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Full Length Article

Dopamine activates NF-κB and primes the NLRP3 inflammasome in primary human macrophages

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ABSTRACT

Induction of innate immune genes in the brain is thought to be a major factor in the development of addiction to substances of abuse. As the major component of the innate immune system in the brain, aberrant activation of myeloid cells such as macrophages and microglia due to substance use may mediate neuroinflammation and contribute to the development of addiction. All addictive drugs modulate the dopaminergic system and our previous studies have identified dopamine as a pro-inflammatory modulator of macrophage function. However, the mechanism that mediates this effect is currently unknown. Inflammatory activation of macrophages and induction of cytokine production is often mediated by the transcription factor NF-κB, and prior studies have shown that dopamine can modulate NF-κB activity in T-cells and other non-immune cell lines. Here we demonstrated that dopamine can activate NF-κB in primary human macrophages, resulting in the induction of its downstream targets including the NLRP3 inflammasome and the inflammatory cytokine IL-1β. These data also indicate that dopamine primes but does not activate the NLRP3 inflammasome in human macrophages. Activation of NF-κB was required for dopamine-mediated increases in IL-1β, as an inhibitor of NF-κB was able to abrogate the effects of dopamine on production of these cytokines. Connecting an increase in extracellular dopamine to NF-κB activation and inflammation suggests specific intracellular targets that could be used to ameliorate the inflammatory impact of dopamine in neuroinflammatory conditions associated with myeloid cell activation such as addiction.

1. Introduction

Drug abuse is a major public health issue, with more than 250 million people around the world using illicit drugs in 2015 (UNODC, 2017). The use of psychostimulants, such as methamphetamine and cocaine, is widely prevalent and contributes substantially to this burden (Degenhardt et al., 2014; Favrod-Coune and Broers, 2010).In the US, In the US, around 1 in 5 individuals over the age of 12 used an illicit drug in 2018 (SAMHSA (2019)). The annual health costs associated with substance abuse are difficult to determine (Pacula et al., 2009), but are estimated to be tens of billions of dollars (NIDA, 2018). A substantial amount of this cost is due to the development of drug-associated health issues including neurologic complications and psychiatric problems (Favrod-Coune and Broers, 2010; Bachi et al., 2017; Glasner-Edwards and Mooney, 2014; Riezzo et al., 2012; Sanchez-Ramos, 2015; Whiteford et al., 2010). Chronic drug use leads to progressive changes in the brain and behavior that promote drug seeking and drug dependence via poorly understood mechanisms (Everitt et al., 2001; Hogarth et al., 2013; Volkow et al., 2016). Neuroplastic changes induced by addictive substances may be in part mediated by neuroinflammatory processes, as many addictive drugs are known to both initiate and exacerbate neuroinflammation (Clark et al., 2013; Kousik et al., 2012; Sekine et al., 2008). Immune activation has also been correlated with the development and maintenance of addiction, and induction of innate immune genes by addictive drugs in the frontal and limbic cortices may contribute to loss of behavioral control and negative limbic affect that supports continued substance dependence (Crews et al., 2011). Neuroinflammation has also been implicated in the pathophysiology of neuropsychiatric disorders including depression, anxiety, schizophrenia, and neurodegenerative disease (Brook et al., 2002; Fontenelle et al., 2011; Ross and Peselow,

Abbreviations: BBB, blood-brain barrier; BG, basal ganglia; DA, dopamine; DR, dopamine receptor; DAMP, damage-associated molecular pattern; hMDM, human monocyte derived-macrophages; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern; M-CSF, macrophage colony stimulating factor; NLRP3, nod-like receptor Family Pyrin Domain Containing 3; PBMC, peripheral blood mononuclear cells; TLR, toll-like receptor; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells.

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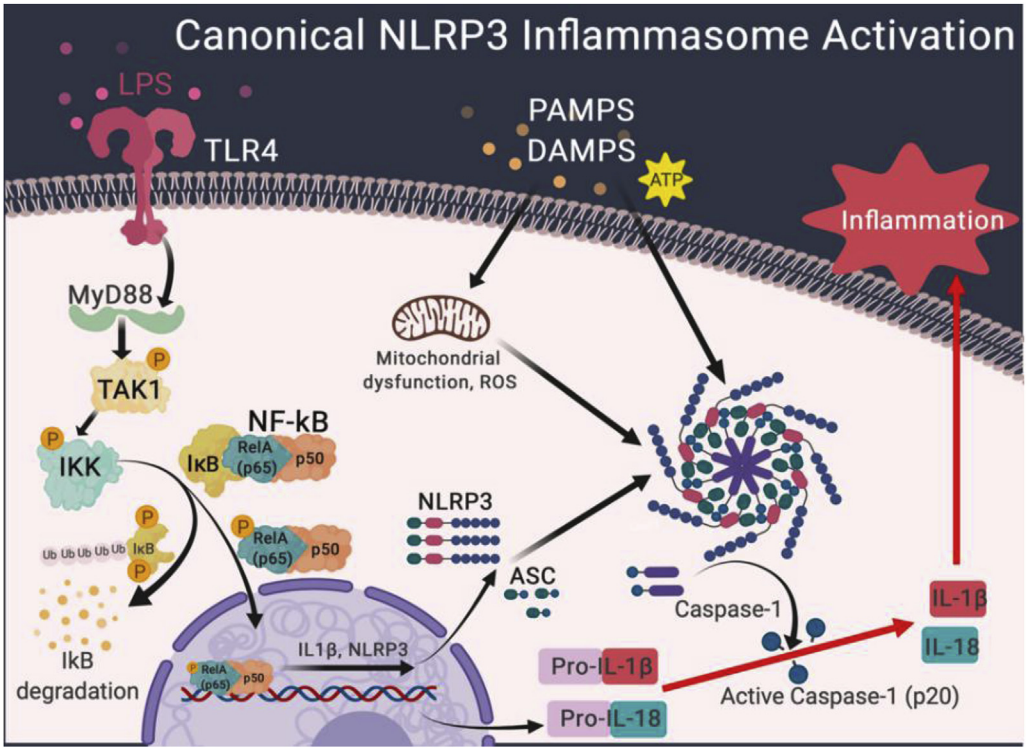

Fig. 1. The canonical activation pathway for the NLRP3 inflammasome. Activation starts with ligand binding to TLR4, which initiates a signaling cascade that releases NF-κB to translocate to the nucleus. Once in the nucleus, NF-κB mediates the transcription of NLRP3, ASC, pro-IL-1β, and pro-IL-18. Once these are transcribed, the inflammasome is considered primed. A second signal, from either a pattern or damage activated molecular pathogen (PAMPS or DAMPs), then initiates assembly of the inflammasome complex, which then activates caspase-1. Active caspase-1 then proteolytically cleaves the pro-forms of the cytokines, enabling release of the mature cytokines to promote inflammation.

2012; Callaghan et al., 2012; Curtin et al., 2015). Thus, substance-induced neuroinflammation may contribute to the development or aggravation of neuropsychiatric dysfunction that has been particularly associated with addiction to psychostimulant drugs (Fernandez-Serrano et al., 2012; Rusyniak, 2013; Taylor et al., 2013). A better understanding of the neuroinflammatory mechanisms associated with stimulant abuse is critical to develop more effective strategies to treat addiction, neuropsychiatric and neurodegenerative disorders, and inflammatory disease associated with substance abuse.

The precise mechanisms mediating increased neuroinflammation and immune activity associated with stimulant use are not clear. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a key mediator of innate immune gene expression, regulating the production of many inflammatory cytokines (Roulston et al., 1995; Brasier, 2010; Cui et al., 2014; Herman and Pasinetti, 2018). This protein has also been linked to numerous behavioral and cognitive pathologies including addiction (Nitkiewicz et al., 2017; Caviedes et al., 2017; Mattson and Camandola, 2001). An important link between these processes could be NF-κB controlled production of IL-1β, a master regulator of inflammation that is linked to neuronal injury as well as the development of behavioral and cognitive dysfunction in a variety of neurologic disorders (Brabers and Nottet, 2006; Burm et al., 2016; Festa et al., 2015; Lippai et al., 2013; Rizzo et al., 2018). Maturation and secretion of IL-1β and other IL-1 family cytokines is controlled by the multi-protein nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome complex in a two-step process requiring an initial "priming" step preceding a second activation step (Fig. 1). The priming step occurs in response to diverse stimuli, including Toll-like receptor (TLR) agonists such as LPS, leading to the transcription of inflammasome genes. The activation step occurs when the NLRP3 protein senses pathogen- or damage-associated molecular patterns (PAMPS or DAMPS) to trigger inflammasome assembly, autoproteolysis of caspase-1, and cleavage of pro-cytokines to their mature forms that are then released from the cell. This two-step pathway, which starts with NF-κB activation, maintains tight control over the release of the IL-1 cytokines in order to prevent unwanted inflammation (Herman and Pasinetti, 2018; He et al., 2016; Bauernfeind et al., 2009; Jo et al., 2016).

Activation of NF-κB is apparent in the brain following acute and chronic drug use, and is associated with increased cytokine expression and addictive behaviors in animal models (Lippai et al., 2013; Asanuma and Cadet, 1998; Mulligan et al., 2006; Okvist et al., 2007; Zhang et al., 2011a). Furthermore, animal studies have shown that inhibition of NF-κB reduces drug-associated neuroinflammation, neuroplasticity, and drug-seeking behavior (Periyasamy et al., 2018; Russo et al., 2009; Zhang et al., 2011b). In particular, these studies implicate brain myeloid cells as the primary effectors of drug-induced NF-κB activation. Psychostimulants in particular can impact innate immune signaling in the brain via activation of mononuclear phagocytes, such as microglia, perivascular macrophages, and monocytes (Clark et al., 2013; Sekine et al., 2008; Crews et al., 2011; Periyasamy et al., 2018; Du et al., 2019; Frank et al., 2016; Li et al., 2017; Liu et al., 2012; Atluri et al., 2016; Brown et al., 2018; Cearley et al., 2011; Nair et al., 2001; LaVoie et al., 2004; Little et al., 2009; Lopez-Pedrajas et al., 2015). Both cocaine and methamphetamine have been shown to activate these cells, resulting in increased inflammatory cytokine production in dopamine-rich regions such as the striatum, nucleus accumbens and ventral tegmental area following drug exposure (Frank et al., 2016; Brown et al., 2018; Cearley et al., 2011; Zhu et al., 2018a; Northcutt et al., 2015; Goncalves et al., 2017). These effects are not seen *ex vivo* in isolated microglia (Frank et al., 2016; Cearley et al., 2011), suggesting that endogenous CNS factors, such as neuronally released dopamine, are required to mediate the inflammatory effects of methamphetamine carried out by myeloid cells.

All stimulants exert their addictive effects via modulation of the dopaminergic system, and both cocaine and methamphetamine greatly increase CNS levels of dopamine, a key mediator of frontal and limbic lobe function (Del Arco and Mora, 2009). Emerging data suggests that dopamine also has an immunomodulatory role in brain, particularly via its effects on CNS myeloid cells, which express all five subtypes of dopamine receptors. Furthermore, exposing these cells to dopamine alters their immune function and can exacerbate the release of certain inflammatory mediators (Gaskill et al., 2009, 2012; Nickoloff-Bybel et al., 2019; Nolan et al., 2019; Mastroeni et al., 2009; Pinoli et al., 2017).

Thus, exposure to the concentrations of dopamine induced by stimulant use may mediate myeloid associated neuroinflammation by acting on NF-κB and the NLRP3 inflammasome. Our previous data support this hypothesis, demonstrating that dopamine increases the production of a number of NF-κB regulated cytokines, including IL-1β, in primary human macrophages (Nolan et al., 2019).

