.025934276
<i>Birc3</i>	baculoviral IAP repeat-containing 3	↑	1.85	0.000249888	0.0180785

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Plaur</i>	plasminogen activator, urokinase receptor	↑	1.84	0.000372616	0.024027265
<i>Adm</i>	adrenomedullin	↑	1.83	0.000412153	0.025791711
<i>C5ar2</i>	complement component 5a receptor 2	↑	1.82	0.000168154	0.015299921
<i>Tgpl*</i>	T cell specific GTPase 1	↑	1.81	0.00115296	0.048440665
<i>Mkl</i>	mixed lineage kinase domain-like	↑	1.81	0.000657386	0.033596569
<i>Tgfp2*</i>	T cell specific GTPase 2	↑	1.81	0.001178572	0.048961902
<i>Cd300lf</i>	CD300 antigen like family member F	↑	1.81	0.001183609	0.049033829
<i>Cd52</i>	CD52 antigen	↑	1.79	0.000534611	0.029807573
<i>Cs2rb2</i>	colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage)	↑	1.79	0.000923022	0.042121064
<i>Fcgr3</i>	Fc receptor, IgG, low affinity III	↑	1.78	5.03E-07	0.000257089
<i>Pigs2</i>	prostaglandin-endoperoxide synthase 2	↑	1.77	2.95E-05	0.004345051
<i>Ctla2a*</i>	cytotoxic T lymphocyte-associated protein 2 alpha	↑	1.76	0.000291616	0.020116107
<i>Gbp9*</i>	guanylate-binding protein 9	↑	1.76	0.000878369	0.040709675
<i>Hspa1a</i>	heat shock protein 1A	↑	1.76	0.000242993	0.0180525
<i>Map3k8</i>	mitogen-activated protein kinase kinase kinase 8	↑	1.76	4.32E-05	0.005519554
<i>Bcl3</i>	B cell leukemia/lymphoma 3	↑	1.76	0.000766635	0.037035702
<i>Apobec3</i>	apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3	↑	1.76	0.000266281	0.018805747
<i>Nlr5*</i>	NLR family, CARD domain containing 5	↑	1.76	0.001064565	0.045763941
<i>Rac2</i>	RAS-related C3 botulinum substrate 2	↑	1.75	0.000258464	0.018518239
<i>Ccr5</i>	chemokine (C-C motif) receptor 5	↑	1.74	8.70E-06	0.002014954
<i>Osmr*</i>	oncostatin M receptor	↑	1.74	5.31E-06	0.001431624
<i>Tlr7</i>	toll-like receptor 7	↑	1.73	2.70E-05	0.004083134
<i>Ponc</i>	pro-opiomelanocortin-alpha	↑	1.73	0.001001447	0.044601992
<i>Hspb1</i>	heat shock protein 1	↑	1.71	0.000623632	0.032567179
<i>Spil</i>	spleen focus forming virus (SFFV) proviral integration oncogene	↑	1.70	4.14E-05	0.005519554
<i>Themis2</i>	thymocyte selection associated family member 2	↑	1.70	0.000262915	0.018675116
<i>Irgm1*</i>	immunity-related GTPase family M member 1	↑	1.69	0.001159624	0.048583023
<i>Irgb2*</i>	integrin beta 2	↑	1.69	6.15E-05	0.007074965
<i>Lcp1</i>	lymphocyte cytosolic protein 1	↑	1.69	4.17E-05	0.005519554
<i>Syk</i>	spleen tyrosine kinase	↑	1.66	1.37E-05	0.002622998

