Published in final edited form as:

Eur J Neurosci. 2017 November ; 46(9): 2519–2533. doi:10.1111/ejn.13711.

Inflammation alters AMPA-stimulated calcium responses in dorsal striatal D2 but not D1 spiny projection neurons

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Abstract

Neuroinflammation precedes neuronal loss in striatal neurodegenerative diseases and can be exacerbated by the release of proinflammatory molecules by microglia. These molecules can affect trafficking of AMPARs. The preferential trafficking of calcium-permeable versus impermeable AMPARs can result in disruptions of $[Ca^{2+}]_i$ and alter cellular functions. In striatal neurodegenerative diseases, changes in $[Ca^{2+}]_i$ and L-type voltage-gated calcium channels (VGCCs) have been reported. Therefore, the present study sought to determine whether a proinflammatory environment alters AMPA-stimulated $[Ca^{2+}]_i$ through calcium-permeable AMPARs and/or L-type VGCCs in dopamine-2 and dopamine-1 expressing striatal spiny projection neurons (D2 and D1 SPNs) in the dorsal striatum. Mice expressing the calcium indicator protein, GCaMP in D2 or D1 SPNs, were utilized for calcium imaging. Microglial activation was assessed by morphology analyses. To induce inflammation, acute mouse striatal slices were incubated with lipopolysaccharide (LPS). Here we report that LPS treatment potentiated AMPA responses only in D2 SPNs. When a nonspecific VGCC blocker was included, we observed a decrease of AMPA-stimulated calcium fluorescence in D2 but not D1 SPNs. The remaining agonist induced $[Ca^{2+}]_i$ was mediated by calcium-permeable AMPARs because the responses were completely blocked by a selective calcium-permeable AMPAR antagonist. We

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Author contributions:

CDW, KMZ, and SV conceived and designed the study; CDW, NW, ASR performed the experiments, analyzed data, and prepared figures; CDW, KMZ, and SV wrote the manuscript. All authors approved the final version of the manuscript.

Conflicts of Interest:

The authors declare no competing financial interests.

Data Accessibility Statement:

Data will be made available on the journal's Figshare page (insert URL)

used isradipine, the highly selective L-type VGCC antagonist to determine the role of L-type VGCCs in SPNs treated with LPS. Isradipine decreased AMPA-stimulated responses selectively in D2 SPNs after LPS treatment. Our findings suggest that dorsal striatal D2 SPNs are specifically targeted in proinflammatory conditions and that L-type VGCCs and calcium-permeable AMPARs are important mediators of this effect.

Graphical Abstract Text

Representative schematic of a corticostriatal slice illustrating the experimental procedure and the main finding that LPS treatment increases AMPA-stimulated calcium responses in indirect pathway striatal projecting neurons. The red line indicates the average of all responding cells.

Keywords

SPN; calcium-imaging; VGCCs; neuroinflammation; calcium-permeable AMPARs

Introduction

The control and refinement of voluntary movement is the result of precise neurotransmitter convergence on striatal medium spiny neurons (SPNs), (for review (Calabresi *et al.*, 2014)). SPNs are the principal projection neurons (~95%) of the striatum and are divided into two populations based on dopamine receptor expression and output targets (Gerfen *et al.*, 1990; Surmeier *et al.*, 1996; Doig *et al.*, 2010; Kravitz *et al.*, 2010). Specifically, D1 expressing SPNs are part of the “direct” pathway and facilitate movement whereas D2 expressing SPNs are part of the “indirect” pathway and inhibit movement (Doig *et al.*, 2010; Kravitz *et al.*, 2010). Recently, however, it was suggested that these pathways are more complex and integrated (Jaidar *et al.*, 2010; Cui *et al.*, 2013; Calabresi *et al.*, 2014).

Firing rates of SPNs are determined by the integration and modulation of excitatory and inhibitory signals (Kreitzer, 2009; Doig *et al.*, 2010; Jaidar *et al.*, 2010). Disruptions of SPN activity and glutamatergic signaling are observed in striatal neurodegenerative disorders such Huntington’s disease (HD), HIV-associated dementia (HAND), and Parkinson’s disease (PD). For example, SPNs in HD animal models show potentiation of glutamate currents (Chen *et al.*, 1999; Li *et al.*, 2003; Li *et al.*, 2004), increased sensitivity to glutamate and slightly depolarized resting membrane potentials (Levine *et al.*, 1999), and presynaptic (Cepeda *et al.*, 2003) and postsynaptic (Li *et al.*, 2003; Li *et al.*, 2004) glutamatergic alterations. Similarly, animal models of HIV-infection exhibit potentiation through glutamate receptors (Fitting *et al.*, 2014) and reduced glutamate uptake in the synapse (Melendez *et al.*,

2016). This may underlie the abnormal subcortical electrical activity observed during motor movements of HAND patients (Baldeweg and Gruzelier, 1997). Although PD is not classified as a striatal neurodegenerative disorder, alterations in glutamatergic signaling and SPN activity are observed. For example, PD animal models demonstrate remodeling of corticostriatal glutamatergic synapses (Stephens *et al.*, 2005; Smith *et al.*, 2009; Villalba and Smith, 2010; Pienaar *et al.*, 2012; Matikainen-Ankney *et al.*, 2016), increased excitatory neurotransmission (Matikainen-Ankney *et al.*, 2016), and increased SPN firing (Zold *et al.*, 2012; Escande *et al.*, 2016). In accordance with animal studies, PD patients demonstrate increased SPN firing frequency (Singh *et al.*, 2016), although D1 or D2 SPNs have not been differentiated.

Calcium dysregulation is also observed in striatal neurodegenerative disorders. For example, HD and HIV-infected animal models demonstrate increased calcium flux through calcium-permeable glutamate receptors (Lipton, 1994; Lannuzel *et al.*, 1995; Chen *et al.*, 1999; Bonavia *et al.*, 2001; Haughey and Mattson, 2002; Fan *et al.*, 2012; Fitting *et al.*, 2014; Hu, 2016). Furthermore, increased $[Ca^{2+}]_i$ is detected in PD, HD, and HIV-infection models (Lipton, 1991; Kruman *et al.*, 1998; Bonavia *et al.*, 2001; Haughey and Mattson, 2002; Mattson *et al.*, 2005; Day *et al.*, 2006; Day *et al.*, 2008; Rom *et al.*, 2009; Miller and Bezprozvanny, 2010; Vigont *et al.*, 2014; Hu, 2016; Meeker *et al.*, 2016). Increased $[Ca^{2+}]_i$ could be a result of potentiated calcium conductance through VGCCs as these channels are affected in PD, HD, and HAND (Watson *et al.*, 1988; Lipton, 1994; Bonavia *et al.*, 2001; Haughey and Mattson, 2002; Gelman *et al.*, 2004; Day *et al.*, 2006; Romero *et al.*, 2008; Wu *et al.*, 2008; Chan *et al.*, 2010; Ilijic *et al.*, 2011; Kang *et al.*, 2012; Brimblecombe *et al.*, 2015).