However, the increases in IL-1β were intracellular, and only minimal concentrations were detected in supernatant under any treatment condition, suggesting that dopamine initiated the first, or "priming" step in inflammasome activation, without activating the NLRP3 complex required for IL-1β secretions. To test this hypothesis, we used primary human monocyte-derived macrophages (hMDM) to examine the impact of dopamine on activation of NF-κB, transcription of NLRP3-related genes, and triggering of NLRP3 activation via caspase-1. Our data show that dopamine activates NF-κB and primes the inflammasome without triggering the final activation step needed to induce secretion of IL-1β. This effect was greater in hMDM from individuals infected with cytomegalovirus (CMV), suggesting that dopamine can additively increase inflammasome priming in individuals with pre-existing inflammation. Further, hMDM primed with dopamine had a more robust inflammatory response, releasing a larger amount of IL-1β from cells when stimulated with a second activating signal. Together, these data identify a key pathway that could mediate dopamine-induced activation of the innate immune system in the brain during psychostimulant abuse and suggest that these elevated dopamine levels could pre-dispose individuals to a more robust inflammatory response and negatively impact brain function.

2. Materials & methods

2.1. Reagents

RPMI-1640 medium and penicillin/streptomycin (P/S) were from Invitrogen (Carlsbad, CA, USA). LPS from E. Coli 055:B5, hydroxyethyl piperazineethanesulfonic acid (HEPES), β-mercaptoethanol, Tween 20 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fetal calf serum (FCS) and human AB serum were from Lonza (Basel, Switzerland). Macrophage colony stimulating factor (M- CSF) was from Peprotech (Rocky Hill, NJ, USA). TaqMan Master Mix, and PCR assay probes for IL-1β and NLRP3 genes and 18s were purchased from Applied Biosystems (Foster City, CA, USA). The NF-κB inhibitor, BAY 11–7082 (Selleckchem, Houston, TX) was diluted in DMSO and stored at −80 °C. Pre-treatment with BAY 11–7082 was performed for 45 min at a concentration of 10^{-5} M (Juliana et al., 2010; Lee et al., 2012), which was not cytotoxic in our hMDM cultures (Fig. 6C). Dopamine hydrochloride (DA) was also obtained from Sigma-Aldrich. Dopamine was resuspended in dH_2O at a stock concentration of 10 mM, and then aliquoted and frozen for two months or until use, whichever occurred first. New aliquots were prepared every two months. All dopamine preparations and treatments were performed in the dark, with dopamine treatment occurring immediately after aliquot thawing.

2.2. Cell isolation and culture

Human peripheral blood mononuclear cells (PBMC) were separated from blood obtained from de-identified healthy donors (New York Blood Center, Long Island City, New York) by Ficoll-Paque (GE Healthcare, Piscataway, NJ, USA) gradient centrifugation. After isolation, the percentage of monocytes in the PBMC was quantified using a monocyte isolation kit (MACS, Miltenyi Biotechnology) and PBMC's were plated at a density of approximately 1×10^5 monocytes/cm^2 to obtain a pure culture of human monocyte-derived macrophages (hMDM) via adherence isolation. PBMC were cultured in RPMI-1640 with 10% FBS, 5% human AB serum, 10 mM HEPES, 1% P/S, and M-CSF (10 ng/mL) for 3 days, washed with fresh media to remove non-adherent cells, and cultured another 3 days in fresh media containing M-CSF. After 6 days in culture cells are considered to be mature hMDM. All experiments were performed on day 6 or 7.

2.3. Quantitative RT-PCR

Total RNA was extracted from MDM using TRIzol™ reagent and chloroform extraction. Purity and concentration of RNA were determined using a NanodropOne spectrophotometer (Nanodrop technologies, Wilmington, DE, USA). Synthesis of cDNA was performed on 500 ng of RNA from each donor using the High Capacity cDNA Reverse Transcriptase synthesis kit (Applied Biosystems, Foster City, CA). Taqman 20x gene expression assays for IL-1β (Hs01555410_m1) and NLRP3 (Hs00918082_m1) (Thermo Fisher Scientific, Waltham, MA) were used to determine gene expression levels. Analysis of 18S using a Taqman 20x gene expression assay (Hs99999901_s1, Thermo Fisher Scientific, Waltham, MA) was used as an internal control. Gene expression levels were expressed as $2^{-\Delta Ct}$ where $\Delta C_T = [C_T \text{ (sample)} - C_T \text{ (housekeeping gene)}]$. Human Brain Total RNA was used as a positive control (Life Technologies, Carlsbad, CA) and samples containing no cDNA served as the negative controls. Samples with a C_T value above 37 were considered to have no amplification. Taqman primers were validated using 5-point standard curves to assure optimal amplification efficiency.

2.4. Western blotting

Primary human MDM cultured at 1×10^5 cells per cm^2 were incubated with dopamine (10^{-6} M or 10^{-8}) or the positive control LPS (10 ng/mL) for the indicated timepoint. At the indicated timepoints, cells were washed in 1X PBS and lysed with M-PER mammalian protein extraction reagent (Thermo Fisher Scientific, Waltham, MA), containing 1% Halt Protease and Phosphatase Inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA). After lysing, cells were sonicated with a Q125 sonicator (Qsonica, Newtown, CT) at 25% power for 5 s and then spun down at 13,000 RPM for 10 min at 4 °C. To generate nuclear and cytoplasmic extracts, hMDM were washed in 1X PBS and lysed with NE-PER Nuclear and Cytoplasmic Extraction Kit according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA), in the presence of 1% Halt Protease and Phosphatase Inhibitor cocktails (Thermo Fisher Scientific, Waltham, MA). Whole cell lysates, nuclear and cytoplasmic fractions were stored at 4 °C until the protein quantification by Bicinchoninic acid assay (BCA) using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Whole cell lysates were then diluted to a concentration of 1–2.5 μg/mL, while nuclear and cytoplasmic fractions were left neat. All lysates were stored at −80 °C until analyzed by Western blot.

Protein lysates were run on Bolt Bis-Tris Plus 10% precast gels (Life Technologies, Carlsbad CA) in MOPS/SDS running buffer in a mini gel tank (Life Technologies) for 90 min at 100 V to examine levels of NF-κB, IκB, H3 and GAPDH. Proteins were then transferred onto an Immobilon PVDF membrane from EMD Millipore (Temecula, CA) at 25 V for 60 min. For analysis of NLRP3, proteins were run at 100 V for 1 h and transferred at 90 V for 2 h. Total protein stain was performed using Revert Total Protein Stain (LI-COR Biosciences, Lincoln, NE) according to the manufacturer's instructions. After imaging and removing the total protein stain, membranes were then blocked in 50% Odyssey Blocking Buffer (TBS) for 1 h (Licor Biosciences, Lincoln, NE) prior to overnight incubation at 4 °C with the appropriate primary antibody. Following primary incubation, blots were washed in TBS-0.1% Tween. Blots were stained with IRDye 800CW Donkey Anti-Rabbit or Anti-Mouse IgG secondary antibody diluted in secondary antibody solution (TBS-0.2% Tween + SDS 0.01%) at a 1:20,000 dilution and membranes were incubated in the dark at room temperature for 1 h.

Membranes were then washed and imaged using the Odyssey Fc Imaging System (Licor Biosciences, Lincoln, NE). Blots were analyzed using Image Studio Lite (Licor Biosciences, Lincoln, NE). Target bands were normalized to the loading control GAPDH in cytoplasmic fractions,

Table 1
Parameters for Colocalization using High Content Analysis of Immunocytochemistry.

Condition	Value
Ch1: Smoothing	1
Ch1: Thresholding (Fixed)	600
Object.Ch1.Area.Ch1	120.009–800.588
Object.Ch1.Avg.Intensity.Ch1	501.49–6000
Ch2: Smoothing	2
Ch2: Thresholding (Fixed)	600
Ch2: Segmentation (Intensity)	100
Object.Ch1.Area.Ch2	22.722–7255.283
Object.Ch1.Avg.Intensity.Ch2	200–2800
Object.Ch1.Var.Intensity.Ch2	0–1750
Ch3: Smoothing	1
Ch3: Thresholding (Fixed)	550
Ch3: Segmentation (Shape)	2
Object.Ch1.Area.Ch3	1.649–3328.196
Object.Ch1.Avg.Intensity.Ch3	50–6000
Object.Ch1.Total.Intensity.Ch3	0–140000
ROIA.Mask.Ch	Channel 1 (DAPI)
ROIA.TargetI	Channel 3 (NF-κB)

H3 in nuclear fractions, and to total protein stain in whole-cell lysates. Each condition was compared to the untreated control to determine fold-change in expression or phosphorylation. Antibodies from Cell Signaling Technology (CST, Danvers, MA) were phospho–NF–κB p65 (Ser536, CST3033, 1:1000), NF-κB p65 (CST8242, 1:1000), IκB (CST4814, 1:1000), NLRP3 (CST15101, 1:1000), cleaved caspase-1 (CST4199, 1:1000), total caspase-1 (p50, CST3866, 1:1000), and H3 (CST4499, 1:2000). GAPDH (Santa Cruz Biotechnology, Dallas, TX, sc-47724) was used at a concentration of 1:5000.

2.5. Immunocytochemical staining

Human macrophages were cultured in Nunc™ MicroWell™ 96-well optical-bottom plates (Thermo Fisher Scientific, Waltham, MA) and treated with dopamine (10^{-6} M) for 1 h (NF-κB experiments) or 24 h (IL-1β experiments), fixed with 4% paraformaldehyde for 10 min and permeabilized with 0.1% Triton X-100 in PBS for 5 min. Treatment with LPS (10 ng/mL) for 1 h or 24 h was used as a positive control. Cells were blocked for 30 min in 1% BSA and 300 mM glycine in 0.1% Tween-20 in PBS. Primary antibodies used were mouse monoclonal CD68 (Thermo Fisher, KP1, MA5-13324, 1:400), rabbit monoclonal NF-κB (CST8242, 1:400), and rabbit polyclonal IL-1β (Abcam, ab9722 1:100) diluted in blocking solution and incubated at 4 °C overnight. Alexa Fluor 488 goat anti-rabbit and Alexa Fluor 546 goat anti-mouse secondary antibodies (Thermo Fisher, 1:1000) were used for detection, and nuclei were stained with DAPI (0.2 μg/mL). Images were acquired on a Cell Insight CX7 automated 7-channel confocal scanning microscope and analyzed using the HCS studio software and the Cellomics Colocalization bio-application (Cellomics, ThermoFisher, Pittsburgh, PA). 12 field images were taken per well using a 40x objective with 2–4 wells per condition (30 total wells, 360 total fields). Images were acquired with an exposure time of 0.1 s, with a smart focus search range of 352 μm and intra-well autofocusing at every field.

Each image went through background correction (manual, donor-specific levels alterations in Image Settings). Objects were identified using smoothing, thresholding, and segmentation. DAPI served as a nuclear stain objects identified by DAPI were gated using area and fluorescence intensity (Object.Ch1.Area.Ch1 and Object.Ch1.AvgIntensity.Ch1). The CD68 fluorescent marker identified MDM based on fluorescence intensity (Object.Ch1.AvgIntensity.Ch2 and Object.Ch1.Var.Intensity.Ch2) and an area-based mask modification (Object.Ch1.Area.Ch2). NF-κB staining in Channel 3 was masked using area and intensity (Object.Ch1.-Area.Ch3, Object.Ch1.AvgIntensity.Ch3, and Object.Ch1.Total.Intensity.Ch3). Parameters used for each condition are described in Table 1. After each condition had been scanned, the colocalization of NF-κB (Ch 3) and DAPI (Ch 1) was quantified using (Mean_ROI_A_Target_I_Object_Count). Counts were normalized from the 2–4 fold increase from untreated to LPS treated (10 ng/m) groups for each donor based on Western blot data (Fig. 4).

Table 2
Parameters for Live Dead Assay using High Content Analysis of Immunocytochemistry.