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Pgylvp1*</i>	peptidoglycan recognition protein 1	↑	1.66	1.51E-06	0.000561269
<i>Kcnj8</i>	potassium inwardly-rectifying channel, subfamily J, member 8	↑	1.65	7.87E-06	0.001882344
<i>Ifitm2</i>	interferon induced transmembrane protein 2	↑	1.63	0.000505586	0.028619616
<i>Ikzf1</i>	IKAROS family zinc finger 1	↑	1.61	0.001076374	0.046004904
<i>Zbb16*</i>	zinc finger and BTB domain containing 16	↑	1.61	9.69E-06	0.002176541
<i>Nod2</i>	nucleotide-binding oligomerization domain containing 2	↑	1.61	3.44E-05	0.004849278
<i>Nostrin</i>	nitric oxide synthase trafficker	↑	1.60	1.71E-05	0.003088282
<i>Bcl6b</i>	B cell CLL/lymphoma 6, member B	↑	1.60	0.000268213	0.018852472
<i>Clec5a</i>	C-type lectin domain family 5, member a	↑	1.60	0.001024552	0.04495601
<i>Lcp2</i>	lymphocyte cytosolic protein 2	↑	1.60	0.000207807	0.0171499
<i>Clec2d</i>	C-type lectin domain family 2, member d	↑	1.60	6.74E-05	0.007687459
<i>Tnfrsf1b</i>	tumor necrosis factor receptor superfamily, member 1b	↑	1.59	1.87E-06	0.000661649
<i>Kremen1</i>	kringle containing transmembrane protein 1	↑	1.58	5.03E-05	0.006168173
<i>Zfp36</i>	zinc finger protein 36	↑	1.58	0.000576625	0.030852116
<i>Vcam1</i>	vascular cell adhesion molecule 1	↑	1.58	0.000232666	0.017803663
<i>Slc11a1*</i>	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	↑	1.57	0.000955187	0.043322241
<i>Nod1</i>	nucleotide-binding oligomerization domain containing 1	↑	1.55	0.000420586	0.025934276
<i>Myo1f</i>	myosin IF	↑	1.54	0.000464259	0.027107957
<i>Serpinh1</i>	serine (or cysteine) peptidase inhibitor, clade H, member 1	↑	1.53	0.000471669	0.027325452
<i>Slc19a3</i>	solute carrier family 19, member 3	↑	1.53	1.78E-05	0.003110677
<i>Ackr1</i>	atypical chemokine receptor 1 (Duffy blood group)	↑	1.53	1.29E-05	0.002559713
<i>Abcc9*</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	↑	1.52	2.56E-07	0.000157988
<i>Ncf1</i>	neutrophil cytosolic factor 1	↑	1.51	0.00054815	0.030104231
<i>Hspa5</i>	heat shock protein 5	↑	1.51	7.29E-07	0.00033795
<i>Ier3</i>	immediate early response 3	↑	1.50	0.000188989	0.016487626
<i>Ipt1l</i>	inositol 1,4,5-trisphosphate receptor 1	↑	1.45	0.000191707	0.016530279
<i>Nfkbi2</i>	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta	↑	1.45	0.000747804	0.03648252
<i>Xbp1</i>	X-box binding protein 1	↑	1.45	3.48E-06	0.001054445
<i>Nfe2l2</i>	nuclear factor, erythroid derived 2, like 2	↑	1.45	0.000248905	0.0180785
<i>Slc2a1*</i>	solute carrier family 2 (facilitated glucose transporter), member 1	↑	1.44	2.53E-05	0.003945444