Despite having different etiologies and clinical presentations, patients with HD, PD, and HAND demonstrate neuroinflammation as indicated by increased glial activation and elevated proinflammatory molecule release (Yoshioka *et al.*, 1995; Adamson *et al.*, 1996; Giovannoni *et al.*, 1998; Adamson *et al.*, 1999; Knott *et al.*, 2000; Sapp *et al.*, 2001; Imamura *et al.*, 2003; Croisier *et al.*, 2005; Rostasy *et al.*, 2005; Brabers and Nottet, 2006; Tai *et al.*, 2007b, a; Hahn *et al.*, 2010; Maguire-Zeiss and Fedoroff, 2010; Edison *et al.*, 2013; Abassi *et al.*, 2016). Importantly, neuroinflammation coincides with disease progression (Sapp *et al.*, 2001; Ouchi *et al.*, 2005; Tai *et al.*, 2007a; Politis *et al.*, 2011; Iannaccone *et al.*, 2013; Terada *et al.*, 2016) and correlates with disease and symptom severity (Imamura *et al.*, 2003; Croisier *et al.*, 2005; Ouchi *et al.*, 2009; Edison *et al.*, 2013; Lin *et al.*, 2015). Furthermore, microglial activation can precede diagnosis as in the case of HD (Bjorkqvist *et al.*, 2008) and HAND (An *et al.*, 1999; Kolson, 2002; Fischer-Smith and Rappaport, 2005), with early disruptions in motor symptoms years before diagnosis (Tai *et al.*, 2007b; Paulsen *et al.*, 2008; Politis *et al.*, 2011). Notably, changes in corticostriatal glutamatergic transmission in SPNs can be detected prior to overt motor symptoms (Albin *et al.*, 1990; Levine *et al.*, 1999; Milnerwood and Raymond, 2007; Fan *et al.*, 2012; Deng *et al.*, 2014). Activation of microglia early in disease can perpetuate these alterations by release of the proinflammatory molecules, TNF α and nitric oxide, which affect trafficking of calcium-permeable glutamate receptors such as AMPARs (Albensi and Mattson, 2000; Beattie *et al.*, 2002; Stellwagen *et al.*, 2005; Lai *et al.*, 2006; Stellwagen and Malenka, 2006; Rameau *et*

al., 2007; Serulle et al., 2007; Balosso et al., 2009; Lee et al., 2010; Lewitus et al., 2014; Lewitus et al., 2016).

AMPA receptors are glutamatergic ion channels that are either permeable (CP) or impermeable to calcium. Although most AMPARs are edited to be impermeable to calcium in the adult brain (Isaac *et al.*, 2007), SPNs retain CP-AMPA receptors (Bernard *et al.*, 1997; Cull-Candy *et al.*, 2006; Deng *et al.*, 2007). CP-AMPA receptors may play an overlooked but important role for SPN calcium regulation given that CP-AMPA receptors can conduct calcium when NMDARs are inactivated (Carter and Sabatini, 2004). Therefore we hypothesized that a proinflammatory environment could alter AMPAR-induced calcium influx and investigated if D2 or D1 SPNs were affected similarly. We investigated our hypothesis by using calcium imaging of GCaMP transgenic mice to distinguish changes in D2 and D1 SPNs. Our findings suggest a role for both CP-AMPA receptors and L-type VGCCs in the presence of LPS and that this effect is selective for D2 SPNs.

Materials and Methods

Chemicals

Studies employed commercially available chemicals as follows: S-AMPA (Abcam, #AB120005), cyclothiazide (Abcam, #AB120061), (R)-CPP (Abcam, #AB120159), TTX (Abcam, #AB120054), philanthotoxin-74 (Alomone Labs, #P-120), and isradipine (Tocris, #2004). Stocks were prepared in artificial cerebral spinal fluid (aCSF) or DMSO as the manufacturer recommended. Lipopolysaccharide (LPS; *Escherichia coli* 0.55:B5) was purchased from Sigma-Aldrich (#L6529) and resuspended in dH₂O. Stocks were stored at -20 °C.

Mice with genetically encoded Ca²⁺ indicator in D2 and D1 SPNs

Cre-dependent GCaMP3 (JAX, #014538) or GCaMP6f (#024105) floxed mice were purchased from The Jackson Laboratory. These animals were bred with mice that express Cre recombinase under the control of the *drd2* (GENSAT, ER44), *adora2A* (JAX, #010685), or *drd1* (GENSAT, EY217) promoter. The presence of Cre and GCaMP3 or GCaMP6 in pups from this breeding strategy was confirmed using automated genotyping (Transnetyx, Inc., Cordova, TN). Homozygous CX3CR1^{gfp/gfp} mice were also purchased from the Jackson Laboratory (#005582). These animals were bred with C57/Bl6 mice to produce CX3CR1^{gfp/+} mice, which maintain one functional CX3CR1 allele.

Acute slice preparation and treatment

Acute coronal corticostriatal slices were prepared from male and female mice at postnatal days (P18–P23) as previously described (Janssen *et al.*, 2009; Janssen *et al.*, 2011; Partridge *et al.*, 2014). Calcium responses and basal levels of calcium are considerably more reliable at the age selected than in older animals, due to the greater difficulty in tissue preservation upon slicing. The age chosen was a compromise to allow for as long as possible striatal development and minimal tissue damage due to experimentation. Briefly, mice were subjected to rapid decapitation to minimize pain and distress. Acute coronal slices (250 μm) were prepared in aCSF containing (in mM): NaCl (124), NaHCO₃ (26), dextrose (10), KCl

(4.5), CaCl_2 (2.0), Na_2HPO_4 (1.2) and MgCl_2 (1), at an osmolarity of 325 mOsm. After recovery, slices were hemisected and incubated for two hours with either vehicle or LPS at 31 °C in a 5% CO_2 incubator (Fig. 2A). Slices were washed in aCSF before being subjected to calcium imaging. All animal experimental procedures were performed in accordance with the guidelines and explicit approval of the Georgetown University Animal Care and Use Committee.

