



# The Endocannabinoid System as a Window Into Microglial Biology and Its Relationship to Autism

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### Specialty section:

This article was submitted to  
Cellular Neurophysiology,  
a section of the journal  
Frontiers in Cellular Neuroscience

**Received:** 13 June 2019

**Accepted:** 03 September 2019

**Published:** 18 September 2019

### Citation:

Araujo DJ, Tjoa K and Saijo K  
(2019) The Endocannabinoid System  
as a Window Into Microglial Biology  
and Its Relationship to Autism.  
*Front. Cell. Neurosci.* 13:424.  
doi: 10.3389/fncel.2019.00424

Microglia are the resident, innate immune cells of the central nervous system (CNS) and are critical in managing CNS injuries and infections. Microglia also maintain CNS homeostasis by influencing neuronal development, viability, and function. However, aberrant microglial activity and phenotypes are associated with CNS pathology, including autism spectrum disorder (ASD). Thus, improving our knowledge of microglial regulation could provide insights into the maintenance of CNS homeostasis as well as the prevention and treatment of ASD. Control of microglial activity is in part overseen by small, lipid-derived molecules known as endogenous cannabinoids (endocannabinoids). Endocannabinoids are one component of the endocannabinoid system (ECS), which also includes the enzymes that metabolize these ligands, in addition to cannabinoid receptor 1 (CB<sub>1</sub>) and 2 (CB<sub>2</sub>). Interestingly, increased ECS signaling leads to an anti-inflammatory, neuroprotective phenotype in microglia. Here, we review the literature and propose that ECS signaling represents a largely untapped area for understanding microglial biology and its relationship to ASD, with special attention paid to issues surrounding the use of recreational cannabis (marijuana). We also discuss major questions within the field and suggest directions for future research.

**Keywords:** microglia, endocannabinoids, autism, neuroinflammation, neurodevelopment

## INTRODUCTION

Microglia represent a self-sustaining population of cells that originates from the yolk sac and colonizes the brain *in utero* (Alliot et al., 1999; Ginhoux et al., 2010; Bruttger et al., 2015; Askew et al., 2017; Huang et al., 2018). Microglia are the resident immune cells of the central nervous system (CNS) and thus are the first line of defense against CNS infection and injury. For example, they phagocytize debris and pathogens as well as initiate neuroinflammatory responses through release of cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF- $\alpha$ ) (Yang et al., 2010; Janda et al., 2018). They also recruit natural killer cells, macrophages, and lymphocytes to sites of infection and injury (Yang et al., 2010). Moreover, microglia influence the health of their local environment through release of neurotrophic and neurotoxic factors (Nakajima et al., 2001; Srinivasan et al., 2004; Parkhurst et al., 2013).

In addition to the aforementioned roles, microglia carry out other functions essential for CNS homeostasis (Saijo and Glass, 2011; Butovsky and Weiner, 2018; Lenz and Nelson, 2018). Specifically, these cells oversee neurogenesis by both phagocytizing and directing the migration

of newborn neurons (Sierra et al., 2010; Ribeiro Xavier et al., 2015). Microglia also regulate neuronal connections by engulfing excessive synaptic structures through use of the classical complement cascade (Stevens et al., 2007). Lastly, microglia modulate neuronal plasticity via release of neurotrophins such as brain-derived neurotrophic factor (BDNF) (Parkhurst et al., 2013). Early wiring of the brain requires tight control of these processes and therefore microglia critically impact CNS development (Paolicelli et al., 2011; Bialas and Stevens, 2013; Shigemoto-Mogami et al., 2014).

Due to their wide-ranging contributions to CNS homeostasis and development, microglia displaying irregular activity and/or phenotypes can lead to disorders of the CNS (Salter and Stevens, 2017; Butovsky and Weiner, 2018). In this review, we focus on microglial dysfunction as it relates to autism spectrum disorder and associated conditions. We also discuss the role of the endogenous cannabinoid system in modulating microglial involvement in these disorders.