Condition	Value
Ch1: Smoothing	1
Ch1: Thresholding (Isodata)	−0.808
Ch1: Segmentation (Shape)	2
Object.Ch1.Area.Ch1	0–1327.17
Object.Ch1.Avg.Intensity.Ch1	33.94–1952.94
Ch2: Smoothing	3
Ch2: Thresholding (Isodata)	−0.08
Ch2: Segmentation (Shape)	3
Object.Ch1.Area.Ch2	0–353.02
Object.Ch1.Avg.Intensity.Ch2	268.58–5652.18
ROIA.Mask.Ch	Channel 1 (Live Cells)
ROIA.TargetI	Channel 2 (Cell Death)

2.6. Live dead assay

hMDM cultured in Nunc™ MicroWell™ 96-well optical-bottom plates (Thermo Fisher Scientific, Waltham, MA) were treated with vehicle or BAY 11–7082 at 10^{-4}-10^{-7} M for 24 h. As a positive control, 3 wells were treated with 100% methanol for 30 min prior to analysis for cell viability. Cell viability was then determined using the LIVE/DEAD Viability/Cytotoxicity Kit (Thermo Fisher Scientific, Waltham, MA) modified for 96-well plates according to the manufacturer's instructions. Images were acquired on a Cell Insight CX7 automated 7-channel confocal scanning microscope and analyzed using the HCS Studio software and the Cellomics Colocalization bio-application. Three field images were taken per well at a 10x objective with 3 wells per condition (6 total wells, 180 total fields). All images were taken with an exposure time of 6.5139 s and a smart focus search range of 198 μm. At every field change there was intra-well autofocusing.

Each image went through background correction (manual, donor-specific Levels alterations in Image Settings). Cells were identified using smoothing, thresholding, and segmentation via Channel 1. Channel 1 served as a live-cell stain, and objects were gated using area and fluorescence intensity (Object.Ch1.Area.Ch1 and Object.Ch1.AvgIntensity.Ch1). The second channel identified cell death and was gated based on fluorescence intensity (Object.Ch1.AvgIntensity.Ch2) and area (Object.Ch1.-Area.Ch2). The colocalization of the area of cell death (Channel 2) and live cells (Channel 1) was quantified using Mean_ROI_A_Target_I_%OverlapArea, with ROIA identified as channel 1 and Target 1 as Channel 2 (Table 2). These percentages enumerated the percentage of cells dying upon image.

2.7. Cytokine and Caspase-1 secretion

Primary hMDM cultured at 1×10^5 cells per cm^2 in 48-well plates (BD Falcon) were incubated for 24 h with dopamine (10^{-6} M) or the positive control LPS (1 ng/mL) prior to the collection of supernatants and lysates to examine IL-1β production. At 30 min prior to this collection, some plates were stimulated with ATP (2.5 mM) to induce inflammasome activation, and supernatants were collected for the analysis of active caspase-1 and IL-1β secretion. For NF-κB inhibition assays, cells were pretreated for 45 min with BAY 11–7082 (10^{-5} M) prior to dopamine or LPS treatment. After 24 h incubation with dopamine or LPS in the presence of BAY 11–7082, supernatants and lysates were collected for the analysis of IL-1β production. Cytokine concentration was quantified via AlphaLISAs performed according to the manufacturer's protocol (PerkinElmer). The limit of detection was 0.6 pg/mL for IL-1β for AlphaLISA. Caspase-1 concentration in supernatants was determined via

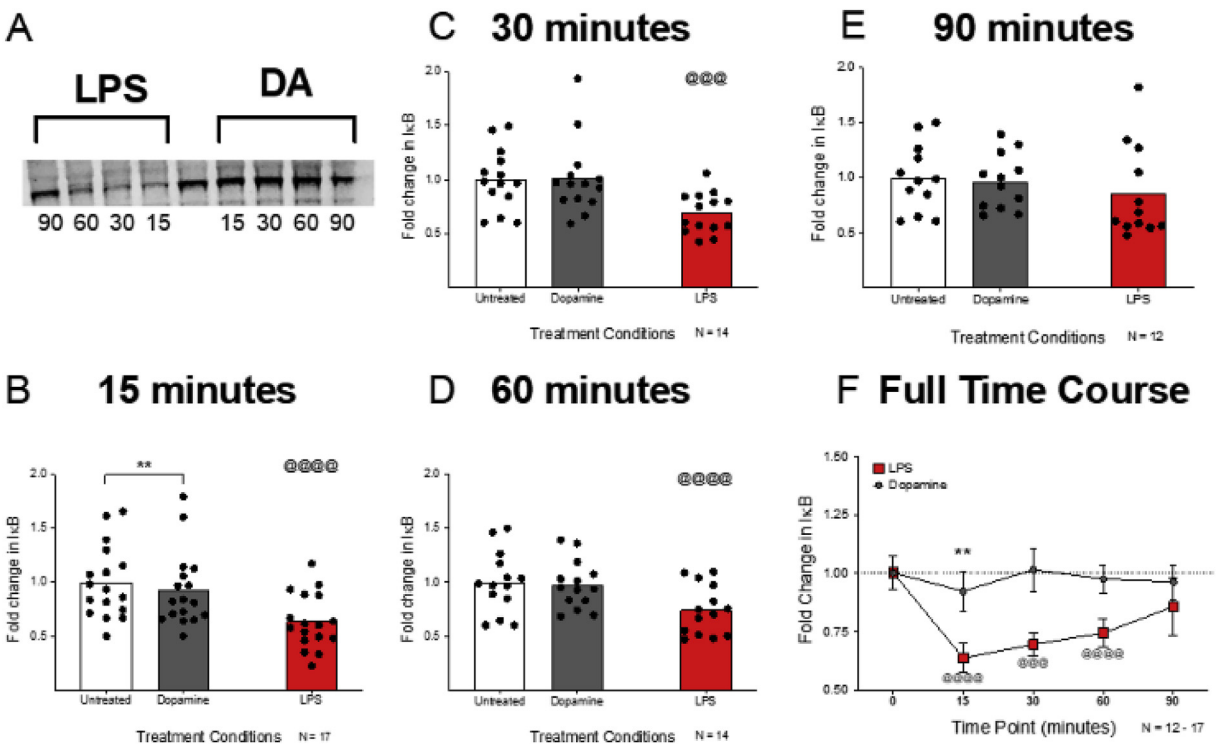

Fig. 2. Dopamine decreases IκB levels transiently in cytoplasmic lysates at 15 min(2A) Representative Western blot of IκB protein level after treatment with either LPS (10 ng/mL) or DA (10^{-6} M) for 15, 30, 60 or 90 min, compared to untreated condition (center) in MDM cells. 2B-E The fold change in IκB protein level is shown at 15, 30, 60 and 90 min for untreated, dopamine-treated conditions [Dopamine, Paired t-tests, n = 12–17, - 15 min, **p = 0.0044, t = 3.312, df = 16; 30 min, p = 0.8289, t = 0.2205, df = 13; 60 min, p = 0.4342, t = 0.8070, df = 13; 90 min, p = 0.4038, t = 0.8683, df = 11] and LPS-treated conditions [LPS, Paired t-tests, n = 12–17 -15 min, @@@@ p > 0.0001, t = 6.512, df = 16; 30 min, @@@ p = 0.0002, t = 5.010, df = 13; 60 min, @@@@ p > 0.0001, t = 5.541, df = 13; 90 min, p = 0.0885, t = 1.869, df = 11]. 2F Graphical representation of fold change in IκB over time at 0, 15, 30, 60 and 90 min, which demonstrates that decrease in IκB in the cytosol at 15 min following dopamine treatment compared to the untreated condition.

Quantikine® ELISA performed according to the manufacturer's protocol, and the limit of detection for this assay was 0.68 pg/mL (R&D systems, Minneapolis, MN).

2.8. Statistical analysis

Statistical analysis was performed using Prism 8.1 (Graphpad, La Jolla, CA, USA), with a $p < 0.05$ considered significant. Prior to analysis, all data were normalized to the mean of the vehicle treated condition. While gene expression data are displayed as $2^{-\Delta\Delta C}{}_T$, all statistical analysis of gene expression data was performed on data normalized to $2^{-\Delta C}{}_T$. To determine the appropriate statistical test for each data set, data were evaluated by analysis of skewness and evaluation of normality and lognormality to determine the distribution of the data. *Post-hoc* analyses were performed when appropriate. While LPS-mediated effects are shown on the same graph as dopamine-mediated changes, LPS was used as a positive control and therefore was not included in the same analyses as those analyzing the impact of dopamine in most figures. The separate tests performed on the LPS-treated samples are denoted by the use of the @ sign, rather than the * used to show significance in the analyses of dopamine-mediated changes.

3. Results

3.1. Dopamine activates NF-κB

Previous work from our lab shows that dopamine treatment increases the production of a number of cytokines and chemokines such as IL-6, IL-1β, IL-18, CCL2, and CXCL8 (Gaskill et al., 2012; Nolan et al., 2019). Transcription of all of these cytokines can be regulated by NF-κB, but the effects of dopamine on this factor are not clear. There are a number of distinct pathways that mediate activation of NF-κB, but all of these pathways require the phosphorylation and nuclear translocation of the RelA (p65) protein in the NF-κB complex. In most pathways, the degradation of the NF-κB inhibitor (IκB) precedes changes in p65 [72]. To better examine the mechanism underlying dopamine regulation of inflammatory cytokine production, hMDM were evaluated for dopamine-mediated changes in the cytoplasmic concentration of IκB and the phosphorylation of cytoplasmic p65 at serine 536. Dopamine induced changes in the nuclear and cytoplasmic concentrations of p65 were also examined. The MDM were exposed to a concentration of dopamine (10^{-6} M) equivalent to that which could be expected in the brain during cocaine or methamphetamine use (Matt and Gaskill, 2019a), for 15, 30, 60 or 90 min. At the indicated time-point, nuclear and cytoplasmic extracts were generated and examined by Western blot to quantify changes in distinct aspects of NF-κB activation pathway. MDM were also treated with LPS (10 ng/mL), a TLR4 agonist known to activate NF-κB (Chow et al., 1999; Zhang and Ghosh, 2000), as a positive control.

Analysis of IκB levels in cytoplasmic lysates demonstrated a slight but significant decrease in IκB at the 15-min dopamine timepoint, with no significant changes at 30, 60 or 90 min [Fig. 2, Dopamine, Paired t-tests, n = 12–17, - 15 min, **p = 0.0044, t = 3.312, df = 16; 30 min, p = 0.8289, t = 0.2205, df = 13; 60 min, p = 0.4342, t = 0.8070, df = 13; 90 min, p = 0.4038, t = 0.8683, df = 11]. A representative Western blot demonstrating these changes at 15 min is shown (Fig. 2A). This is in contrast to the effect of the positive control, LPS, which decreased cytoplasmic IκB at 15, 30 and 60 min [Fig. 2, LPS, Paired t-tests, n = 12–17 -15 min, @@@@ p > 0.0001, t = 6.512, df = 16; 30 min, @@@ p = 0.0002, t = 5.010, df = 13; 60 min, @@@@ p > 0.0001, t = 5.541, df = 13; 90 min, p = 0.0885, t = 1.869, df = 11]. Thus, dopamine and LPS treatment resulted in different patterns of IκB degradation, with dopamine causing a slight, transient decrease in cytoplasmic IκB, while the