Calcium imaging

To determine changes in $[\text{Ca}^{2+}]_i$, striatal slices from *drd1*-, *adora*-, and *drd2*- *GCaMP3/6f* mice were treated with LPS (125 ng/ml or 250 ng/ml as stated in the figure legend) or vehicle as described above and a glutamate receptor agonist cocktail was used to evoke $[\text{Ca}^{2+}]_i$. The agonist cocktail was provided locally with a y-tube applicator (Rabe *et al.*, 2000), to allow rapid perfusion and to minimize the region exposed to the treatment. The agonist cocktail consisted of AMPA (50 μM) to stimulate AMPARs and cyclothiazide (100 μM) to block AMPAR desensitization. It also contained blockers for NMDARs (CPP, 50 μM) and depending on the experimental conditions, blockers for voltage-gated calcium channels (i.e., cadmium chloride, CdCl_2 , 25 μM , isradipine, 5 μM (Brimblecombe *et al.*, 2015)), CP-AMPA (i.e., philanthotoxin-74, 300 μM , (Nilsen and England, 2007; Poulsen *et al.*, 2014)), and voltage-gated sodium channels (i.e., TTX, 1 μM) to block action potentials and neurotransmitter release. Striatal slices were excited with 488 nm light to visualize *GCaMP* expressing neurons using a Nikon GFP HYQ LP filter cube (ex425/475nm, dm480, ba 485LP). The Excite 120LED (Excelitas Technology) was used as a white light source. Imaging was performed using a Rolera-XR FAST 1394 camera (Q imaging) mounted on an upright microscope (E600FN, Nikon) equipped with Nomarski optics and a 20 $\times$ 0.5 NA water-immersion objective lens at room temperature (22–24 °C). Images were acquired at 20 Hz with 20 ms exposure to minimize photobleaching. AMPA-stimulated fluorescence intensity changes over baseline ($\Delta F/F$) were analyzed in manually defined regions of interest (ROIs) corresponding to D2 and D1 SPNs somata using ImageJ (NIH). ROIs were evaluated following subtraction of background, which was estimated by selecting the darkest areas in the neuropil. Background and basal F values were not statistically different between experimental conditions.

Microglial morphology analyses

Images were analyzed using ImageJ software. Images were scaled and cell bodies were isolated using the freehand tracing tool and thresholded to the cell body area. Cells were selected from the middle of each image using the wand tool for area and perimeter measurements. Microglial protrusions were assessed using a modified Sholl analysis from images using ImageJ, NeuronJ, and NeuronStudio software (Langhammer *et al.*, 2010). Briefly, confocal images were stacked and compressed to a single .tif file and converted to 8-bit. The cell body was then isolated from the protrusions using NeuronJ. The files were then converted and translated into NeuronStudio where the protrusions were manually corrected for any software errors. Finally the data were compiled and analyzed using MatLab. The results were verified by an independent blinded observer.

Microglial movement analyses

Vehicle and LPS treated slices derived from CX3CR1^{gpf/+} mice were visualized with a Nikon FN1 upright microscope equipped with a ThorLabs scanning laser confocal system and a Nikon Fluor 60× water-immersion objective. Green fluorescence was excited with a 488 nm laser (1% power) and detected by a photomultiplier (PMT) (50% gain) through a fluorescein isothiocyanate (FITC)-emission bandpass filter. Planes imaged ranged from 8 to 20 μm in depth. 512 × 512 pixel frames were acquired every 10 s for 10 minutes.

ImageJ custom scripts and publicly available plugins were used for image analyses. Specifically, each movie was converted into a single RGB stack that was then assigned a name from a randomized integer list for blinded analysis. Movies with drift in the x or y dimensions were registered with the StackReg plugin using Rigid Body transformation. Each resulting blinded movie was split into individual channel files for further analyses. The number of process elongations of each microglia over the course of the movie was counted for each cell, and divided by the duration of the movie to determine the frequency of process extension for each cell.

Statistics

To reach statistical significance but to minimize animal usage, 2–6 mice were used per experimental condition. For each mouse, all responding cells in 3–5 striatal slices per treatment were analyzed. All data sets were checked for normality using D'Agostino-Pearson omnibus or Shapiro-Wilk tests for the use of parametric statistics. Two-tailed student t-tests, 1-way ANOVA, or 2-way ANOVA were conducted with significance set to $P < 0.05$. Data values in the text and figures are presented as mean ± SEM or box and whiskers plot. Statistical tests and graphical plots were carried out in Prism 7 software (GraphPad). Treatment interactions were assessed by Sidak's or Tukey's post hoc multiple comparisons tests.

Results

LPS treatment of acute striatal slices alters microglial morphology

To induce a proinflammatory environment and mimic a neurodegenerative disease state, we employed the well-established initiator of microglial activation, LPS (Qin *et al.*, 2007; Hunter *et al.*, 2008; Li *et al.*, 2008; Hunter *et al.*, 2009; Zhang *et al.*, 2010; Daniele *et al.*, 2014; Dickens *et al.*, 2014). LPS stimulates microglia to release proinflammatory molecules such as TNFα and nitric oxide, which have been shown to affect trafficking of AMPARs (Albensi and Mattson, 2000; Beattie *et al.*, 2002; Stellwagen *et al.*, 2005; Lai *et al.*, 2006; Stellwagen and Malenka, 2006; Balosso *et al.*, 2009; Lee *et al.*, 2010; Lewitus *et al.*, 2014; Lewitus *et al.*, 2016). To confirm the effects of LPS treatment on acute striatal slices, we prepared slices from transgenic mice expressing green fluorescent protein (GFP) in microglia (CX3CR1^{gpf/+}) and treated them with vehicle or 125 ng/ml LPS. Microglia in vehicle treated slices showed elongated protrusions that extended and retracted from the cell body (Fig. 1A,B) indicative of healthy surveying (Nimmerjahn *et al.*, 2005). In contrast, microglia in LPS treated slices had less dynamic and noticeably retracted protrusions with a classic amoeboid shape (Fig. 1C,D) consistent with LPS-stimulated microglial activation

(Daniele *et al.*, 2014). Movie 1 shows representative time-lapse videos of microglia treated with vehicle or LPS. Specifically, in the vehicle treated slices, microglial protrusions appeared to survey the microenvironment (Movie 1, *left panel*) whereas microglia in LPS treated slices displayed an amoeboid phenotype that moved considerably slower with fewer protrusions (Movie 1, *right panel*). To further support our qualitative interpretation, we quantified the complexity of microglial protrusions and length of protrusions as previously described (Langhammer *et al.*, 2010). Using a modified Sholl analysis, LPS treatment decreased the complexity of microglial protrusions (Fig. 1E; 2-way ANOVA, $F_{(1,294)} = 55.08$, $P < 0.001$, $P < 0.01$, $P < 0.05$). Similarly, LPS treatment significantly decreased the length of microglial protrusions (Fig. 1F; Students *t*-test, $t_{(42)} = 2.56$, $P = 0.0143$) and the total number of protrusions (Fig. 1G; Students *t*-test, $t_{(42)} = 3.14$, $P = 0.0031$). Examples of skeletonized microglia in the vehicle and LPS conditions are shown in Figure 1H–K respectively. Furthermore, we quantified the dynamics of the microglia processes and found that LPS treatment decreased the frequency of process extension from the soma compared to vehicle conditions (Fig. 1L–N; Students *t*-test, $t_{(23)} = 8.037$, $P < 0.0001$). Therefore, two hour LPS treatment of acute striatal slices effectively induced morphological changes indicative of activated microglia.