## MICROGLIA AND AUTISM SPECTRUM DISORDER

The Autism and Developmental Disabilities Monitoring Network estimates the current prevalence of autism spectrum disorder (ASD) to be 1 in 59 among children in the United States (Baio et al., 2018). ASD denotes a collection of heterogeneous neurodevelopmental disorders defined by (1) repetitive, restricted behaviors and interests and (2) abnormalities in socio-communication (American Psychiatry Association, 2013). Thus, ASD is an umbrella term and it encompasses several disorders including autism, Asperger's syndrome, pervasive developmental disorder not otherwise specified, and childhood disintegrative disorder (American Psychiatry Association, 2013). Conditions frequently comorbid with ASD include intellectual disability, attention-deficit/hyperactivity disorder, epilepsy, perturbed sleep patterns, aggression, anxiety, and altered sensory perception (Leitner, 2014; Srivastava and Schwartz, 2014; Fakhoury, 2015; Park et al., 2016; Postorino et al., 2016). These associated conditions can vary in severity and be more or less common within patient subsets. Finally, due to the lack of available therapeutics for ASD, there is a continuous, pressing need for investigation into the causes and progression of the disorder.

Given their role in CNS development and neuroinflammation, microglia are poised to influence the pathogenesis of ASD (Edmonson et al., 2016; Salter and Stevens, 2017; Lenz and Nelson, 2018). Evidence for microglial involvement in ASD comes from both post-mortem- and positron-emission tomography (PET)-imaging studies which show increased neuroinflammation and numbers of activated microglia in brains of ASD patients (Vargas et al., 2005; Morgan et al., 2010; Suzuki et al., 2013). More recently, a large-scale analysis of transcriptomic datasets from post-mortem cerebral cortex has revealed a distinct microglial signature in ASD brains (Gandal et al., 2018). This is concordant with previous observations of

microglial activation-related gene enrichment in ASD brain-derived gene networks (Voineagu et al., 2011).

Altered synaptic density is observed in post-mortem ASD brain tissue (Hutsler and Zhang, 2010) and ASD mouse models (Comery et al., 1997; Tang et al., 2014; Wang et al., 2017). These alterations are presumably due to deficits in developmental synaptic pruning (Hansel, 2019). Indeed, current thinking posits that microglia can exert control over the progression of ASD through synaptic pruning dysregulation (Di Marco et al., 2016; Lenz and Nelson, 2018). This hypothesis is supported by the finding that inhibiting microglial autophagy leads to increased synaptic density and reduced sociability in mice (Kim et al., 2017). Moreover, mice with loss of microglia-enriched fractalkine receptor CX3C-chemokine receptor 1 (CX3CR1) display impaired synaptic pruning and reduced social interactions (Zhan et al., 2014). Additional support for the involvement of microglia in ASD pathogenesis comes from studies on mouse models of Rett syndrome (RTT), a syndromic form of ASD caused by mutations in the gene encoding methyl-CpG binding protein 2 (MECP2) (Lombardi et al., 2015). In one model of RTT, neuronal, but not microglial, loss of *Mecp2* leads to excessive synaptic engulfment by microglia in later stages of the disease (Schafer et al., 2016). This suggests that neuronal loss of MECP2 is sufficient to induce aberrant microglial activity and it is consistent with the observation that deletion of *Mecp2* using a *Cx3cr1-Cre* line does not produce RTT-like symptoms (Wolf et al., 2017). Furthermore, *Mecp2*-null microglia produce toxic levels of glutamate that damage post-synaptic structures *in vitro* (Maezawa and Jin, 2010). Still, due to the phenotypic and genetic heterogeneity of ASD, it remains to be seen if these findings in RTT models are representative of autism etiology in general.

Finally, children born to mothers who experience infections or autoimmune disease during their pregnancies are more likely to develop ASD (Jiang et al., 2016; Careaga et al., 2017). This phenomenon, known as maternal immune activation (MIA), has been phenocopied in rodent models (Shi et al., 2003; Patterson, 2011; Careaga et al., 2017; Salter and Stevens, 2017). While embryonic microglia may mediate the neuroinflammatory consequences of MIA (Salter and Stevens, 2017), how this underlies ASD remains unclear.

Considered together, the aforementioned findings implicate microglia as targets for the treatment of ASD. Due to its anti-inflammatory effects, the endogenous cannabinoid (endocannabinoid) system represents a promising tool for modulating microglial involvement in ASD. We next provide a brief overview of the endocannabinoid system and then summarize the evidence linking microglial-endocannabinoid signaling to ASD, with attention paid to issues surrounding the use of recreational cannabis.