Gene Symbol	Gene Name	Direction	Fold change	pvalue	padj
<i>Il17ra</i>	interleukin 17 receptor A	↑	1.43	0.000532623	0.029807573
<i>Htr2a</i>	5-hydroxytryptamine (serotonin) receptor 2A	↑	1.43	0.000915986	0.041928973
<i>Ezr</i>	ezrin	↑	1.41	1.78E-06	0.000644428
<i>Clqc</i>	complement component 1, q subcomponent, C chain	↑	1.32	0.001169468	0.048720184
<i>Kazn</i>	kazrin, periplakin interacting protein	↑	1.27	0.000771733	0.037160936
<i>Adcy9</i>	adenylate cyclase 9	↑	1.25	0.000548252	0.030104231
<i>Dapk1</i>	death associated protein kinase 1	↑	1.25	3.80E-06	0.001127402
<i>Cd163</i>	CD163 antigen	↑	1.23	0.000848315	0.040068045
<i>Dnajc3</i>	DnaJ heat shock protein family (Hsp40) member C3	↑	1.21	0.000481134	0.027657747
<i>Mil2</i>	myeloid leukemia factor 2	↑	1.21	2.34E-05	0.003801418
<i>Lzsl1</i>	leucine zipper, putative tumor suppressor 1	↑	1.18	0.000666227	0.033596569
<i>Ddx3x</i>	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked	↓	0.88	1.60E-05	0.002936785
<i>Rfx5</i>	regulatory factor X, 5 (influences HLA class II expression)	↓	0.79	3.47E-05	0.004849278
<i>Hmgb3</i>	high mobility group box 3	↓	0.77	0.000422766	0.025934276
<i>Podxl2</i>	podocalyxin-like 2	↓	0.74	0.000578095	0.030852116
<i>Ecel1</i>	endothelin converting enzyme-like 1	↓	0.71	0.000598238	0.031574611
<i>Adcy8</i>	adenylate cyclase 8	↓	0.71	0.000829787	0.039444134
<i>Tspan6</i>	tetraspanin 6	↓	0.69	0.000216119	0.0171499
<i>Isl1</i>	ISL1 transcription factor, LIM/homeodomain	↓	0.68	0.000216799	0.0171499
<i>Grik1</i>	glutamate receptor, ionotropic, kainate 1	↓	0.65	0.000708151	0.034662015
<i>Mc3r</i>	melanocortin 3 receptor	↓	0.61	0.000297137	0.020308023
<i>Gpc3</i>	glypican 3	↓	0.60	0.000232885	0.017803663
<i>Gtla1</i>	glycine receptor, alpha 1 subunit	↓	0.54	0.000491955	0.028062222
<i>Cd83</i>	CD83 antigen	↓	0.51	3.20E-11	7.91E-08
<i>Igsf1</i>	immunoglobulin superfamily, member 1	↓	0.47	4.84E-06	0.00132888
<i>Ngfr</i>	nerve growth factor receptor (TNFR superfamily, member 16)	↓	0.42	8.84E-07	0.000356587

Table 3

A: List of inflammation /immune related genes examined with qPCR in the dorsal striatum.

B: List of inflammation /immune related genes examined with qPCR in the ventral striatum.

A. Confirmation of significant changes in selected inflammation/immune related genes in dorsal striatum using qPCR; 6 genes studied of the 55 found to be changed by RNA-seq				
Gene Symbol	Gene Name	p-value, qPCR	qPCR confirmation *	Direction of change $\pm$
<i>Cybb</i>	cytochrome b-245, beta polypeptide	0.0199	Yes	↑
<i>Psmb9</i>	proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2)	0.0348	No	↑
<i>Irgm1</i>	immunity-related GTPase family M member 1	0.0996	Yes	↑
<i>Tap1</i>	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	0.2662	No	↑
<i>Slc2a1</i>	solute carrier family 2 (facilitated glucose transporter), member 1	0.3444	Yes	↑
<i>Socs3</i>	suppressor of cytokine signaling 3	0.3444	No	↑

B. Confirmation of significant changes in selected inflammation/immune related genes in ventral striatum using qPCR; 11 genes studied of the 141 found to be changed by RNA-seq				
Gene Symbol	Gene Name	p-value, qPCR	qPCR confirmation *	Direction of change $\pm$
<i>Ccr5</i>	chemokine (C-C motif) receptor 5	0.0140	Yes	↑
<i>Itpr1</i>	inositol 1,4,5-trisphosphate receptor 1	0.0175	Yes	↑
<i>Icam1</i>	intercellular adhesion molecule 1	0.0175	Yes	↑
<i>Cybb</i>	cytochrome b-245, beta polypeptide	0.0406	Yes	↑
<i>Socs3</i>	suppressor of cytokine signaling 3	0.0678	No	↑
<i>Ngfr</i>	nerve growth factor receptor (TNFR superfamily, member 16)	0.1578	No	↓
<i>Cd83</i>	CD83 antigen	0.2068	No	↓
<i>Robo3</i>	roundabout homolog 3 (Drosophila)	0.2426	No	↑
<i>Slc2a1</i>	solute carrier family 2 (facilitated glucose transporter), member 1	0.3955	No	↑
<i>C3</i>	complement component 3	0.8558	No	↑
<i>Tlr7</i>	toll-like receptor 7	0.9455	No	↑

Genes are listed alphabetically by gene symbol

* "Yes" indicates that the qPCR replication result was statistically significant and in the same direction. "No" indicates that the qPCR replication result was not statistically significant.