Calcium imaging in *drd2*- and *drd1*- GCaMP mice

To investigate the effect of LPS on AMPA-stimulated calcium changes in SPNs, we prepared slices from cre-dependent reporter mice that express the green calcium sensor protein GCaMP3 or GCaMP6f in D2 and D1 SPNs. The experimental paradigm is shown in Figure 2A. The dorsal striatum is easily visualized at low magnification (Fig. 2B) and $450 \times 450 \mu\text{m}$ areas (Fig. 2C) for calcium imaging were randomly selected. In order to prevent any secondary effects of broad agonist stimulation via bath application, we used a y-tube delivery system to deliver a small amount of the agonist cocktail to a precise region of cells (Fig. 2B). As seen in Figure 2C, there are a number of GCaMP3 expressing SPNs with fluorescence in the baseline condition, reflecting uninduced $[\text{Ca}^{2+}]_i$ levels. After application of the agonist solution we observed increased fluorescence in SPNs expressing GCaMP3 (Fig. 2D), indicative of agonist-induced rising $[\text{Ca}^{2+}]_i$ levels. ROIs corresponding to responding cells and the time dependent changes in fluorescence intensity were measured and plotted as $\Delta F/F$ versus time for vehicle and LPS treated slices (Fig. 2E). The traces illustrating the agonist-induced effects were quantified for peak amplitude to capture increases in maximum fluorescence intensity. Representative examples of AMPA-stimulated peak fluorescence responses from individual slices are shown in Figure 2E with the cumulative response distributions illustrated in Figure 2F.

Effect of LPS treatment on SPNs

We initially tested three different concentrations of LPS (i.e., 125, 250, and 500 ng/ml). We found that the two-hour treatment of 500 ng/ml of LPS resulted in damaged slices as observed with DIC such that individual cells could not be identified (data not shown). Therefore, we omitted this concentration and more closely compared 125 ng/ml and 250 ng/ml LPS with vehicle treatment (Fig. 3A–C). Incubation of slices with 125 ng/ml of LPS did not appear to change the morphology of SPNs compared to the vehicle condition (Fig.

3A,B). However, 250 ng/ml of LPS caused morphologically appearing unhealthy SPNs with enlarged nuclei and membrane puckering (Fig. 3C, white arrows).

Further analyses included quantification of the number of cells with high basal fluorescence suggesting high $[Ca^{2+}]_i$ levels produced by cellular damage. As neuroinflammatory conditions can increase apoptosis due to increased $[Ca^{2+}]_i$ (Annunziato *et al.*, 2003), we reasoned that SPNs with high basal fluorescence were unhealthy and would be unresponsive to agonist stimulation (defined as a nonresponder). Indeed, 250 ng/ml LPS resulted in a significantly higher percent of D2 nonresponders (Fig. 3D top, 1-way ANOVA, $F_{(2,51)} = 10.07$, $P = 0.0083$) and cells with higher basal fluorescence (Fig. 3D bottom, 1-way ANOVA, $F_{(2,2355)} = 157.80$, $P < 0.0001$) when compared to vehicle treated D2 SPNs. Similarly, 250 ng/ml LPS also significantly increased the percent of D1 nonresponders (Fig. 3E top, 1-way ANOVA, $F_{(2,70)} = 4.816$, $P = 0.035$) and cells with higher basal fluorescence (Fig. 3E bottom, 1-way ANOVA, $F_{(2,326)} = 18.74$, $P < 0.0001$) when compared to vehicle treated D1 SPNs. In contrast, 125 ng/ml LPS did not result in a higher proportion of D2 or D1 nonresponders and did not increase basal fluorescence when compared to vehicle treated SPNs (Fig. 3D). Representative slices used for analyses are shown in Figure 3F–H. For example, treatment with 250 ng/ml LPS (Fig. 3H) leads to a greater proportion of SPNs being unresponsive to agonist stimulation with high basal fluorescence, while vehicle (Fig. 3F) and LPS 125 ng/ml treated (Fig. 3G) SPNs show relatively low basal fluorescence prior to agonist stimulation.

For further verification that treatment with the lower concentration of LPS does not change the morphology of SPNs, we fixed *drd2*- and *drd1-GCaMP3* slices following a two-hour incubation with vehicle or 125 ng/ml LPS. Confocal imaging demonstrated that the morphology of the processes and somas of D2 (Fig. 3I,J) and D1 SPNs (Fig. 3K,L) were intact and visibly unchanged due to LPS treatment. Therefore we selected 125 ng/ml of LPS as an ideal concentration given that it did not increase the percent of nonresponders or basal fluorescence of SPNs, nor did it result in discernable morphological changes when compared to vehicle.

LPS augments AMPA mediated $[Ca^{2+}]_i$ in D2 but not D1 SPNs

Because animal models of PD, HD, and HAND demonstrate increased neuroinflammation as well as disruptions in glutamate and calcium signaling, we sought to understand the functional consequences of selective CP-AMPA modulation in dorsal striatal SPNs when subjected to a proinflammatory environment. We studied the effects of glutamatergic-mediated calcium disruptions separately in D2 and D1 SPNs by using calcium imaging to assess changes in $[Ca^{2+}]_i$. To elicit the calcium response, the agonist cocktail included: AMPA, cyclothiazide, and CPP. We found that LPS treatment increased the peak amplitude of the AMPA-stimulated fluorescence response in D2 SPNs (Fig. 4A; Students *t*-test, $t_{(28)} = 5.234$, $P < 0.0001$). In contrast, LPS did not significantly change the peak amplitude of AMPA-stimulated fluorescence response in D1 SPNs (Fig. 4B; Students *t*-test, $t_{(15)} = 0.3808$, $P = 0.38$). These data suggest that LPS increases AMPA-stimulated $[Ca^{2+}]_i$ selectively in D2 SPNs.

To determine how much of the AMPA-stimulated calcium signal was through activation of SPNs, resulting in subsequent action potentials and neurotransmitter release, we blocked voltage-gated sodium (TTX) and calcium channels (CdCl_2). In contrast to findings in the absence of blockers, D2 SPNs in LPS treated slices demonstrated a decrease in the peak amplitude of the AMPA response with blockade of neuronal activity (Fig. 4C; Students t -test, $t_{(15)} = 9.478$, $P < 0.0001$). However, LPS treated D1 SPNs did not respond differently under the same conditions (Fig. 4D; Students t -test, $t_{(21)} = 0.6411$, $P = 0.53$).

The observation that combining CdCl_2 and TTX reversed the action of LPS in D2 SPNs, suggests a role for neuronal activation and possibly neurotransmitter release in the proinflammatory environment. To further explore this finding in D2 SPNs, we isolated the contributions of CdCl_2 and TTX separately to determine if they could attenuate the LPS-mediated potentiation of AMPA-stimulated $[\text{Ca}^{2+}]_i$. The inclusion of TTX alone did not reverse the LPS-mediated potentiation of the AMPA responses in D2 SPNs (Fig. 5A; Students t -test, $t_{(9)} = 7.89$, $P < 0.0001$), suggesting that the effect of LPS was not due to neuronal activation. We next tested AMPA-stimulated $[\text{Ca}^{2+}]_i$ in the presence of CdCl_2 alone in the agonist cocktail. The inclusion of CdCl_2 was sufficient to reverse the LPS-mediated potentiation of the AMPA responses in D2 SPNs (Fig. 5B; Students t -test, $t_{(13)} = 12.72$, $P < 0.0001$), suggesting that the potentiation of AMPA-stimulated responses with LPS treatment was dependent on VGCCs. Taken together these findings support a D2 SPN specific alteration of AMPA-stimulated $[\text{Ca}^{2+}]_i$ due to activation of VGCCs in inflammatory conditions.