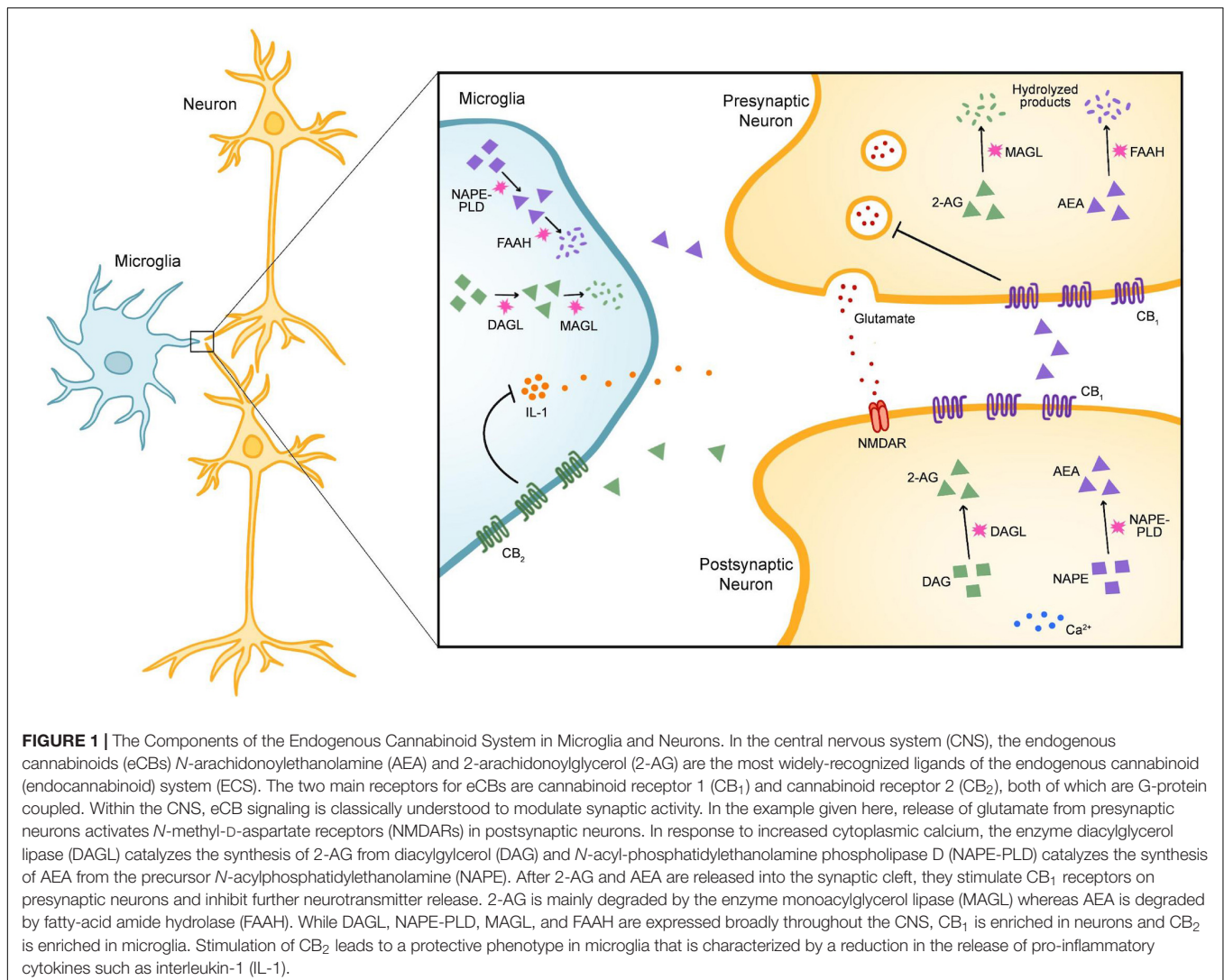
## THE ARCHITECTURE OF THE ENDOCANNABINOID SYSTEM

The endocannabinoid system (ECS) exerts control over microglial activity and therefore shows promise for treating

CNS dysfunction (Benito et al., 2008; Stella, 2009; Lisboa et al., 2016). The ECS consists of three major components: (1) small, lipid-derived endocannabinoids (eCBs), (2) the enzymes responsible for synthesizing and degrading eCBs, and (3) the metabotropic receptors that recognize eCBs (Lutz et al., 2015). The most well-known eCBs in the brain are *N*-arachidonylethanolamine (AEA or anandamide) and 2-arachidonoylglycerol (2-AG) (Lutz et al., 2015; Parsons and Hurd, 2015). In response to increased cytoplasmic calcium, 2-AG and AEA are synthesized on demand from lipid precursors by the enzymes diacylglycerol lipase (DAGL) and *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), respectively (Alger and Kim, 2011; Lutz et al., 2015; Parsons and Hurd, 2015). The enzyme primarily responsible for degrading 2-AG is monoacylglycerol lipase (MAGL), whereas AEA is catabolized by fatty acid amide hydrolase (FAAH). In the CNS, these components are expressed in neurons, microglia, astrocytes, and oligodendrocytes (Figure 1; Stella, 2009; Lutz et al., 2015; Ilyasov et al., 2018). The two