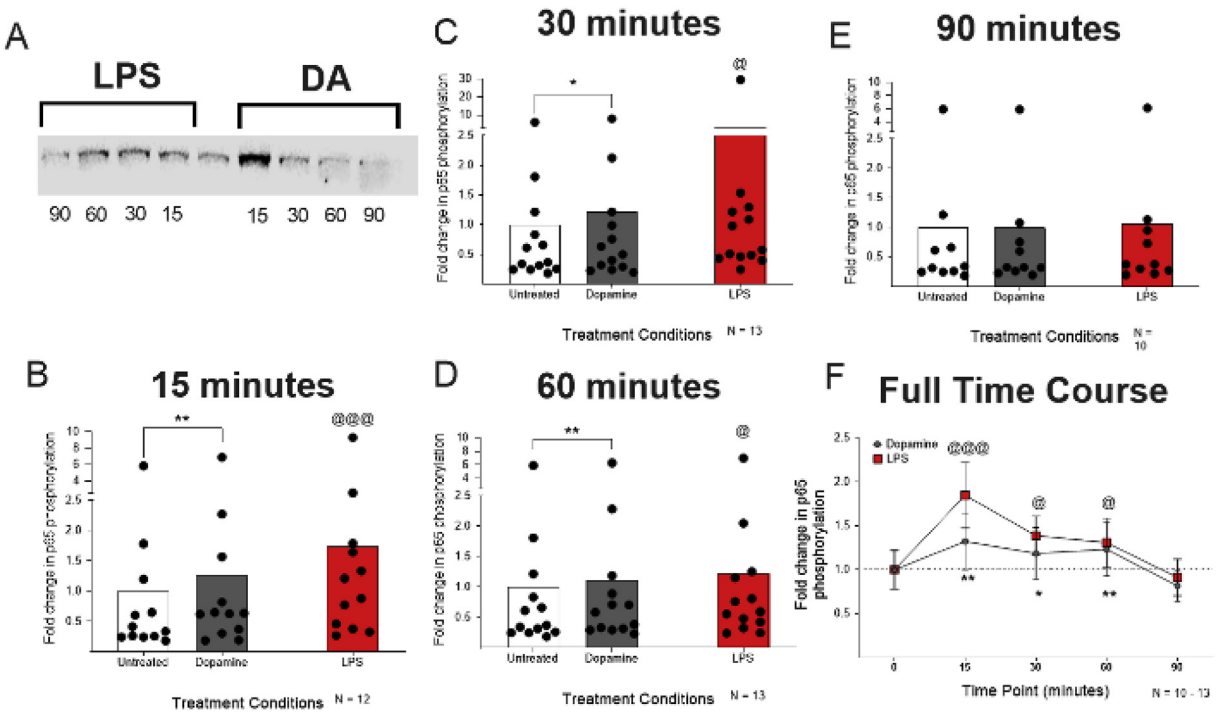

Fig. 3. Dopamine increases phosphorylated p65 levels at 15, 30 and 60 min 3A Representative Western blot of p-p65 protein level after treatment with either LPS (10 ng/mL) or DA (10^{-6}) treatment for either 15, 30, 60 or 90 min, compared to untreated condition (center) in MDM cells. 2B-E The fold change in p-p65 level is shown at 15, 30, 60 and 90 min for untreated, dopamine-treated and LPS-treated conditions [Wilcoxon tests, n = 10–13; 15 min, DA, **p = 0.0049, sum of (+,-) ranks 63, -3, LPS, @@@ p = 0.0005, sum of (+,-) ranks 78, 0; 30 min, DA, *p = 0.0479, sum of (+,-) ranks 74, -17, LPS, @ p = 0.0266, sum of (+,-) ranks 77,-14; DA, **p = 0.0024, sum of (+,-) ranks 86, -5, LPS, @ p = 0.0215, sum of (+,-) ranks 78, -13; DA, p = 0.7695, sum of (+,-) ranks 24, -31, LPS, p = 0.1309, sum of (+,-) ranks 43, -12]. 2F Graphical representation of fold change in p-p65 at 0, 15, 30, 60 and 90 min, showing an increase in nuclear p65 following dopamine treatment at 15, 30 and 60 min.

decrease in response to LPS was more robust and sustained.

Degradation of IκB releases the NF-κB p65 subunit, enabling the phosphorylation of this protein (Karin and Ben-Neriah, 2000). Therefore, p65 phosphorylation should occur rapidly following loss of IκB. As expected based on the kinetics of IκB degradation, both dopamine and LPS significantly increased phosphorylation of the NF-κB p65 subunit at 15, 30 and 60 min. However, the pattern of activation was distinct, with LPS showing a greater, more sustained phosphorylation and dopamine showing a smaller, more transient effect [Fig. 3, Wilcoxon tests, n = 10–13; 15 min, DA, **p = 0.0049, sum of (+,-) ranks 63, -3, LPS, $^{@@@}$ p = 0.0005, sum of (+,-) ranks 78, 0; 30 min, DA, *p = 0.0479, sum of (+,-) ranks 74, -17, LPS, $^{@}$ p = 0.0266, sum of (+,-) ranks 77,-14; 60 min, DA, **p = 0.0024, sum of (+,-) ranks 86, -5, LPS, $^{@}$ p = 0.0215, sum of (+,-) ranks 78, -13; 90 min, DA, p = 0.7695, sum of (+,-) ranks 24, -31, LPS, p = 0.1309, sum of (+,-) ranks 43, -12]. A representative Western blot demonstrating these changes at 15 min is shown (Fig. 3A). Phosphorylation of p65 precedes NF-κB translocation into the nucleus, so we examined the amount of NF-κB in cytoplasmic and nuclear fractions after 60 min of dopamine treatment. As expected, both dopamine and LPS significantly increased the amount of nuclear p65 after 60 min [Fig. 4A and B, Wilcoxon test, n = 19; DA, **p = 0.0020, sum of (+,-) ranks 168, -22, LPS, $^{@@@}$ p = 0.0002, sum of (+,-) ranks 179, -11]. These increases in nuclear p65 were accompanied by decreases in cytoplasmic p65 [Fig. 4C and D, Paired t-tests, n = 9; DA, *p = 0.0268, t = 2.706, df = 8, LPS, $^{@@}$ p = 0.0053, t = 3.796, df = 8] indicating nuclear translocation of NF-κB.

3.2. Dopamine increases NF-κB nuclear translocation

To confirm that dopamine activates NF-κB, and to more precisely examine how dopamine affects p65 nuclear expression, MDM were treated with dopamine or LPS for 60 min, stained for NF-κB (green), cell nuclei (DAPI, blue) and the macrophage marker CD68 (red) and examined using high content fluorescent microscopy (Fig. 5A). To specifically analyze NF-κB present within the nucleus, we generated a nuclear mask based on the DAPI stain and analyzed the average intensity of NF-κB staining in the nuclei of all MDM in each population. Between 1000 and 2000 cells were analyzed in each well, with 2–4 wells per treatment (Fig. 5A, white arrows indicating nuclear NF-κB staining). Analysis of the average intensity confirmed our Western blotting data, showing that both dopamine and LPS significantly increased the amount of nuclear NF-κB expression, although the dopamine mediated increase was not of the same magnitude as seen by Western Blot [Fig. 5B, n = 12, paired *t*-test; DA, **p = 0.0071, t = 3.302, df = 11; LPS **p = 0.0029, t = 3.801, df = 11]. Visual examination of the differently treated populations indicates that the increase in nuclear NF-κB does not occur evenly across the population, suggesting that the discrepancy in the magnitude of the dopamine effect could be due to different effects within the population. To examine this discrepancy, the percentage of hMDM in the untreated and dopamine-treated population in which there were high levels of nuclear NF-κB was evaluated. The level of nuclear NF-κB considered high was determined by setting an intensity threshold based on the LPS response as described in the Methods. Analyses show that dopamine treatment significantly increases the percentage of hMDM in which there are high levels of nuclear NF-κB [Fig. 5C, Wilcoxon test, n = 12, ***p = 0.001, sum of (+,-) ranks 77, -1], indicating that dopamine mediates its effects by increasing the nuclear NF-κB in a subpopulation of hMDM rather than through effects on the entire population.

To examine the potential clinical impact of dopamine on disease-associated inflammation, the hMDM from the donors analyzed in Fig. 5C were divided into two groups that were either positive or negative for cytomegalovirus (CMV). This virus is common in the adult population, and induces systemic inflammation (Pawelec, 2014; Yurochko and Huang, 1999) through activation of NF-κB (Hiscott et al., 2001; DeMeritt

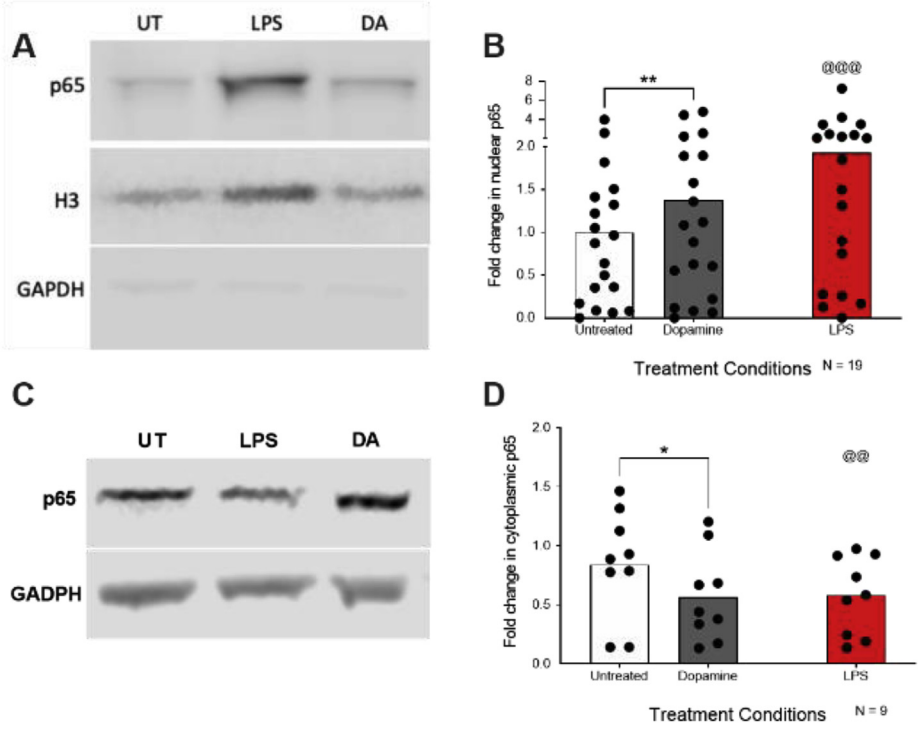

Fig. 4. Dopamine increases nuclear and decreases cytoplasmic p65 levels at 60 min 4A A representative Western blot of nuclear p65 protein levels in MDM after 60 min of treatment with either LPS (10 ng/mL) or Dopamine (10^{-6} M), compared to untreated MDMs. 4B Fold change of nuclear p65 in either untreated, dopamine-treated or LPS-treated MDMs for 60 min [Fig. 4A and B, Wilcoxen test, n = 19; DA, **p = 0.0020, sum of (+,-) ranks 168, -22, LPS, @@@ p = 0.0002, sum of (+,-) ranks 179, -11]. 4C A representative Western blot of cytoplasmic p65 protein levels in MDM after 60 min of treatment with either LPS or Dopamine, compared to untreated MDMs. 4D Fold change of cytoplasmic p65 in either untreated, dopamine-treated or LPS-treated MDMs for 60 min [Fig. 4C and D, Paired t-tests, n = 9; DA, *p = 0.0268, t = 2.706, df = 8, LPS, @@ p = 0.0053, t = 3.796, df = 8]. Taken together, these figures demonstrate that at 60 min, nuclear p65 levels are increased while cytoplasmic p65 levels are decreased.

et al., 2004). These groups were then reanalyzed for the interaction of dopamine on NF-κB in hMDM from donors with and without CMV. Reanalysis showed a significant main effect of both dopamine and CMV on NF-κB nuclear translocation, with no interaction between these conditions [Fig. 5E, mixed effects model (REML), n = 7 without CMV, n = 5 with CMV; CMV, **p = 0.0098, F(1,10) = 10.13; DA, **p = 0.0027, F (1,10) = 15.58]. Taken together, these results demonstrate that dopamine significantly increases the activation of NF-κB by increasing the