Intriguingly, the peak amplitude of fluorescence responses in the vehicle treated slices was consistently higher in the experiments that combined TTX and CdCl_2 (Fig. 4C). This effect was significant in both D2 and D1 SPNs (Fig. 4A,C; $P < 0.0001$). We reasoned that this difference could be due to changes in the background (Fb), which would artificially inflate F/F. To assess if there was artificial inflation of F/F, we compared the backgrounds (Fb) of all analyzed slices from the two treatment variables (LPS or vehicle) in both experimental conditions (i.e., with or without TTX and CdCl_2). We found no effect of treatment (2-way ANOVA; $F(1,43) = 0.030$; $P = 0.86$) or experimental conditions (2-way ANOVA; $F(1,43) = 1.904$; $P = 0.17$) on the background fluorescence in slices prepared from *drd2-GCaMP3* mice (Fig. 5C). Similarly, there was no change in background fluorescence of *drd1-GCaMP3* slices (Fig. 5D) as a result of treatment (2-way ANOVA; $F(1,36) = 3.817$; $P = 0.06$) or experimental conditions (2-way ANOVA; $F(1,36) = 2.337$; $P = 0.14$). These data suggest that the changes in vehicle treated slices between the experimental conditions were a result of blocking the activation of VGCCs and/or voltage-gated sodium channels and will be investigated in future experiments.

Philanthotoxin blocks AMPA-stimulated Ca^{2+} response in D2 and D1 SPNs

We next investigated the role of CP-AMPA receptors in AMPA-stimulated $[\text{Ca}^{2+}]_i$ in proinflammatory conditions. We hypothesized that the AMPA response could be attenuated by the inclusion of the GluA1/CP-AMPA receptor blocker philanthotoxin with activity blockade. Pretreatment with philanthotoxin combined with TTX and CdCl_2 prior to AMPA cocktail exposure completely reduced the fluorescence responses in both D2 (Fig. 6A; 1-way

ANOVA, $F(3,51) = 302.4$, $P < 0.001$) and D1 SPNs (Fig. 6B; 1-way ANOVA, $F(3,69) = 224.9$, $P < 0.001$) regardless of the microenvironment (i.e., proinflammatory or control). These results strongly suggest that the calcium response elicited by the AMPA cocktail after activity blockade is mediated by CP-AMPA receptors because it could be completely blocked by the GluA1 antagonist. Specifically, the inclusion of TTX and CdCl₂ reveals the contribution of CP-AMPA receptors because the complete blockade of the calcium response with philanthotoxin indicates that there are not additional modes of AMPA-stimulated calcium entry. Figure 6 demonstrates that there is a decrease in AMPA-stimulated [Ca²⁺]_i through CP-AMPA receptors in D2 SPNs with LPS treatment. These data corroborate and extend previous findings (Lewitus et al., 2014) by showing that this effect is specific to D2 SPNs.

LPS potentiates AMPA-stimulated Ca²⁺influx through L-type voltage-gated calcium channels in D2 SPNs

As shown in Fig 4A, LPS potentiated AMPA-stimulated calcium entry in D2 SPNs. This calcium entry can be due to AMPA-mediated depolarization and activation VGCCs in SPNs (Perrier et al., 2002; Stanika et al., 2015) as well as direct permeation through CP-AMPA receptors. However, Figure 6A shows that LPS results in decreased calcium entry via CP-AMPA receptors in D2 SPNs, suggesting that the potentiation of AMPA-stimulated calcium responses was through VGCCs. We hypothesized that this potentiation was specifically through L-type VGCCs because they are implicated in striatal neurodegenerative disorders (Guzman et al., 2010; Ilijic et al., 2011; Surmeier et al., 2011; Surmeier et al., 2016), and blockade of L-type VGCCs *in vitro* protects against LPS-induced inflammation (Li et al., 2009). We tested this hypothesis using isradipine, a highly selective L-type VGCC blocker (Sinnegger-Brauns et al., 2009; Ilijic et al., 2011; Kang et al., 2012). As we lost the *drd2* mouse strain for these experiments, we used *adora-GCaMP6f* mice (Chen et al., 2013), where the GCaMP sensor is targeted to D2 SPNs based on the specificity of adenosine receptor A2a gene regulatory elements (Dobbs et al., 2016; Lemos et al., 2016). First, we conducted a series of experiments to test if similar responses to LPS could be seen in this strain of mice. Slices were prepared from *adora-GCaMP6f* mice and exposed to an AMPA cocktail in the absence of CdCl₂ and TTX. Similar to slices from *drd2-GCaMP3* mice (Fig. 4A), LPS treated slices prepared from *adora-GCaMP6f* mice demonstrated an increase in the peak amplitude of fluorescence response (Fig. 7A; Students *t*-test, $t_{(22)} = 4.479$, $P = 0.002$).

Next, we replicated the neuronal activity blockade experiments in the *adora-GCaMP6f* mice to assess whether D2 SPNs in these mice continue to respond in a similar fashion as *drd2-GCaMP3* SPNs. We observed that activity blockade reversed the LPS directed increase as shown by the reduction in peak amplitude fluorescence responses (Fig. 7B; Students *t*-test, $t_{(16)} = 6.076$, $P < 0.0001$). In addition, the different strains were statistically compared to validate our observations of the similarity in their responses. Indeed, we found that the *adora-GCaMP6f* mice responded comparably to the *drd2-GCaMP3* mice in both experimental conditions. Specifically, there was no significant difference between *adora-GCaMP6f* D2 SPNs and *drd2-GCaMP3* SPNs when exposed to AMPA-cocktail without TTX and CdCl₂ (Fig. 7C; 2-way ANOVA, $F_{(1,50)} = 3.788$, $P = 0.10$). The same outcome was detected in our experiments with TTX and CdCl₂ as we observed no difference between the two strains of mice with activity blockade (Fig. 7D; 2-way ANOVA, $F_{(1,31)} = 1.663$, $P =$

0.21). This formally supports our observation that *adora-GCaMP6f* mice respond similarly to the *drd2-GCaMP3* mice in our experimental conditions.

To gain more insights on the role of L-type VGCCs in the effect of LPS, we prepared slices from *adora-GCaMP6f* and *drd1-GCaMP6* mice and incubated them in LPS or vehicle. Calcium imaging was performed in the presence of the AMPA cocktail that now included TTX and the L-type VGCC blocker, isradipine. TTX was included to block neuronal activity in the slice induced by the AMPA cocktail. We observed an isradipine-mediated decrease of the peak amplitude of fluorescence response in D2 (Fig. 7E; Students t-test, $t_{(16)} = 5.31$, $P < 0.0001$) but not D1 SPNs (Fig. 7F; Students t-test, $t_{(11)} = 0.3456$, $P = 0.74$). These data support the proposal that L-type VGCCs play a role in AMPA-stimulated calcium influx selectively in dorsal striatal D2 SPNs under inflammatory conditions.