main receptors for eCBs include cannabinoid receptors 1 (CB<sub>1</sub>) and 2 (CB<sub>2</sub>), both of which are G protein-coupled (Parsons and Hurd, 2015). Finally, while CB<sub>1</sub> is enriched in neurons, CB<sub>2</sub> expression is primarily restricted to microglia (Stella, 2009).

Acute consumption of *Cannabis sativa* (marijuana) yields wide-ranging effects on memory, cognition, appetite, and mood in both humans and rodents (Curran et al., 2016; Kendall and Yudowski, 2016). These effects result from action of the phytocannabinoid (or plant-derived cannabinoid)  $\Delta^9$ -tetrahydrocannabinol (THC) on CB<sub>1</sub> within the brain (Panagis et al., 2014; Curran et al., 2016; Kendall and Yudowski, 2016). Yet, long-term consequences of cannabis use have been poorly studied, especially with regards to microglia and their impact on neuronal circuitry. Notably, increased eCB signaling is associated with an anti-inflammatory, protective phenotype in microglia (Benito et al., 2008; Stella, 2009; Lisboa et al., 2016). For example, pharmacological inhibition of FAAH decreases microglial activation marker expression,



cytokine production, and synaptic plasticity deficits, in the hippocampi of aged rats (Murphy et al., 2012). Additionally, stimulation of CB<sub>2</sub> inhibits microglial activation and increases striatal neuron survival and motor coordination in a model of Huntington's disease excitotoxicity (Palazuelos et al., 2009). Moreover, exposure to anti-inflammatory cytokines increases eCB production and CB<sub>2</sub> expression in microglia (Mecha et al., 2015). These findings cast the ECS as an attractive target for influencing microglial activity (Dhopeswarkar and Mackie, 2014; Lisboa et al., 2016; Cassano et al., 2017; Donvito et al., 2018). However, the consequences of manipulating eCB signaling on ASD risk and pathogenesis are largely uncharacterized. The increasing legality and use of cannabis currently seen throughout the world therefore requires a better understanding of eCB signaling in microglia as it relates to ASD.

## MICROGLIAL-ENDOCANNABINOID SIGNALING AND ASD

### Cannabis Use and ASD Risk

Approximately 2.5–5% of people between the ages of 15–64 years old consume cannabis, making it the most popular illicit drug in the world (Gunn et al., 2016). THC readily crosses the fetal-placental barrier (Wu et al., 2011) and is also secreted in breast milk (Perez-Reyes and Wall, 1982). As of now, there is no strong link between prenatal cannabis use and an increased risk of ASD in offspring. Prenatal exposure to cannabis does correlate with negative outcomes in child development, including growth restriction (Zuckerman et al., 1989; El Marroun et al., 2009; Gunn et al., 2016) and decreased cognitive performance (Richardson et al., 2002; Goldschmidt et al., 2004; Gunn et al., 2016). Still, few studies have been carried out on this topic and these observations are not always reproducible (Wu et al., 2011; El Marroun et al., 2018). Since rates of cannabis use in both pregnant and non-pregnant women are steadily increasing (Brown et al., 2017), there is an unmet need for clarifying the relationship between prenatal cannabis exposure and CNS development. Thus, future clinical and pre-clinical investigations should focus on elucidating the long-lasting effects of prenatal cannabis exposure and if these effects are linked to ASD pathogenesis. Such studies should be paired with efforts to determine if prenatal cannabis exposure impacts microglial synaptic pruning and thereby neurodevelopment in general. These experiments could also take place in the context of pathogen-induced MIA to better recapitulate environmental risks for ASD.