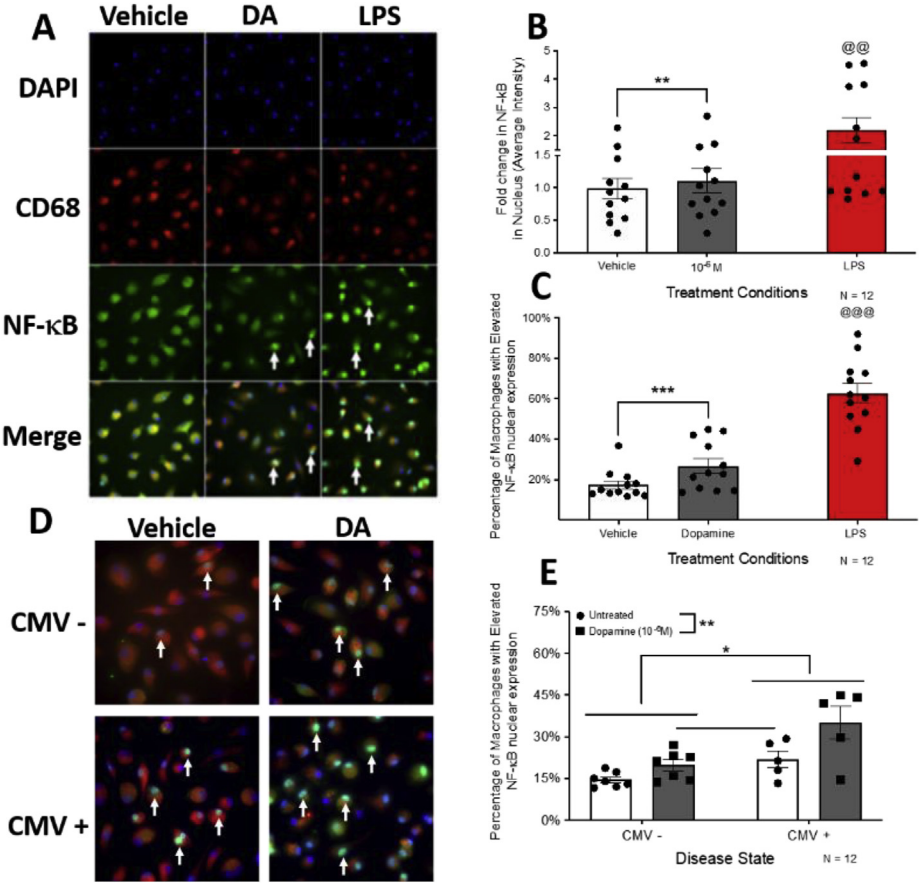

Fig. 5. Dopamine increases the number of cells with high levels of nuclear NF-κB. (5A) Representative images of nuclear translocation of NF-κB (green) in hMDM in untreated, dopamine-treated or LPS-treated conditions for 60 min. White arrow indicates nuclear NFκB staining. (5B) Fold change in the average intensity of nuclear NF-κB in control and treated-conditions, [Fig. 5B, Wilcoxon test, n = 12, ***p = 0.001, sum of (+,-) ranks 77, -1] (5C) As the increase in average intensity shown in 5B was not the same magnitude as what was seen in Western blot (not shown), the percent of hMDM that expressed high levels of nuclear NF-κB increased in dopamine-treated and LPS-treated hMDMs was compared to controls [Fig. 5C, Wilcoxon test, n = 12, ***p = 0.001, sum of (+,-) ranks 77, -1]. hMDMs treated with dopamine displayed an increased percentage of cells with a high NFκB level. (5D) Representative images of nuclear translocation of NF-κB in vehicle or DA treated hMDM from donors with or without CMV infection. Note the increased amount of NF-κB in the dopamine-treated cells with CMV. (5E) Percentage of cells with elevated NF-κB nuclear expression in hMDM increased when pre-existing CMV infection was present [Fig. 8B, mixed effects model (REML), n = 7 without CMV, n = 6 with CMV; CMV, **p = 0.0037, F(1,11) = 13.44; DA, **p = 0.0020, F (1,11) = 16.18]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

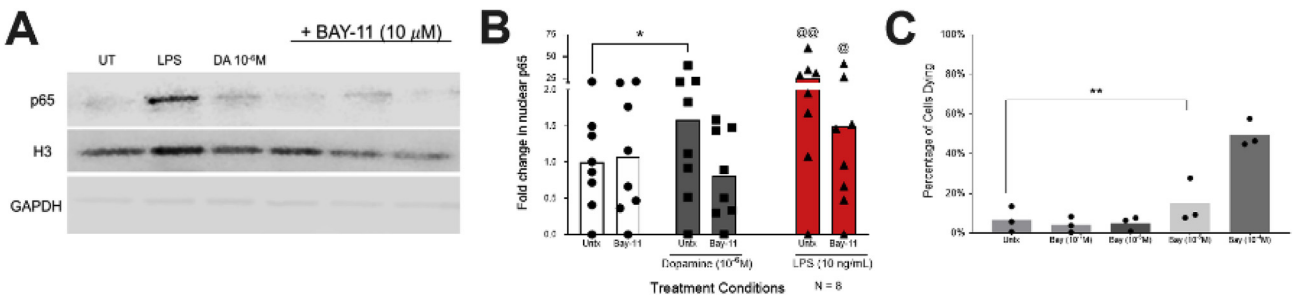

Fig. 6. Dopamine-mediated effects on NF-κB p65 nuclear translocation were inhibited by BAY-11. 6A Representative Western blot of nuclear lysates that were either untreated or treated with NF-κB inhibitor, BAY-11 (10^{-5} M), under untreated, dopamine-treated (10^{-6} M) or LPS-treated (10 ng/mL) conditions. 6B Fold change in nuclear NF-κB levels in the presence or absence of BAY-11 (10^{-5} M) either untreated, dopamine-treated (10^{-6} M) or LPS-treated (10 ng/mL) conditions, showing increased nuclear p65 in dopamine-treated conditions that is diminished when BAY-11 is present [Figs. 6B and 2 × 2 ANOVA, n = 8, BAY x dopamine, F (1,7) = 12.20, *p = 0.0101] 6C A live/dead assay was performed to analyze the percentage of dying cells via two-channel colocalization, confirming that BAY-11 (10 μM) is not significantly killing the cells [Fig. 6C, n = 3, one-way rmANOVA, F(1.049, 2.097) = 31.57, p = 0.0272; Holm-Sidak's, untx vs. BAY (10-4M), ** p = 0.003].

percentage of macrophages with elevated nuclear NF-κB across the population. Further, these data suggest that dopamine acts additively with chronic inflammation to increase the nuclear translocation of NF-κB in a larger number of cells, suggesting that exposure to dopamine could exacerbate inflammation.

3.3. Dopamine acts directly on the NF-κB pathway

To determine whether dopamine stimulation of NF-κB mediates the increase in the production of inflammatory cytokines, dopamine-induced cytokine production was examined in hMDM pretreated with the NF-κB inhibitor BAY 11–7082 (BAY). This compound specifically inhibits the activation of IκB kinase, preventing the phosphorylation and subsequent degradation of IκB and thereby blocking the phosphorylation and internalization of the NF-κB p65 protein (Pierce et al., 1997). To confirm that BAY is able to inhibit NF-κB activation in hMDM, these cells were pre-treated with BAY (10^{-5} M) for 45 min, then treated with dopamine (10^{-6} M) and LPS (10 ng/mL) for 60 min. To ensure that the concentration of BAY (10^{-5} M) used is not cytotoxic to hMDM, a LIVE/DEAD assay was performed. This assay demonstrated that even after 24 h of treatment, the use of BAY (10^{-5} M) did not alter hMDM viability, with only the highest concentration of BAY tested (10^{-4} M) inducing significant cell death compared to vehicle-treated cells [Fig. 6C, n = 3, one-way rmANOVA, F(1.049, 2.097) = 31.57, *p = 0.0272; Holm-Sidak, untx vs. BAY (10^{-4} M), **p = 0.003].

Nuclear lysates from the 60-min time point were examined for nuclear translocation of p65 (Fig. 6A and B), showing a significant interaction between BAY and dopamine with no main effects of either BAY or dopamine alone [Figs. 6B and 2 × 2 ANOVA, n = 8, BAY x dopamine, F (1,7) = 12.20, * p = 0.0101]. *Post-hoc* analysis showed a significant increase in nuclear translocation in hMDM that were exposed to dopamine, and this was lost when cells were pre-treated with BAY [Dunnett's, Untx:Untx vs. Untx:Dopamine, *p = 0.0425, BAY:Untx vs. BAY:Dopamine, p = 0.4843]. As in previous experiments, the effect of LPS was more robust than that of dopamine, as we found a significant main effect of both LPS and BAY, and a significant interaction between BAY and LPS [Figs. 6B and 2 × 2 ANOVA, n = 8, LPS, F (1,7) = 8.518, $^{@}$ p = 0.0224; BAY, F (1,7) = 9.084, $^{@}$ p = 0.0196, LPS x BAY, F (1,7) = 9.696, $^{@}$ p = 0.0170]. *Post-hoc* analysis confirmed that LPS significantly increased nuclear translocation in both untreated and BAY pre-treated cells [Tukey's, Untx:Untx vs. Untx:LPS, $^{@@}$ p = 0.0022, BAY:Untx vs. BAY:LPS, $^{@@}$ p = 0.0184]. In all experiments, BAY alone had no effect on p65 nuclear translocation. These experiments confirmed that BAY was able to block the dopamine-mediated activation of NF-κB in hMDM.

3.4. Dopamine primes, but does not activate the NLRP3 inflammasome

The production of IL-1β is tightly regulated by inflammasomes, including the NLRP3 inflammasome. Together with the data showing dopamine activates the NF-κB pathway, this suggests that impact of dopamine on IL-1β is at least partially mediated by its effects on this complex. To define more precisely the impact of dopamine on the inflammasome, hMDM were treated with dopamine (10^{-6} M) or LPS (10 ng/mL) for 3 and 6 h and examined for expression of NLRP3 and IL-1β mRNA, as well as expression of NLRP3 protein. After 3 h of dopamine treatment, there is a significant increase in NLRP3 and IL-1β mRNA [Fig. 7A – NLRP3, n = 11, paired *t*-test, *p = 0.0308, t = 2.513, df = 10; Fig. 7D - IL-1β, n = 16, Wilcoxon test, *p = 0.0386, sum of (+,-) ranks 108, -28] and a slight but significant increase in NLRP3 expression [Fig. 7B, C, n = 12, Paired *t*-test, **p = 0.0030, t = 3.786, df = 11]. As with other experiments, LPS significantly increased expression of all targets at 3 h [Fig. 7A – NLRP3, n = 11, paired *t*-test, $^{@}$ p = 0.0445, t = 2.297, df = 10, 7B – NLRP3 protein, Wilcoxon test, $^{@@@}$ p = 0.005, sum of (+,-) ranks 78, 0; 7D - IL-1β, n = 16, Wilcoxon test, $^{@}$ p = 0.0034, sum of (+,-) ranks 122, -14;]. The increases in NLRP3 gene and protein expression are in line with those seen with LPS in other studies (Budai et al., 2013; Kankkunen et al., 2010). Further, even small increases in the level of NLRP3 can impact the sensitivity of inflammasome activation (Guarda et al., 2011), suggesting that the increases mediated by dopamine could effectively prime the inflammasome. Additionally, production of intracellular IL-1β, as evaluated by AlphaLISA analysis of cell lysate, was significantly increased by dopamine [Fig. 7E, n = 41, Wilcoxon, DA - **p = 0.0043, sum of (+,-) ranks 647.0,-214.0; LPS - @@@@ p = <0.0001, sum of (+,-) ranks 861.0, 0.000], but the secretion of IL-1β into culture supernatant was unchanged [7F, n = 7, paired *t*-test, p = 0.8237, t = 0.2327, df = 6]. The lack of secretion of IL-1β in response to treatment with dopamine indicates that the inflammasome is only primed, as full activation induces the activation of caspase-1 and proteolytic cleavage of IL-1β into its mature, secreted form (Eder, 2009; Lu and Wu, 2015; Martinon et al., 2002).