$\pm$ "Direction of change" from RNA-seq results.

Table 3

Enriched molecular function categories in GO analysis

Biological processes (groups) enriched with genes with significant differences in expression between mice that had self-administered oxycodone compared to yoked saline controls (Tables 1 and 2).

GO ID	Groups	Count	p-value	Bonferroni	Benjamini	FDR
GO:0006955	Immune response	38	2.41E-13	3.97E-10	3.97E-10	4.05E-10
GO:0006952	Defense response	36	1.34E-12	2.21E-09	1.11E-09	2.26E-09
GO:0002252	Immune effector process	16	1.84E-08	3.03E-05	1.01E-05	3.09E-05
GO:0050830	Defense response to Gram-positive bacterium	8	1.68E-07	2.76E-04	6.91E-05	2.82E-04
GO:0009617	Response to bacterium	16	3.49E-07	5.76E-04	1.15E-04	5.87E-04
GO:0042742	Defense response to bacterium	13	1.07E-06	1.75E-03	2.93E-04	1.79E-03
GO:0007159	Leukocyte adhesion	6	4.37E-06	7.18E-03	1.03E-03	7.35E-03
GO:0002443	Leukocyte mediated immunity	11	7.71E-06	1.26E-02	1.59E-03	1.30E-02
GO:0006954	Inflammatory response	16	2.99E-05	4.81E-02	5.46E-03	5.02E-02
GO:0001817	Regulation of cytokine production	12	7.60E-05	1.18E-01	1.24E-02	1.28E-01
GO:0002819	Regulation of adaptive immune response	8	9.24E-05	1.41E-01	1.38E-02	1.55E-01
GO:0002822	Regulation of adaptive immune response based on Somatic recombination of immune receptors built from immunoglobulin superfamily domains	8	9.24E-05	1.41E-01	1.38E-02	1.55E-01
GO:0002449	Lymphocyte mediated immunity	9	1.02E-04	1.54E-01	1.38E-02	1.71E-01
GO:0051241	Negative regulation of multicellular organismal process	10	1.29E-04	1.91E-01	1.62E-02	2.16E-01
GO:0009611	Response to wounding	19	1.33E-04	1.96E-01	1.55E-02	2.23E-01
GO:0032680	Regulation of tumor necrosis factor production	6	1.44E-04	2.11E-01	1.57E-02	2.41E-01
GO:0051607	Defense response to virus	5	1.54E-04	2.24E-01	1.57E-02	2.58E-01
GO:0032101	Regulation of response to external stimulus	10	1.61E-04	2.34E-01	1.55E-02	2.71E-01
GO:0001819	Positive regulation of cytokine production	8	1.78E-04	2.54E-01	1.61E-02	2.98E-01
GO:0002250	Adaptive immune response	9	2.06E-04	2.88E-01	1.77E-02	3.45E-01
GO:0002460	Adaptive immune response based on somatic Recombination of immune receptors built from immunoglobulin superfamily domains	9	2.06E-04	2.88E-01	1.77E-02	3.45E-01
GO:0048585	Negative regulation of response to stimulus	8	2.40E-04	3.26E-01	1.96E-02	4.02E-01
GO:0042330	Taxis	10	2.48E-04	3.36E-01	1.93E-02	4.16E-01
GO:0006935	Chemotaxis	10	2.48E-04	3.36E-01	1.93E-02	4.16E-01
GO:0045321	Leukocyte activation	14	3.20E-04	4.10E-01	2.37E-02	5.36E-01

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GO ID	Groups	Count	p-value	Bonferroni	Benjamini	FDR
GO:0045807	Positive regulation of endocytosis	6	4.44E-04	5.19E-01	3.13E-02	7.43E-01
GO:0002274	Myeloid leukocyte activation	6	5.10E-04	5.68E-01	3.44E-02	8.53E-01
GO:0002683	Negative regulation of immune system process	8	6.29E-04	6.46E-01	4.06E-02	1.05E+00
GO:0002684	Positive regulation of immune system process	13	6.39E-04	6.51E-01	3.97E-02	1.07E+00
GO:0050727	Regulation of inflammatory response	7	7.57E-04	7.13E-01	4.52E-02	1.27E+00