### Cannabinoid Signaling as a Target for ASD Treatment

Pre-clinical evidence supporting the role of ECS signaling in ASD comes from research on rodent models of MIA and neuroinflammation. For instance, in response to the innate immunostimulant polyinosinic:polycytidylic acid [poly(I:C)], MIA-based production of IL-17 induces abnormal cortical

development and ASD-like sociability deficits in mouse offspring (Choi et al., 2016). Interestingly, administration of AEA decreases IL-17 production and increases expression of the anti-inflammatory cytokine IL-10 in a mouse model of immune hypersensitivity (Jackson et al., 2014). Treatment with the innate immunostimulant lipopolysaccharide (LPS) during adolescence leads to increased AEA tone and FAAH activity in the amygdala, as well as decreased sociability, in mice (Doenni et al., 2016). These alterations are attenuated with administration of an FAAH inhibitor (Doenni et al., 2016). Still, the contribution of microglia to either the initiation or resolution of these neuroinflammatory effects is unknown and should be the focus of future endeavors.

Cannabidiol (CBD), the second major phytocannabinoid in cannabis (Atakan, 2012), has gained attention as a possible treatment for ASD (Salgado and Castellanos, 2018). Indeed, three clinical reports have recently established that CBD alleviates major symptoms associated with ASD, including seizures, sleeplessness, and anxiety, in children (Barchel et al., 2018; Aran et al., 2019; Bar-Lev Schleider et al., 2019). In addition, because CBD possess a weak affinity for CB<sub>1</sub> and CB<sub>2</sub>, it has no psychoactive effects and may even prevent some of the harmful consequences of THC (Zuardi et al., 2012; Morales et al., 2017; Mouro et al., 2019). Lastly, a commercially available, oral CBD extract (Epidiolex) has recently gained FDA-approval for treatment of drug-resistant epilepsy (Sekar and Pack, 2019), which can be an additional burden faced by ASD patients (Sansa et al., 2011; Kokoszka et al., 2017; Long et al., 2019). Nevertheless, because exposure to other cannabinoids negatively affects the development of the adolescent brain in rats (Cha et al., 2006; Schneider and Koch, 2007; Quinn et al., 2008), parents and physicians should practice extreme caution when recommending the use of cannabinoids to treat ASD (Atakan, 2012).

Subsequent work in this field must emphasize replicating the usefulness of ECS signaling in ASD via paradigms that include larger and more diverse populations. If these results hold, it will be important to establish if abatement of ASD symptoms is due to eCB signaling in microglia. For example, CBD blocks microglial activation (Martin-Moreno et al., 2011) and neuroinflammation (Elliott et al., 2018; Maroon and Bost, 2018), both of which are linked to seizure susceptibility (Rana and Musto, 2018; Zhao et al., 2018). Consequently, it will be beneficial to establish if CBD-based reduction of epilepsy in ASD patients is reliant on microglial-based mechanisms. Utilizing mice with microglia-specific loss of ECS components in combination with ASD-relevant mouse models could shed light on this area.

## CONCLUSION

Microglia are indispensable orchestrators of CNS development and homeostasis and are therefore likely involved in the pathogenesis of ASD. Microglial activity can be modulated by eCB signaling, which makes the ECS a potentially forceful tool in the prevention and management of CNS dysfunction.



Future work must focus on detailing the mechanisms by which altered eCB signaling in microglia yields protective and detrimental effects in the CNS, particularly as it relates to the effects of chronic cannabis use. Answering these questions could provide improved therapeutics for ASD and its associated conditions.

## AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. KT and DA designed the figure.

## REFERENCES

- Alger, B. E., and Kim, J. (2011). Supply and demand for endocannabinoids. *Trends Neurosci.* 34, 304–315. doi: 10.1016/j.tins.2011.03.003
- Alliot, F., Godin, I., and Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res. Dev. Brain Res.* 117, 145–152. doi: 10.1016/s0165-3806(99)00113-3
- American Psychiatry Association, (2013). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*, 5th Edn. Arlington, VA: American Psychiatry Association.
- Aran, A., Cassuto, H., Lubotzky, A., Wattad, N., and Hazan, E. (2019). Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems—a retrospective feasibility study. *J. Autism Dev. Disord.* 49, 1284–1288. doi: 10.1007/s10803-018-3808-2
- Askew, K., Li, K., Olmos-Alonso, A., Garcia-Moreno, F., Liang, Y., Richardson, P., et al. (2017). Coupled proliferation and apoptosis maintain the rapid turnover of microglia in the adult brain. *Cell Rep.* 18, 391–405. doi: 10.1016/j.celrep.2016.12.041
- Atakan, Z. (2012). Cannabis, a complex plant: different compounds and different effects on individuals. *Ther. Adv. Psychopharmacol.* 2, 241–254. doi: 10.1177/2045125312457586
- Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., et al. (2018). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2014. *MMWR Surveill. Summ.* 67, 1–23. doi: 10.15585/mmwr.ss6706a1
- Barchel, D., Stolar, O., De-Haan, T., Ziv-Baran, T., Saban, N., Fuchs, D. O., et al. (2018). Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and Co-morbidities. *Front. Pharmacol.* 9:1521. doi: 10.3389/fphar.2018.01521
- Bar-Lev Schleider, L., Mechoulam, R., Saban, N., Meiri, G., and Novack, V. (2019). Real life experience of medical cannabis treatment in autism: analysis of safety and efficacy. *Sci. Rep.* 9:200. doi: 10.1038/s41598-018-37570-y
- Benito, C., Tolon, R. M., Pazos, M. R., Nunez, E., Castillo, A. I., and Romero, J. (2008). Cannabinoid CB2 receptors in human brain inflammation. *Br. J. Pharmacol.* 153, 277–285.
- Bialas, A. R., and Stevens, B. (2013). TGF- $\beta$  signaling regulates neuronal C1q expression and developmental synaptic refinement. *Nat. Neurosci.* 16, 1773–1782. doi: 10.1038/nn.3560
- Brown, Q. L., Sarvet, A. L., Shmulewitz, D., Martins, S. S., Wall, M. M., and Hasin, D. S. (2017). Trends in marijuana use among pregnant and nonpregnant reproductive-aged women, 2002–2014. *JAMA* 317, 207–209.
- Bruttger, J., Karram, K., Wortge, S., Regen, T., Marini, F., Hoppmann, N., et al. (2015). Genetic cell ablation reveals clusters of local self-renewing microglia in the mammalian central nervous system. *Immunity* 43, 92–106. doi: 10.1016/j.immuni.2015.06.012
- Butovsky, O., and Weiner, H. L. (2018). Microglial signatures and their role in health and disease. *Nat. Rev. Neurosci.* 19, 622–635. doi: 10.1038/s41583-018-0057-5
- Careaga, M., Murai, T., and Bauman, M. D. (2017). Maternal immune activation and autism spectrum disorder: from rodents to nonhuman and human primates. *Biol. Psychiatry* 81, 391–401. doi: 10.1016/j.biopsych.2016.10.020