To confirm the lack of inflammasome activation, hMDM treated with dopamine and LPS were analyzed for the expression of active caspase-1. Although caspase-1 activation has commonly been identified by quantification of the p20 form of this enzyme, recent data suggest that caspase-1 activation is more complex than previously described (Boucher et al., 2018). This research indicates that in macrophages, caspase-1 (p46) clusters on the inflammasome as a dimer, undergoing self-cleavage to form an active p33/p10 dimer on the inflammasome surface before undergoing a final cleavage event to destabilize the active form into a p20/p10 tetrameric protein that is released from the inflammasome complex. The active form of caspase-1, p33/p10, is formed preceding the proteolysis of pro-IL-1β, and exists transiently until it is deactivated and released as p20/p10 (Boucher et al., 2018). Therefore, 3 and 6 h hMDM lysates from 8 donors were examined by Western blot for dopamine-mediated changes in the expression of active caspase-1 (p33). Analysis of these data show that neither dopamine nor LPS increased the

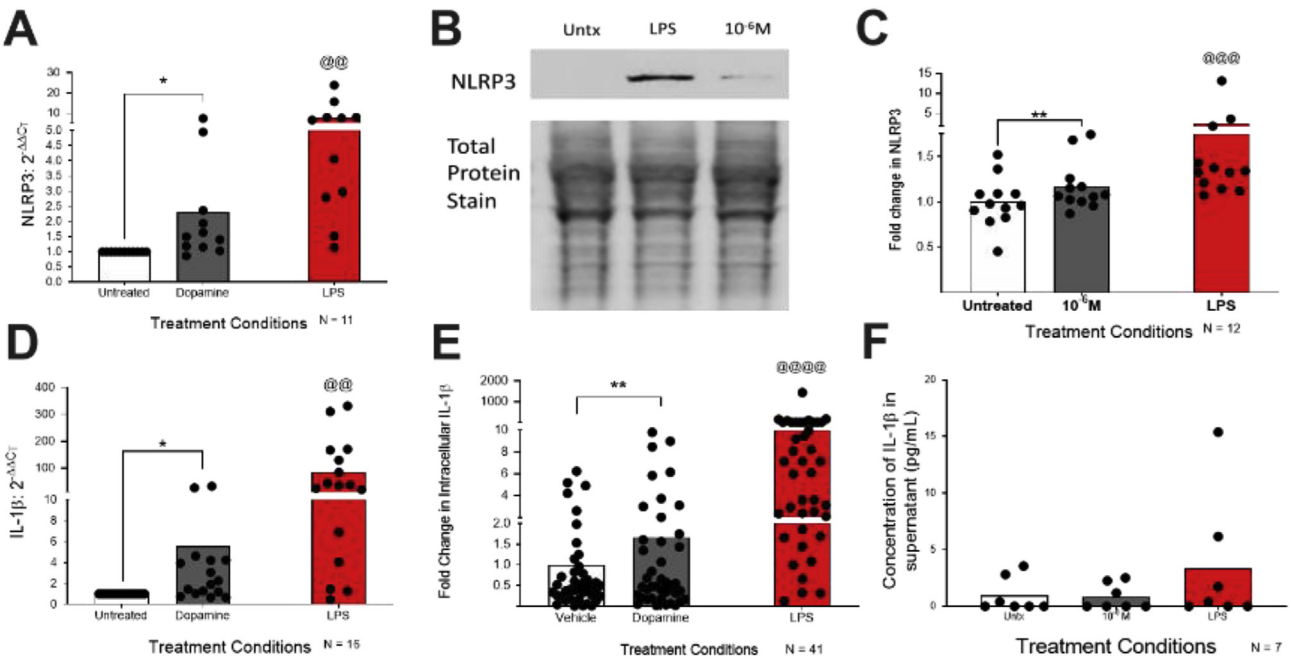

Fig. 7. Dopamine primes the NLRP3 inflammasome by increasing NLRP3 and IL-1β expression levels in a non-oxidative process. (7A) Expression of NLRP3 mRNA increased when hMDMs are treated with dopamine (10^{-6}M) [6A – NLRP3, n = 11, paired *t*-test, *p = 0.0308, t = 2.513, df = 10]. (7B) Representative Western blot of NLRP3 in untreated, dopamine-, or LPS-treated conditions, and the total protein stain is provided to confirm protein was added to each well. (7C) Fold change of NLRP3 protein expression, showing a slight increase in NLRP3 protein expression following dopamine treatment [6C – NLRP3 protein, n = 12, Paired *t*-test, **p = 0.0030, t = 3.786, df = 11]. (7D) mRNA expression of IL-1β in either untreated, dopamine- or LPS-treated conditions, displaying increased IL-1β expression following dopamine treatment [6D - IL-1β, n = 16, Wilcoxon test, *p = 0.0386, sum of (+,-) ranks 108, -28] (7E) Fold change of protein expression of IL-1β intracellularly, supporting that increased IL-1β protein expression also occurs after dopamine treatment [Fig. 7E, n = 41 Wilcoxon, DA - **p = 0.0043, sum of (+,-) ranks 647.0,-214.0; LPS - @@@@ p = <0.0001, sum of (+,-) ranks 861.0, 0.000]. (7F) Amount of IL-1β in supernatant shown as concentration in untreated, dopamine-treated and LPS-treated conditions [n = 7, one-way ANOVA, p = 0.3116, F (2, 20) = 1.155], which LPS as a positive control.

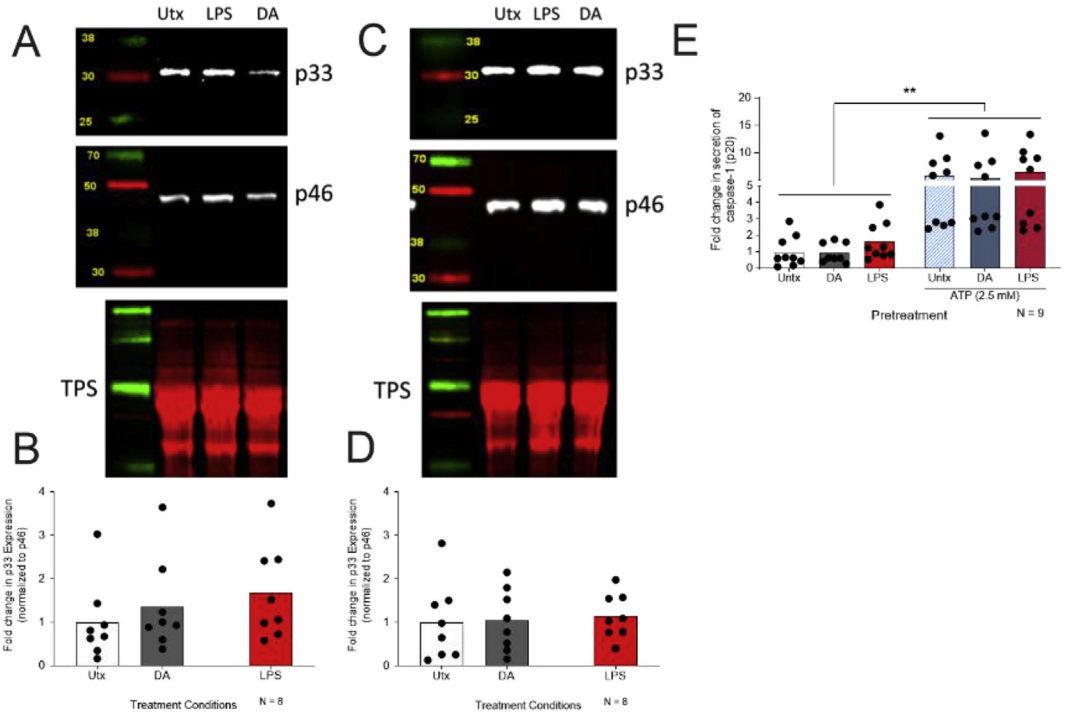

Fig. 8. Dopamine does not increase the amount of active caspase-1. (8A) Representative Western blot of active caspase-1 (p33), total caspase-1 (p46) and total protein stain (TPS) after 3 h treatment. (8B) Percentage of active caspase-1 (p33) relative to total caspase (p46) shown as fold change at 3 h [n = 8: 3 h DA - Wilcoxon test, p = 0.3125, sum of (+,-) ranks 26, -10; 3 h LPS - Wilcoxon test, p = 0.1484, sum of (+,-) ranks 29, -7]. (8C) Representative Western blot of active caspase-1 (p33), total caspase-1 (p46) and total protein stain (TPS) after 6 h treatment. (8D) Percentage of active caspase-1 (p33) relative to total caspase (p46) shown as fold change at 6 h [n = 8: 6 h DA – Paired *t*-test, p = 0.7226, t = 0.3696, df = 7; 6 h LPS - Paired *t*-test, p = 0.4419, t = 0.8150, df = 7]. (8E) Fold change in amount of cleaved caspase-1 in hMDM culture supernatants after 24 h of dopamine or LPS treatment [n = 4, 2 × 3 ANOVA, ATP - F (1,8) = 21.35, **p = 0.0017].

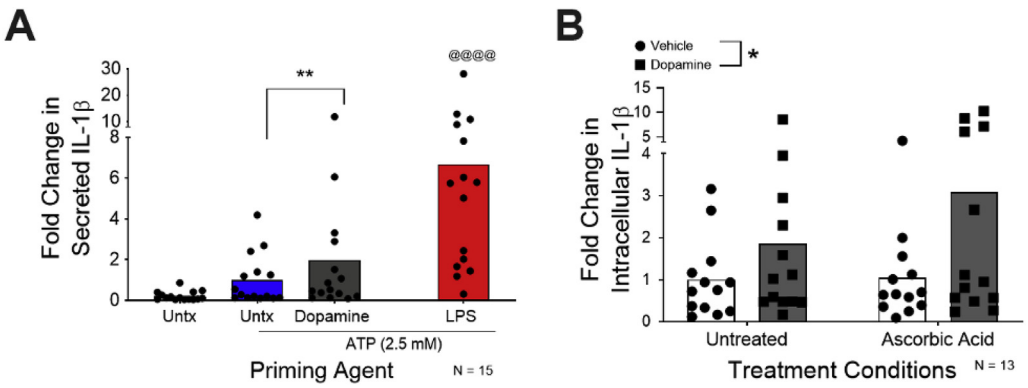

Fig. 9. Dopamine increases IL-1β secretion after inflammasome activation with ATP through a non-oxidative process. (9A) Fold change in secreted IL-1β after activation with ATP in DA primed cultures of hMDM suggests that dopamine increases pro-IL-1β production, which is cleaved and released as IL-1β following ATP stimulation [9A – IL-1β, Wilcoxon matched-pairs signed rank test, n = 15; Untx (ATP) vs. DA, **p = 0.0043, sum (+,-) ranks 108, -12; Untx (ATP) vs. LPS, @@@@ p > 0.0001, sum (+,-) ranks 120, 0]. (9B) Fold change in secreted IL-1β was also evaluated in the presence of ascorbic acid to elevate the contribution of dopamine oxidation, and as dopamine also increased IL-1β in the presence of ascorbic acid, this suggests that the increase in IL-1β is not due to oxidative effects. [2 × 2 ANOVA, Ascorbic Acid x Dopamine, F (1,12) = 2.118, p = 0.1713].

amount of active caspase-1 (p33) relative to full length caspase-1 (p46) [Fig. 8B, n = 8: 3 h DA - Wilcoxon test, p = 0.3125, sum of (+,-) ranks 26, -10; 3 h LPS - Wilcoxon test, p = 0.1484, sum of (+,-) ranks 29, -7; Fig. 8D, n = 8: 6 h DA – Paired *t*-test, p = 0.7226, t = 0.3696, df = 7; 6 h LPS - Paired *t*-test, p = 0.4419, t = 0.8150, df = 7]. Representative Western blots from are shown in Fig. 8A for 3 h and Fig. 8C for 6 h. Additionally, neither dopamine nor LPS increased the total expression of full-length caspase-1 (p46) in cell lysates (data not shown).

As caspase-1 (p20) can be secreted following activation, it is possible that the effects of dopamine were not visible in the cell lysate due to the release of the p20 protein. Therefore, hMDM were treated with either dopamine or LPS for 24 h, with a subset of each treatment also treated with ATP (2.5 mM) 30 min prior to supernatant collection. ATP is a DAMP that triggers the activation and secretion of caspase-1 (p20) and IL-1β after NF-κB activation (Bauernfeind et al., 2009; Keller et al., 2008; Laliberte et al., 1999; Shamaa et al., 2015). Analysis of caspase-1 (p20) release showed no change in caspase-1 (p20) secretion in response to dopamine or LPS, but a significant increase in caspase-1 secretion in all cultures treated with ATP 30 min prior to collection [Fig. 8E, n = 4, 2 × 3 ANOVA, ATP - F (1,8) = 21.35, **p = 0.0017]. Taken together, these data show that while dopamine increases NF-κB activation and nuclear translocation, as well as the transcription and expression of NLRP3 and intracellular IL-1β, it does not initiate activation of caspase-1 nor proteolytic cleavage and secretion of mature IL-1β. This indicates that, in human macrophages, dopamine mediates the initial priming of the NLRP3 inflammasome, but does not fully activate the complex.