Discussion

Multiple neuronal processes in SPNs are regulated by calcium including synaptic strength, cellular excitability, and gene expression (Berridge *et al.*, 2000; West *et al.*, 2002). In addition, SPN firing can be modulated by calcium-activated potassium channels such that calcium flux from action potentials (AP) activates hyperpolarizing potassium channels to control the duration and intervals between APs (Abel *et al.*, 2004; Clements *et al.*, 2013). Synaptic plasticity in striatal SPN has also been shown to be calcium-dependent (Adermark and Lovinger, 2007). Therefore, alterations in calcium influx can have numerous impacts on neuronal function and activity. Furthermore there is evidence of altered $[Ca^{2+}]_i$ in striatal neurodegenerative disorders (Day *et al.*, 2006; Day *et al.*, 2008; Marambaud *et al.*, 2009; Miller and Bezprozvanny, 2010; Vigont *et al.*, 2014), which highlights the importance of investigating potential mechanisms of calcium disruptions in SPNs.

Because CP-AMPA receptors are retained in SPNs throughout adulthood (Bernard *et al.*, 1997; Cull-Candy *et al.*, 2006; Deng *et al.*, 2007), and because proinflammatory molecules like TNF α and NO can alter CP-AMPA trafficking (Albensi and Mattson, 2000; Beattie *et al.*, 2002; Stellwagen *et al.*, 2005; Lai *et al.*, 2006; Stellwagen and Malenka, 2006; Balosso *et al.*, 2009; Lee *et al.*, 2010; Lewitus *et al.*, 2014; Lewitus *et al.*, 2016), CP-AMPA receptors may play an important role in calcium regulation in proinflammatory conditions. Important work by Lewitus *et al.* (2014) demonstrated that the treatment of acute striatal slices with TNF α reduced the ratio of AMPA to NMDA current amplitudes as well as the expression of CP-AMPA receptors in dorsal striatal SPNs. Recently, using a cocaine sensitization model, they discovered a microglial-mediated reduction in AMPA currents which was specific to D1 SPNs in the nucleus accumbens (Lewitus *et al.*, 2016). Therefore the current work sought to elucidate the role of CP-AMPA receptors, VGCCs, and calcium signaling selectively in dorsal striatal D2 and D1 SPNs in a proinflammatory environment. To this end, we utilized GCaMP transgenic animals to assess for the first time calcium changes in dorsal striatal SPNs in acute striatal slices under inflammatory conditions. We observed that without blockade of neuronal activity, LPS potentiated AMPA-stimulated calcium responses in dorsal striatal D2 but not D1 SPNs. These results differ from those in Lewitus *et al.* (Lewitus *et al.*, 2016) perhaps due to brain region (i.e., dorsal versus ventral striatum) and / or the use of different LPS variants.

We next isolated the specific alterations of CP-AMPA and VGCCs as a consequence of LPS by conducting a series of experiments with selective blockers. We found that AMPAR activation of VGCCs appear to be the major targets of the LPS-mediated potentiation in AMPA-stimulated $[Ca^{2+}]_i$ in D2 SPNs. This was supported by the observation that a nonspecific VGCC channel blocker (i.e., $CdCl_2$) but not a voltage-gated sodium channel blocker (i.e., TTX), reversed the LPS-mediated increase in AMPA-stimulated $[Ca^{2+}]_i$. Notably, this effect was selective to D2 SPNs, which provides novel evidence that D2 SPNs in the dorsal striatal are preferentially sensitive in proinflammatory conditions. D2 SPNs have been reported to have higher GluR1/GluR2 ratios (Deng *et al.*, 2007), which could partly explain differential vulnerability in neurodegenerative diseases. In addition, because D2 SPNs are intrinsically more excitable (Day *et al.*, 2006; Day *et al.*, 2008) and because the slicing procedure can cause inflammation (Dzhala *et al.*, 2012), this may prime D2 SPNs to be additionally sensitive to a proinflammatory insult.

CP-AMPA is an important mechanism of AMPA-stimulated calcium entry that may underlie the alteration produced by the proinflammatory environment. Indeed, the CP-AMPA antagonist philanthotoxin, demonstrated that CP-AMPA is present in both D2 and D1 SPNs, as the $[Ca^{2+}]_i$ elicited by the AMPA cocktail could be completely blocked by this GluA1 antagonist in the presence of TTX and $CdCl_2$. However, we found that LPS decreased calcium influx through CP-AMPA, supporting previous work showing that a proinflammatory environment decreased expression and function of CP-AMPA in striatal SPNs (Lewitus *et al.*, 2014). Our study further extends these findings by showing preferential targeting of D2 SPNs as a consequence of LPS treatment. However, our results also suggest that CP-AMPA is not the mechanism underlying the LPS-mediated potentiation of AMPA-stimulated $[Ca^{2+}]_i$ with intact neuronal activity. Instead, we demonstrate that VGCCs are the main target.

In the striatum, there are several subtypes of VGCCs which can be blocked non-selectively with $CdCl_2$. VGCCs are present at presynaptic (Barral *et al.*, 2000) and postsynaptic (Akopian and Walsh, 2002; Olson *et al.*, 2005; Adermark and Lovinger, 2007; Stanika *et al.*, 2015) locations and have distinct functions. In particular, L-type VGCCs have been implicated in striatal neurodegenerative diseases (Guzman *et al.*, 2010; Ilijic *et al.*, 2011; Surmeier *et al.*, 2011; Surmeier *et al.*, 2016). For example, L-type VGCCs genes are altered in HAND patients (Gelman *et al.*, 2004) with evidence of an upregulation and over-activation in SPNs during HIV-infection (Hu, 2016). In support of this, L-type VGCC antagonists can prevent the excitotoxic effects of rising $[Ca^{2+}]_i$ due to HIV viral protein exposure (Lipton, 1991; Lannuzel *et al.*, 1995; Bonavia *et al.*, 2001). In addition, emerging evidence suggests that there is a protective role of blocking L-type VGCCs in animal models (Chan *et al.*, 2010) and in patients with PD (Ritz *et al.*, 2010; Parkinson Study, 2013). Thus, to gain further insights into the central role of L-type VGCCs in a proinflammatory environment, we examined the effect of isradipine, a specific L-type blocker. We found that isradipine had the same effect as cadmium in that it reversed the LPS-mediated potentiation of AMPA-stimulated $[Ca^{2+}]_i$ in D2 SPNs. This strongly implicates a role of L-type VGCCs in a proinflammatory environment. Furthermore, L-type VGCCs are also localized to microglia and VGCC blockers decrease microglia-mediated neurotoxicity (Hashioka *et al.*, 2012).