- Cassano, T., Calcagnini, S., Pace, L., De Marco, F., Romano, A., and Gaetani, S. (2017). Cannabinoid receptor 2 signaling in neurodegenerative disorders: from pathogenesis to a promising therapeutic target. *Front. Neurosci.* 11:30. doi: 10.3389/fnins.2017.00030
- Cha, Y. M., White, A. M., Kuhn, C. M., Wilson, W. A., and Swartzwelder, H. S. (2006). Differential effects of delta9-THC on learning in adolescent and adult rats. *Pharmacol. Biochem. Behav.* 83, 448–455. doi: 10.1016/j.pbb.2006.03.006
- Choi, G. B., Yim, Y. S., Wong, H., Kim, S., Kim, H., Kim, S. V., et al. (2016). The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* 351, 933–939. doi: 10.1126/science.aad0314
- Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., et al. (1997). Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proc. Natl. Acad. Sci. U.S.A.* 94, 5401–5404. doi: 10.1073/pnas.94.10.5401
- Curran, H. V., Freeman, T. P., Mokrysz, C., Lewis, D. A., Morgan, C. J., and Parsons, L. H. (2016). Keep off the grass? Cannabis, cognition and addiction. *Nat. Rev. Neurosci.* 17, 293–306. doi: 10.1038/nrn.2016.28
- Dhopeswarkar, A., and Mackie, K. (2014). CB2 Cannabinoid receptors as a therapeutic target—what does the future hold? *Mol. Pharmacol.* 86, 430–437. doi: 10.1124/mol.114.094649
- Di Marco, B., Bonaccorso, C. M., Aloisi, E., D'Antoni, S., and Catania, M. V. (2016). Neuro-Inflammatory mechanisms in developmental disorders associated with intellectual disability and autism spectrum disorder: a neuro-immune perspective. *CNS Neurol. Disord. Drug Targets* 15, 448–463. doi: 10.2174/1871527315666160321105039
- Doenni, V. M., Gray, J. M., Song, C. M., Patel, S., Hill, M. N., and Pittman, Q. J. (2016). Deficient adolescent social behavior following early-life inflammation is ameliorated by augmentation of anandamide signaling. *Brain Behav. Immun.* 58, 237–247. doi: 10.1016/j.bbi.2016.07.152
- Donvito, G., Nass, S. R., Wilkerson, J. L., Curry, Z. A., Schurman, L. D., Kinsey, S. G., et al. (2018). The endogenous cannabinoid system: a budding source of targets for treating inflammatory and neuropathic pain. *Neuropsychopharmacology* 43, 52–79. doi: 10.1038/npp.2017.204
- Edmonson, C. A., Ziats, M. N., and Rennert, O. M. (2016). A non-inflammatory role for microglia in autism spectrum disorders. *Front. Neurol.* 7:9. doi: 10.3389/fneur.2016.00009
- El Marroun, H., Brown, Q. L., Lund, I. O., Coleman-Cowger, V. H., Loree, A. M., Chawla, D., et al. (2018). An epidemiological, developmental and clinical overview of cannabis use during pregnancy. *Prev. Med.* 116, 1–5. doi: 10.1016/j.jypmed.2018.08.036
- El Marroun, H., Tiemeier, H., Steegers, E. A., Jaddoe, V. W., Hofman, A., Verhulst, F. C., et al. (2009). Intrauterine cannabis exposure affects fetal growth trajectories: the generation R study. *J. Am. Acad. Child Adolesc. Psychiatry* 48, 1173–1181. doi: 10.1097/CHI.0b013e3181bfa8ee
- Elliott, D. M., Singh, N., Nagarkatti, M., and Nagarkatti, P. S. (2018). Cannabidiol attenuates experimental autoimmune encephalomyelitis model of multiple sclerosis through induction of myeloid-derived suppressor cells. *Front. Immunol.* 9:1782. doi: 10.3389/fimmu.2018.01782
- Fakhoury, M. (2015). Autistic spectrum disorders: a review of clinical features, theories and diagnosis. *Int. J. Dev. Neurosci.* 43, 70–77. doi: 10.1016/j.ijdevneu.2015.04.003