3.5. Dopamine treatment potentiates ATP-mediated release of IL-1β

To determine if dopamine priming could affect the function of the NLRP3 inflammasome, the impact of dopamine on cytokine release mediated by inflammasome activation was examined. As shown in Fig. 1, the second step of inflammasome activation is mediated by exposure to PAMPs or DAMPs following an initial priming step (Herman and Pasinetti, 2018; He et al., 2016; Bauernfeind et al., 2009; Jo et al., 2016). Therefore, hMDM were treated with dopamine for 24 h, and treated with either vehicle or ATP (2.5 mM) for 30 min prior to collection of lysates and supernatants, which were analyzed for concentration of IL-1β. Treatment with LPS was again used as a positive control, as LPS primes the inflammasome, increasing the response to activating stimuli such as ATP. Analysis of culture supernatant demonstrates that dopamine priming followed by ATP-mediated activation of the inflammasome induces the secretion of significantly greater amounts of IL-1β relative to secretion from hMDM not primed with dopamine [Fig. 9A, Wilcoxon matched-pairs signed rank test, n = 15; Untx (ATP) vs. DA, **p = 0.0043, sum (+,-) ranks 108, -12; Untx (ATP) vs. LPS, @@@@ p > 0.0001, sum (+,-) ranks 120, 0]. As expected, priming with LPS induced a similar, but much more robust effect.

In addition to its normal degradation pathway, dopamine can also be oxidized, releasing free radicals and forming dopamine quinones (Smythies and Galzigna, 1998; Meiser et al., 2013). This makes it possible that some of the effects mediated by dopamine are due to the involvement of oxidative pathways. To determine if the effects of dopamine on IL-1β were related to dopamine oxidation, hMDM were treated with dopamine for 24 h in the presence or absence of the antioxidant L-ascorbic acid (L-AA, 200 μM), and examined for changes in intracellular IL-1β. In these assays, there was a significant main effect of dopamine [Fig. 9B and 2 × 2 ANOVA, n = 13, *p = 0.0249, F (1,12) = 6.568] but no interaction between dopamine and ascorbic acid [Fig. 9B and 2 × 2 ANOVA, Ascorbic Acid x Dopamine, F (1,12) = 2.118, p = 0.1713]. This corroborates our previous data showing that dopamine oxidation is not the mechanism by which it influences HIV replication in hMDM (Gaskill et al., 2009), instead acting through dopamine receptors (Nickoloff-Bybel et al., 2019; Gaskill et al., 2014). Together, these data demonstrate that dopamine exposure amplifies the secretion of IL-1β induced by inflammasome activation, and that this is not mediated through an oxidative mechanism.

4. Discussion

This study demonstrates that dopamine activates NF-κB in primary human monocyte-derived macrophages, priming the NLRP3 inflammasome and inducing production of IL-1β. Our previous data showed that dopamine exposure promotes a pro-inflammatory phenotype in macrophages, increasing production of inflammatory cytokines through a mechanism that may be mediated by dopamine receptors (Nickoloff-Bybel et al., 2019; Nolan et al., 2019). However, the specific pathways underlying this effect are not well understood, and the overall impact of dopamine on myeloid cell activation remains undefined. This current study expands on our prior work by identifying a key intracellular pathway mediating the pro-inflammatory effects of dopamine. These data in this study show that dopamine acts on several steps of the NF-κB signaling pathway involved in macrophage activation and inflammatory cytokine production (Kopp and Ghosh, 1995; Lenardo and Baltimore,

1989; Monsalve et al., 2009; Vallabhapurapu and Karin, 2009). Specifically, dopamine increased phosphorylation of the NF-κB p65 subunit, and induced p65 nuclear translocation in hMDM. This effect was reduced by IκK-inhibitor BAY 11–7082, suggesting that dopamine acts directly on the NF-κB pathway. Further, these studies also show that dopamine acts on the tightly regulated NLRP3 inflammasome pathway to modulate production and secretion of IL-1β. The impact of dopamine seems to be mediated by modulation of the priming step preceding inflammasome activation, as dopamine increased expression of NLRP3 mRNA and protein, but not expression of activate caspase-1 or the secretion of IL-1β. These findings are consistent with our prior data showing that dopamine increased intracellular expression of cytokines IL-1β and IL-18 without triggering secretion (Nolan et al., 2019). The dopamine-mediated priming of the inflammasome also contributes to the extracellular inflammatory milieu as dopamine exposure produced greater secretion of IL-1β from ATP-stimulated cells. Further, the additive interaction of dopamine and CMV on NF-κB activity suggest that these effects could enhance an existing inflammatory response.

Previous studies have reported dopamine-mediated activation of NF-κB in some cell lines; however, the precise proteins translating dopamine receptor activation to NF-κB activation remain unclear. The D2 agonist quinpirole (10^{-6} M) increases NF-κB DNA binding in NG108-15 neuroblastoma cells, but these studies did not examine IκB degradation, p65 phosphorylation, or nuclear translocation (Takeuchi and Fukunaga, 2003, 2004). Another study found that while very high levels of dopamine (10^{-4} M) increased HIV transcription via modulation of NF-κB binding sites in Jurkat T-cells, it did not affect IκB or induce p65 nuclear translocation (Rohr et al., 1999), suggesting a distinct NF-κB activation mechanism. Activation of NF-κB typically involves the phosphorylation of subunits such as p65, which can be mediated by several kinases such as IκK, GSK3β, and TBK1 (Viatour et al., 2005; Buss et al., 2004; Schwabe and Brenner, 2002), and the most commonly studied pathway activating NF-κB acts via IκK-mediated serine phosphorylation of IκBα, leading to its degradation (Viatour et al., 2005; Hayden and Ghosh, 2008; Gilmore, 2006; Christian et al., 2016).

However, other pathways have been described (Sun, 2011; Hayashi et al., 1993), and an increasing number of studies suggest that IκBα degradation and p65 phosphorylation do not always occur together, and both are not necessary for NF-κB activation (Bohuslav et al., 2004; Sasaki et al., 2005). For example, hydrogen peroxide induces p65 phosphorylation and nuclear translocation independent of IκB degradation, activating IκK by inducing tyrosine rather than serine phosphorylation, which does not induce IκB degradation (Sethi et al., 2007; Takada et al., 2003). Further, the IκB protein can undergo both tyrosine nitration and polyubiquitination, leading to destabilization of newly synthesized IκB (Enesa et al., 2008) or the dissociation of intact IκBα from p65 (Yakovlev et al., 2007).

Our data show that dopamine induces slight but significant IκB degradation in hMDM after 15 min, but it is not clear whether this small change is indicative of the particular pathway(s) involved. As the effects of dopamine on p65 phosphorylation and nuclear translocation are more robust than on IκB degradation, this may indicate that dopamine-mediated p65 phosphorylation promotes nuclear localization of NF-κB, and/or enhances transcriptional activity without inducing IκK-mediated NF-κB activation. In this case, dopamine might act to block new synthesis or decrease the stability of IκB without directly inducing its degradation, leading to the slight decrease observed. Thus, dopamine could induce modifications of IκB, independent of IκK, that may modulate inflammatory activation, suggesting an important avenue of investigation for future research.

Although this study did not define the receptors involved in activation of these inflammatory pathways, our previous study on dopamine-mediated changes in cytokine production suggests that D1-like dopamine receptors mediate pro-inflammatory activity, while D2-like receptors mediate anti-inflammatory activity (Nolan et al., 2019). This hypothesis is supported by a number of studies (Nakano et al., 2011; Devoino et al., 2006; Nakagome et al., 2011a; Zhang et al., 2012, 2015; Zhu et al., 2018b; Kimmel et al., 2005; Laengle et al., 2006), but the specific dopamine receptor subtypes associated with NF-κB and NLRP3 inflammasome activity in human cells are not well understood. In primary murine astrocytes, activation of D2-like dopamine receptors suppressed LPS-induced IκK phosphorylation and NF-κB nuclear translocation (Du et al., 2018), and decreased NLRP3 inflammasome activation through a non-canonical, β-arrestin 2 dependent mechanism (Zhu et al., 2018b). These data suggest that D2-like receptors are anti-inflammatory, however others suggest that D1-like receptors can also mediate anti-inflammatory effects. In RAW264.7 mouse macrophages, p65 phosphorylation and NF-κB nuclear translocation were inhibited by the D1-like agonist fenoldopam (1 μm) (Feketeova et al., 2018), and in human NK lymphocytes, activation of the D5 receptor by dopamine (10^{-9} through 10^{-18} M) blocked the formation of the NF-κB complex (Mikulak et al., 2014). With our data, these studies suggest that dopamine-induced changes in NF-κB may vary by species, cell-type, and inflammatory context.

This may also be true of NLRP3 activation. In mouse bone marrow-derived macrophages (BMDM), acute exposure to high levels of dopamine (1.5×10^{-5} M) inhibited NLRP3-mediated IL-1β production. Production of NLRP3 was also inhibited by high levels of dopamine or the D1-like agonist A68,930 ($2–4 \times 10^{-5}$ M) (Yan et al., 2015). Similar results were seen in rodent models of spinal cord injury and intracerebral hemorrhage, where systemic exposure to A68,930 ($\sim 1.8 \times 10^{-6}$ M), reduced IL-1β production and NLRP3 activity (Jiang et al., 2016; Wang et al., 2018). The data from Yan and colleagues suggests the inhibition of inflammasome activity is mediated by D1-like dopamine receptors acting through a cAMP-dependent pathway, although D1-like receptor mediated increases in cAMP were not defined. Our recent data show dopamine does not increase production of cAMP in human macrophages (Nickoloff-Bybel et al., 2019), suggesting the differences in the effect of dopamine may be due to a distinct pathway for dopamine activity in human cells, or perhaps differential expression of dopamine receptor subtypes between species. Different dopamine-receptor subtypes vary in affinity for dopamine, and it has been suggested that high-affinity receptors (DRD3, DRD4, DRD5) can promote inflammation (Pacheco, 2017). Our data are consistent with this hypothesis, as we have shown that the most highly expressed receptor on primary hMDM is DRD5 (Nickoloff-Bybel et al., 2019; Nolan et al., 2019). Future research should take these potential difference into account, considering species-specific differences in the inflammatory response (Ariffin and Sweet, 2013) and carefully defining the commonalities and differences in the expression and pharmacology of dopamine receptors across different species and cell types.

Irrespective of the dopamine receptors mediating this effect, these data suggest that NF-κB-regulated inflammation could be modulated by changes in dopamine concentrations, either through endogenous activity, drugs of abuse, or dopaminergic therapeutics. The concentration of dopamine used in these studies (10^{-6} M) models the impact of substance abuse in dopamine-rich brain regions, particularly the use of stimulants such as cocaine or methamphetamine, which robustly and acutely increase dopamine levels (Kimmel et al., 2005; Nutt et al., 2015; Jonsson and Gunne, 1972; dela Peña et al., 2015; Carboni et al., 1989; Schiffer et al., 2003; Zachek et al., 2010; Di Chiara and Imperato, 1988; Boileau et al., 2003; Xi et al., 2009). Thus, elevated CNS dopamine levels associated with drug use may pre-dispose substance abusers to more robust inflammatory responses. This is supported by the neuropathology seen in the CNS of drug addicts (Sekine et al., 2008; Oehmichen et al., 1996; Loftis et al., 2011), and by animal models showing increased myeloid cell activity, NF-κB activation, and IL-1β and IL-6 in dopamine-rich brain regions following psychostimulant exposure (Asanuma and Cadet, 1998; Frank et al., 2016; Loftis et al., 2011; Yamaguchi et al., 1991; Goncalves et al., 2008; Crews et al., 2006; Seminerio et al., 2012). The use of stimulants also increases the risk of infection and sterile inflammatory disease indicating that neuroimmune interactions mediated by dopamine

signaling could also impact physical health (Russo et al., 2010; Kovacs, 2012; Caputi and Giron, 2018; Caso et al., 2007).