Neuronal L-type VGCCs can indirectly regulate synaptic transmission, alter membrane excitability, play an important role in mediating somatic calcium signaling, and can influence the timing of APs and EPSPs (Calin-Jageman and Lee, 2008; Striessnig *et al.*, 2014). In addition, L-type VGCCs are activated at lower negative membrane potentials, are responsive to EPSPs (Mermelstein *et al.*, 2000) and can contribute to striatal LTD (Adermark and Lovinger, 2007). Relevant to our work, SPNs maintain critical balances between their “up-states” and “down-states” (Surmeier *et al.*, 2007) and the frequency of up-states can induce upregulation (Carter and Sabatini, 2004) and activation of L-type VGCCs (Cooper and White, 2000). Importantly, dendritic calcium influx occurs in SPN up-states (Kerr and Plenz, 2002) suggesting that if D2 SPNs are driven toward more up-states, this would increase the likelihood of dendritic and somatic calcium influx. Moreover, the activation of L-type channels can induce CREB phosphorylation (Zhang *et al.*, 2006) and cause transcription of immediate early genes, which can turn on or off the intracellular processes necessary to protect neurons from proinflammatory insults. Our results do not exclude a possible role for second messengers. Furthermore, early activation of NMDA receptors can disrupt subsequent Ca^{2+} entry through VGCCs on SPNs in striatal neurodegenerative disorders (Raymond, 2017). Although beyond the scope of the present work, future experiments will examine SPN selectivity in Ca^{2+} entry via NMDA receptors following an inflammatory insult.

Interestingly, we observed higher peak amplitudes of fluorescence responses in both SPN subtypes in vehicle conditions that included TTX and CdCl_2 compared to vehicle conditions without activity blockade. This change was not seen in LPS conditions with or without activity blockade. We ensured that this effect in vehicle treated slices was not due to changes in background fluorescence between experimental conditions. Furthermore, we did not observe this effect in vehicle conditions with TTX alone but did observe it in vehicle conditions with CdCl_2 alone. This finding further supports a modification of VGCCs in a proinflammatory environment and suggests that protective mechanisms regulating VGCCs could be disrupted with LPS treatment. For example, rising $[\text{Ca}^{2+}]_i$ causes inactivation of VGCCs (Evans *et al.*, 2015), which could be altered in proinflammatory conditions through disrupted VGCCs phosphorylation (Oshiro *et al.*, 2004). In addition calcium entry in striatal neurons can induce release of a variety of neurotransmitters including GABA, which can inhibit calcium flux through VGCCs via activation of GABA_A and GABA_B receptors (Mayfield and Zahniser, 1993; Nisenbaum *et al.*, 1993; Smolders *et al.*, 1995; Barral *et al.*, 2000; Perrier *et al.*, 2002; Long *et al.*, 2009). Moreover, GABA_A receptor-mediated $[\text{Cl}^-]_i$ accumulation could induce cell swelling and alterations of $[\text{Ca}^{2+}]_i$. Lastly, VGCC blockers may modulate neuroinflammation by means of reducing oxidative stress (Surmeier *et al.*, 2010) and production of pro-inflammatory cytokines (Huang *et al.*, 2014). These possibilities and the mechanisms of disruption in proinflammatory environments should be investigated further in future studies.

There is also mounting evidence that D2 SPNs are the first affected in striatal neurodegenerative diseases. For example, it is well established that D2 SPNs are preferentially targeted first in HD (Bunner and Rebec, 2016). In addition, some models of PD suggest that D2 SPNs are more excitable as a function of dopamine depletion (Day *et al.*, 2006; Day *et al.*, 2008). HAND patients also demonstrate parkinsonian-like symptoms (Itoh

et al., 2000) and loss of D2 receptors in the striatum (Gelman *et al.*, 2006). Lastly, there are also indications that L-type VGCCs are affected in D2 SPNs in animal models of PD (Day *et al.*, 2006; Martella *et al.*, 2011).

Taken together, our work demonstrates that a proinflammatory environment is sufficient to induce changes in L-type VGCCs and CP-AMPA. Specifically, LPS potentiates AMPA-stimulated $[Ca^{2+}]_i$ through L-type VGCCs selectively in D2 SPNs. In addition, LPS decreases AMPA-stimulated $[Ca^{2+}]_i$ through CP-AMPA selectively in D2 SPNs. However, our findings may only reflect the initial alterations to a proinflammatory environment with additional compensatory changes occurring after chronic exposure as in the case of Huntington's disease (Raymond, 2017). Given that neuroinflammation is observed in striatal neurodegenerative disorders, our findings could suggest a mechanism of disruption to be explored in early disease models. This study further contributes to the field by combining neuronal calcium imaging and quantitative data of microglial morphology and dynamics of movement after LPS treatment. Importantly, our work is bolstered by the reproducibility of responses from striatopallidal SPNs tested in different strains of GCaMP-expressing mice. Most notably, our findings validate the idea that dorsal striatal D2 SPNs may be more vulnerable in proinflammatory conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by National Institutes of Health R01NS083410 (KMZ), 1F31NS092230 (CDW), 5T32NS041218 (CDW) and the Parkinson's and Movement Disorder Foundation (KMZ) with support from CONACyT of the Mexican Government (#381291 to ASR). We also thank Douglas Kim (Janelia Research Campus, HHMI) for helpful advice and a critical reading of the manuscript.

Abbreviations

aCSF	Artificial cerebral spinal fluid
Adora2a	Adenosine A2A receptor
AMPA	AMPA receptors
AP	Action potential
$[Ca^{2+}]_i$	Intracellular calcium
Ca^{2+}	Calcium
$CdCl_2$	Cadmium chloride
CP-AMPA	Calcium-permeable AMPARs
CPP	3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid
CX3CR1	Chemokine/fractalkine receptor 1

D1	Dopamine-1 receptor expressing
D2	Dopamine-2 receptor expressing
DIC	Differential interference contrast microscopy
FITC	Fluorescein isothiocyanate
GCaMP	Genetically encoded calcium indicator protein
GFP	Green fluorescent protein
GluA1	AMPA receptor subunit-1
GluA2	AMPA receptor subunit-2
HAND	HIV-associated dementia
HD	Huntington's disease
LPS	Lipopolysaccharide
NO	Nitric Oxide
PD	Parkinson's disease
PFA	Paraformaldehyde
PhTX	Philanthotoxin-74
PMT	Photomultiplier
ROI	Region of interest
SPNs	Spiny projection neurons (formally known as MSNs: medium spiny neurons)
TTX	Tetrodotoxin
VGCCs	Voltage-gated calcium channels

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Figure 2. Experimental timeline and validation of calcium imaging experiments

A) Schematic of the experimental set up and timeline used. *B)* Representative image of a corticostriatal slice (2×) with the y-tube drug delivery device (white arrow; AMPA cocktail). *C)* D2 SPNs at baseline without exogenous stimulation (20×) magnification. *D)* Increased fluorescence in GCaMP3 expressing D2 SPNs after stimulation (time = 30s) with AMPA cocktail (50 μM AMPA, 100 μM cyclothiazide, 50 μM CPP). Example somata are highlighted with white arrows. Calibration bar = 250 μm for (*B*) and 25 μm for (*C,D*). *E)* Representative traces of AMPA-stimulated calcium responses in vehicle (*left*) and LPS (*right*) treated D2 SPNs. The red line indicates the average of all responding cells in the vehicle (N = 101 cells) and LPS (N = 98 cells) conditions. *F)* Representative cumulative probability distributions of vehicle and LPS treated cells for peak amplitude AMPA-stimulated responses in D2 SPNs.