Furthermore, CNS dopamine could also impact myeloid-mediated processes in many other regions of the body. The immune cells in the lymphatic and glymphatic systems (Aspelund et al., 2015; Louveau et al., 2015; Xie et al., 2013) could be exposed to dopamine and mediate communication between the brain and periphery. Studies show CSF dopamine to be in the picomolar range (Berger et al., 1994; Andersen et al., 2017; Engelborghs et al., 2003; Eldrup et al., 1995), but these samples are often gathered by lumbar puncture, so it may be higher in other brain adjacent regions. Transport within the CSF would allow dopamine to rapidly move across the brain, as it enters the perivascular spaces that surround all penetrating blood vessels. As CSF is continuously produced at a rate to fully replace its volume several times per day in humans (Sakka et al., 2011; Veening and Barendregt, 2010), it is constantly moving new dopamine around the CNS and into the periphery through the cervical lymph nodes. This would allow peripheral immune cells to interact with dopamine originating from the CNS, providing a potential mechanism for coordinated changes in activity across multiple areas. Potential dopaminergic regulation of myeloid cells in the context of these systems remain to be explored but is a promising area of investigation.

In the circulation, most studies report dopamine concentrations in the picomolar range, but nanomolar levels have also been found (Mitchell et al., 2018; Saha et al., 2001; Kavelaars et al., 2005; De Laurentiis et al., 2002). Plasma concentrations seem to vary widely among individuals (Eisenhofer et al., 2005) and are substantially altered during normal activity. Circulating immune cells, including T-cells or monocytes, are likely to encounter large fluctuations in dopamine, with only small regions containing dopamine concentrations high enough to promote inflammatory effects (Pinoli et al., 2017; Gaskill et al., 2013). However, peripheral dopamine synthesis and metabolism may be underestimated, as the bioactivity of circulating dopamine metabolites and conjugated dopamine are not well understood. A number of peripheral organs, such as the kidney, lung, and gastrointestinal tract, also contain significant dopamine concentrations in the nanomolar to micromolar range, which could significantly alter immune activity (Matt and Gaskill, 2019a). The effects of abused substances on peripheral dopamine concentrations are unknown, but abused drugs that increase CNS dopamine, such as cocaine, methamphetamine, and ethanol, are associated with significant inflammatory pathology in the periphery (Dimitrijevic et al., 2008; Lineberry and Bostwick, 2006; Tiwari et al., 2006). Thus, the effects of substance abuse could also drive inflammation in peripheral tissues through modulation of resident macrophages and other immune cells (Roy et al., 2011). These effects might also be mediated by therapeutic agents that increase dopamine or act on dopamine receptors, such as Bromocriptine, L-DOPA, Emsam or Wellbutrin (Brannan et al., 1993; Lamensdorf et al., 1999; Stahl et al., 2004).

Inflammation in the periphery is also linked to the development of neuropsychiatric and neurodegenerative disorders (Fillman et al., 2014; Holmes et al., 2009). Peripheral cytokines, such as IL-1β, IL-6 and TNF-α, are elevated in neuropsychiatric disorders and can stimulate neuroinflammation through a number of pathways, including the vagus nerve or spinal cord (Chavan et al., 2017; Goehler et al., 1999; Lampa et al., 2012; Schneider et al., 2019; Walker et al., 2019). Activation of NF-κB or other inflammatory pathways in peripheral macrophages could also prime cells prior to BBB translocation, inducing a more reactive phenotype that could contribute to neuroinflammation once exposed to inflammasome activating stimuli in the brain. Our data show that dopamine significantly increases the NF-κB nuclear translocation in macrophages from cytomegalovirus (CMV) positive individuals (Fig. 5D and E), and significantly increases ATP-stimulated secretion of IL-1β (Figs. 7E and 9A). This suggests a mechanism by which elevated dopamine could exacerbate disease progression in substance abusers by stimulating myeloid-mediated inflammation. Further support for this mechanism is shown by data suggesting myeloid lineage cells are the main cell types expressing high levels of NLRP3 in the CNS (Guarda et al., 2011; Chaurasia et al., 2018; Kong et al., 2017; Voet et al., 2019; Katuri et al., 2019) and may be primarily responsible for mediating inflammasome-associated neuroinflammation (Jha et al., 2017; Gustin et al., 2015). Myeloid activation is associated with increased IL-1β and downstream inflammatory effects in diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, neuroHIV, depression and other neurological diseases (Chaurasia et al., 2018; Voet et al., 2019; Gold and El Khoury, 2015; Iwata et al., 2013; Tansey and Goldberg, 2010; Wang et al., 2015). For example, in HIV-infected individuals on suppressive cART, NLRP3 inflammasome activation occurs early in microglia (Walsh et al., 2014), and the release of viral proteins, which still occurs during cART treatment (Johnson et al., 2013; Dickens et al., 2017), specifically increases NLRP3 activation in hMDM and both human and rodent microglia (Chivero et al., 2017; Mamik et al., 2017). In Parkinson's disease, neuroinflammation is increased (Tansey and Goldberg, 2010; Wang et al., 2015), but the effect of dopaminergic treatments on the neuroinflammatory state is not clear, although a connection between levodopa and increased neuroinflammation has been postulated (Dorszewska et al., 2014). Thus, the effects of dopamine on myeloid cells may be a link between the dopamine-associated and inflammatory aspects of pathogenesis.

This has broad clinical relevance, as many diseases, including depression, schizophrenia, neuroHIV, Alzheimer's disease, Parkinson's disease and bipolar disorder are treated with dopamine-altering therapeutics (Strange, 2001; Dean and Scarr, 2004; Ashok et al., 2017; Matt and Gaskill, 2019b; Martorana and Koch, 2014; Belujon and Grace, 2017), and show increases in neuroinflammation during treatment (Brites and Fernandes, 2015; Hurley and Tizabi, 2013; Monji et al., 2013; Doorduin et al., 2009; Goldstein et al., 2009; Kim et al., 2016; Najjar et al., 2013; Hamdani et al., 2013). A recent study found that microglia activation was not only increased in patients with schizophrenia, but also in individuals at ultra-high risk of psychosis and was associated with symptom severity (Bloomfield et al., 2016). Both schizophrenia and psychosis are associated with overactivation of the dopaminergic system, which could subsequently increase inflammatory activation of microglia and macrophages, creating a cycle that exacerbates mesocortical dysfunction and accelerates disease progression. Recent studies indicate that increased cortical immune activation in schizophrenia is mediated by NF-κB activation (Volk et al., 2019), suggesting that dopamine-mediated activation of myeloid NF-κB is a potential link between dysregulated dopamine signaling and inflammation in psychotic disorders. This type of bidirectional interaction could also play a role in neuroplasticity that is corrupted during the development of neuropsychiatric disease and may indicate that microglial activation is tied to behavioral impairment and disease progression via these dopaminergic interactions.

These effects are also relevant in the treatment of psychosis, as individuals with psychotic symptoms often show increased striatal activity and excessive D2 receptor stimulation (Kesby et al., 2018). Our lab and others suggest that activation of D1-like receptors can promote inflammatory activity while D2-like receptors mediate anti-inflammatory activity (Nolan et al., 2019; Nakano et al., 2011; Nakagome et al., 2011b). Many first-generation antipsychotics, such as haloperidol, potently block D2-like dopamine receptors, so the use of these drugs may exacerbate inflammation. Indeed, antipsychotics can trigger myeloid inflammatory responses both *in vitro* and *in vivo*, in humans and rodents (Duarte et al., 2018; Holmes et al., 2016; Cotel et al., 2015). PET imaging of microglial activation using the 18 kDa translocator protein (TSPO) showed no evidence for increased TSPO availability in antipsychotic-free patients compared with healthy controls, but TSPO availability was significantly elevated in medicated patients (Holmes et al., 2016). Use of clozapine, a second-generation anti-psychotic with lower D2R potency than haloperidol, reduces the TLR-4/NF-κB-mediated inflammatory response in microglia, suggesting that distinct interactions with myeloid dopamine receptors could modulate inflammation in different ways (Jeon et al.,

2018). Further, the inflammatory effects of some antipsychotics may also be dependent on glial activation state (Obuchowicz et al., 2017), suggesting elevated dopamine levels that increase glial inflammation could reduce the effectiveness of some medications. This is supported by studies showing that higher levels of inflammation are associated with poorer response to antipsychotics (Mondelli et al., 2015; Zhang et al., 2005; Maes et al., 2000). These data suggest that dopamine-altering drugs should be contraindicated, or at least considered, when used to treat diseases that have an inflammatory component in both substance abusers and the general population. Further, investigating the impact of dopamine on microglial functions would be a novel avenue to study the idiopathic of neuropsychiatric disorders.

Special consideration should also be given to the aging population, as older individuals are at substantially increased risk for neurological diseases (Feigin et al., 2019) that are often treated with dopamine-altering drugs (Matt and Gaskill, 2019b; Klein et al., 2019). These drugs are frequently given in combination (polypharmacy), which is associated with an increase in inflammation and neuropsychiatric complications (Ersoy and Engin, 2018). In addition, alternative therapies for older patients with neuropsychiatric diseases such as depression are often dopamine-altering medications. For example, atypical antipsychotics, which often impact the dopamine system, are frequently given with SSRIs to optimize therapeutic efficiency in patients with treatment-resistant depression (Zhou et al., 2015), and this type of depression is more common in the elderly population (Knöchel et al., 2015). Further, during aging, microglia become more reactive to stimuli and can overproduce inflammatory cytokines such as IL-1β (Matt and Johnson, 2016; Norden and Godbout, 2013). Thus, aging individuals with an aberrant dopaminergic state due to either substance abuse or therapeutics may be particularly susceptible to dopamine-mediated changes in NF-κB signaling and cytokine production. Overall, this suggests that dopamine-induced increases in myeloid NF-κB and inflammasome activity may be an important factor in the development and progression of many diseases, not just those associated with drug abuse.

Myeloid cells are also essential to neuronal health and function, facilitating synapse formation and dissolution, modulating neurotransmission and providing neuronal protection (Herz et al., 2017). Thus, increases in myeloid NLRP3 inflammasome activation induced by substance abuse or therapeutics could interfere with neuronal function by dysregulating the bidirectional communication between CNS myeloid cells and neurons. For example, changes in the expression of IL-1β are linked to reductions in dendritic spine density in cultured cortical neurons (Festa et al., 2015). As reductions in dendritic spine density in the prefrontal cortex have been linked to deficits in executive functioning, this could affect self-control mechanisms that might otherwise inhibit drug use (Dumitriu et al., 2010; Holmes, 1990; Rasakham et al., 2014). Dendritic remodeling mediated by cytokines in other brain regions has also been proposed to impact emotional affect or reward memory that could also promote drug seeking (Russo et al., 2010; Kovacs, 2012; Arezoomandan et al., 2016). Thus, in addition the impact on neuropathogenesis, dopamine-associated increases in NRLP3 inflammasome activity could result in behavioral changes associated with the development of addiction.

In summary, the data in this study show that dopamine activates the NF-κB pathway in primary human macrophages, and that this process primes the NLRP3 inflammasome and is necessary for dopamine-induced inflammatory cytokine production. Further, this process potentiates the NF-κB activity associated with existing inflammation induced by cytomegalovirus infection. This suggests that increases in dopamine induced by substance abuse could promote or exacerbate the development of a number of inflammatory diseases by enhancing the inflammatory response through both NF-κB and the NLRP3 inflammasome. These effects may be an important mechanism for neuroplasticity in dopaminergic brain regions, that alters the development of addiction behavior. Thus, both substance abusers and those using dopaminergic therapeutics are at greater risk for the development of neuroinflammation and associated pathologies due to an aberrantly robust myeloid inflammatory response. A better understanding of these processes could highlight new pathways or targets that could be used to specifically treat inflammatory disease in populations with elevated dopamine. More broadly, determining the specific crosstalk between dopamine signaling and NF-κB/NLRP3 inflammasome signaling could reveal novel factors associated with the general regulation of inflammation. Therefore, future studies defining more precisely the role of dopamine in neuroimmune activity, and in myeloid mediated inflammation in particular, is an important step in developing more effective, targeted treatments for CNS disease.

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Declaration of competing interest

All the authors of this manuscript declare that they have no conflicts of interest either directly or indirectly related to the content of this manuscript.

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