Figure 3. Dose response of LPS

A) An example of the morphology of SPNs with vehicle treatment with differential interference contrast microscopy (DIC) imaging. B) LPS 125 ng/ml does not result in morphological changes of SPNs as observed with DIC. C) LPS 250 ng/ml causes unhealthy appearing SPNs as is indicated by expanded nuclei and shrunken membranes (white arrows). Calibration bar = 15 μ m for (A–C). D) 250 ng/ml LPS causes significantly more non-responding D2 SPNs (*top*) with higher basal fluorescence of non-responding D2 SPNs (*bottom*), (** $P < 0.01$, **** $P < 0.0001$). $N = 3$ –5 animals per treatment and 95 slices. E) 250 ng/mL LPS causes significantly more non-responding D1 SPNs (*top*) with higher basal fluorescence (*bottom*), (* $P < 0.05$, **** $P < 0.0001$). $N = 3$ –5 animals per treatment condition and 77 slices. Values represent means $\pm$ S.E.M. Examples of vehicle treated (F), 125 ng/ml LPS treated (G), and 250 ng/ml treated (H) slices illustrating the proportion of D2 non-responding SPNs and their higher basal fluorescence. Calibration bar = 25 μ m (F–H). Slices were prepared from *drd2*- and *drd1*-GCaMP3 mice and treated with vehicle (I,K) or 125 ng/ml LPS (J,L) and fixed with 4% PFA for qualitative observations of general health and preservation of processes (100 $\times$). Blue = DAPI, Green = GFP. As demonstrated, fixation

of slices allows for visualization of GCaMP expression without immunohistochemistry.
Calibration bar = 60 μm (*I-L*).

Figure 4. LPS treatment increases AMPA mediated fluorescence in D2 SPNs

Corticostriatal coronal sections were pretreated with 125 ng/ml LPS or vehicle for two hours. D2 and D1 SPNs were stimulated with an AMPA cocktail (50 μ M AMPA, 100 μ M cyclothiazide, and 50 μ M CPP). *A*) Peak amplitude of fluorescence response in D2 SPNs (**** $P < 0.0001$). $N = 4$ animals per group and 30 slices. *B*) Peak amplitude of fluorescence response in D1 SPNs ($P = 0.38$). $N = 3$ animals per group and 16 slices. SPNs were pretreated via y-tube with activity blockers (+ 1 μ M TTX and 25 μ M CdCl₂) and subjected to delivery of the AMPA cocktail including activity blockers. *C*) Peak amplitude of fluorescence response in D2 SPNs (**** $P < 0.0001$). $N = 4$ animals per group and 17 slices. *D*) Peak amplitude of fluorescence response in D1 SPNs ($P = 0.53$). $N = 4$ animals per group and 23 slices.

Figure 5. LPS treatment increases AMPA mediated fluorescence in D2 SPNs via VGCCs
Corticostriatal coronal sections from *drd2-GCaMP3* mice were treated with 125 ng/ml LPS or vehicle for two hours. D2 SPNs were pretreated via y-tube with either TTX (1 μ M) or CdCl₂ (25 μ M) before being stimulated with the AMPA cocktail (50 μ M AMPA, 100 μ M cyclothiazide, and 50 μ M CPP). A) Peak amplitude of fluorescence response in D2 SPNs pretreated with TTX and stimulated with the AMPA cocktail + TTX (**** $P < 0.0001$), $N = 11$ slices. B) Peak amplitude of fluorescence responses in D2 SPNs pretreated with CdCl₂ and stimulated with the AMPA cocktail + CdCl₂ (**** $P < 0.0001$), $N = 3$ per group and 15 slices. C) Background fluorescence (F_b) of D2 SPNs does not differ between vehicle and

LPS treatment ($P = 0.86$) or experimental conditions (i.e., with or without TTX & CdCl_2); ($P = 0.17$). $N = 44$ slices. D) Background fluorescence (Fb) of D1 SPNs does not differ between treatment ($P = 0.06$) or experimental conditions ($P = 0.14$). $N = 4$ per group and 41 slices. F/F values represent means $\pm$ S.E.M.

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Figure 6. CP-AMPA receptors are present in SPNs and LPS selectively decreases AMPA-stimulated $[Ca^{2+}]_i$ through CP-AMPA receptors in D2 SPNs

Cortico-striatal coronal sections were treated with 125 ng/ml LPS or vehicle for two hours. D2 or D1 SPNs were pretreated via y-tube application with philanthotoxin-74 (300 μ M), TTX, and CdCl₂, before exposure to the AMPA cocktail + philanthotoxin, TTX, and CdCl₂. A) Peak amplitude of fluorescence response in D2 SPNs (**** $P < 0.0001$), $N = 3$ animals per group and 39 slices. B) Peak amplitude of fluorescence response in D1 SPNs (**** $P < 0.0001$), $N = 3$ animals per group and 49 slices.

Figure 7. L-type VGCCs mediate LPS induced changes in AMPA stimulated $[Ca^{2+}]_i$
 Corticostriatal coronal sections prepared from *adora-GCaMP6f* mice were treated with 125 ng/ml LPS or vehicle for two hours. D2 SPNs were subjected to delivery of AMPA cocktail. A) Peak amplitude of fluorescence response in D2 SPNs (*** $P < 0.001$), $N = 3$ animals per group and 24 slices. B) Peak amplitude of fluorescence response in D2 SPNs following pretreatment of activity blockers (+ 1 μ M TTX and 25 μ M CdCl₂); (**** $P < 0.0001$), $N = 3$ animals per group and 18 slices. C,D) Comparison of slices prepared from *drd2-GCaMP3* and *adora-GCaMP6f* mice demonstrate no significant differences in peak amplitude of AMPA-mediated responses without TTX or CdCl₂ (C) or in the presence of TTX and CdCl₂ (D).

(D). E) Slices were pretreated with isradipine (5 μ M) and TTX before exposure to the AMPA cocktail + isradipine. Peak amplitude of fluorescence response in D2 SPNs decreases with blockade of the L-type VGCCs (**** $P < 0.0001$), $N = 3$ animals per group and 18 slices. F) Peak amplitude of fluorescence response in D1 SPNs ($P = 0.74$), $N = 3$ animals per group and 13 slices. F/F values represent means $\pm$ S.E.M.