## FUNDING

This work was supported by a National Institutes of Health (NIH) postdoctoral training grant (T32AI100829-06A1 to DA) and an NIH research project grant (1R01HD092093 to KS).

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Marissa Co for providing invaluable comments in this manuscript.

- Gandal, M. J., Haney, J. R., Parikshak, N. N., Leppa, V., Ramaswami, G., Hartl, C., et al. (2018). Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. *Science* 359, 693–697. doi: 10.1126/science.aad6469
- Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., et al. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330, 841–845. doi: 10.1126/science.1194637
- Goldschmidt, L., Richardson, G. A., Cornelius, M. D., and Day, N. L. (2004). Prenatal marijuana and alcohol exposure and academic achievement at age 10. *Neurotoxicol. Teratol.* 26, 521–532. doi: 10.1016/j.ntt.2004.04.003
- Gunn, J. K., Rosales, C. B., Center, K. E., Nunez, A., Gibson, S. J., Christ, C., et al. (2016). Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. *BMJ Open* 6:e009986. doi: 10.1136/bmjopen-2015-009986
- Hansel, C. (2019). Deregulation of synaptic plasticity in autism. *Neurosci. Lett.* 688, 58–61. doi: 10.1016/j.neulet.2018.02.003
- Huang, Y., Xu, Z., Xiong, S., Sun, F., Qin, G., Hu, G., et al. (2018). Repopulated microglia are solely derived from the proliferation of residual microglia after acute depletion. *Nat. Neurosci.* 21, 530–540. doi: 10.1038/s41593-018-0090-8
- Hutsler, J. J., and Zhang, H. (2010). Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res.* 1309, 83–94. doi: 10.1016/j.brainres.2009.09.120
- Ilyasov, A. A., Milligan, C. E., Pharr, E. P., and Howlett, A. C. (2018). The endocannabinoid system and oligodendrocytes in health and disease. *Front. Neurosci.* 12:733. doi: 10.3389/fnins.2018.00733
- Jackson, A. R., Nagarkatti, P., and Nagarkatti, M. (2014). Anandamide attenuates Th-17 cell-mediated delayed-type hypersensitivity response by triggering IL-10 production and consequent microRNA induction. *PLoS One* 9:e93954. doi: 10.1371/journal.pone.0093954
- Janda, E., Boi, L., and Carta, A. R. (2018). Microglial phagocytosis and its regulation: a therapeutic target in Parkinson's disease? *Front. Mol. Neurosci.* 11:144. doi: 10.3389/fnmol.2018.00144
- Jiang, H. Y., Xu, L. L., Shao, L., Xia, R. M., Yu, Z. H., Ling, Z. X., et al. (2016). Maternal infection during pregnancy and risk of autism spectrum disorders: a systematic review and meta-analysis. *Brain Behav. Immun.* 58, 165–172. doi: 10.1016/j.bbi.2016.06.005
- Kendall, D. A., and Yudowski, G. A. (2016). Cannabinoid receptors in the central nervous system: their signaling and roles in disease. *Front. Cell Neurosci.* 10:294. doi: 10.3389/fncel.2016.00294
- Kim, H. J., Cho, M. H., Shim, W. H., Kim, J. K., Jeon, E. Y., Kim, D. H., et al. (2017). Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. *Mol. Psychiatry* 22, 1576–1584. doi: 10.1038/mp.2016.103
- Kokoszka, M. A., McGoldrick, P. E., La Vega-Talbot, M., Raynes, H., Palmese, C. A., Wolf, S. M., et al. (2017). Epilepsy surgery in patients with autism. *J. Neurosurg. Pediatr.* 19, 196–207. doi: 10.3171/2016.7.PEDS1651
- Leitner, Y. (2014). The co-occurrence of autism and attention deficit hyperactivity disorder in children - what do we know? *Front. Hum. Neurosci.* 8:268. doi: 10.3389/fnhum.2014.00268
- Lenz, K. M., and Nelson, L. H. (2018). Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. *Front. Immunol.* 9:698. doi: 10.3389/fimmu.2018.00698
- Lisboa, S. F., Gomes, F. V., Guimaraes, F. S., and Campos, A. C. (2016). Microglial cells as a link between cannabinoids and the immune hypothesis of psychiatric disorders. *Front. Neurol.* 7:5. doi: 10.3389/fneur.2016.00005
- Lombardi, L. M., Baker, S. A., and Zoghbi, H. Y. (2015). MECP2 disorders: from the clinic to mice and back. *J. Clin. Invest.* 125, 2914–2923. doi: 10.1172/JCI78167
- Long, S., Zhou, H., Li, S., Wang, T., Ma, Y., Li, C., et al. (2019). The clinical and genetic features of co-occurring epilepsy and autism spectrum disorder in chinese children. *Front. Neurol.* 10:505. doi: 10.3389/fneur.2019.00505
- Lutz, B., Marsicano, G., Maldonado, R., and Hillard, C. J. (2015). The endocannabinoid system in guarding against fear, anxiety and stress. *Nat. Rev. Neurosci.* 16, 705–718. doi: 10.1038/nrn4036
- Maewawa, I., and Jin, L. W. (2010). Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J. Neurosci.* 30, 5346–5356. doi: 10.1523/JNEUROSCI.5966-09.2010
- Maroon, J., and Bost, J. (2018). Review of the neurological benefits of phytocannabinoids. *Surg. Neurol. Int.* 9:91. doi: 10.4103/sni.sni\_45\_18
- Martin-Moreno, A. M., Reigada, D., Ramirez, B. G., Mechoulam, R., Innamorato, N., Cuadrado, A., et al. (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Mol. Pharmacol.* 79, 964–973. doi: 10.1124/mol.111.071290
- Mecha, M., Feliu, A., Carrillo-Salinas, F. J., Rueda-Zubiaurre, A., Ortega-Gutierrez, S., de Sola, R. G., et al. (2015). Endocannabinoids drive the acquisition of an alternative phenotype in microglia. *Brain Behav. Immun.* 49, 233–245. doi: 10.1016/j.bbi.2015.06.002
- Morales, P., Hurst, D. P., and Reggio, P. H. (2017). Molecular targets of the phytocannabinoids: a complex picture. *Prog. Chem. Org. Nat. Prod.* 103, 103–131. doi: 10.1007/978-3-319-45541-9\_4
- Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., et al. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol. Psychiatry* 68, 368–376. doi: 10.1016/j.biopsych.2010.05.024
- Mouro, F. M., Miranda-Lourenco, C., Sebastiao, A. M., and Diogenes, M. J. (2019). From cannabinoids and neurosteroids to statins and the ketogenic diet: new therapeutic avenues in rett syndrome? *Front. Neurosci.* 13:680. doi: 10.3389/fnins.2019.00680
- Murphy, N., Cowley, T. R., Blau, C. W., Dempsey, C. N., Noonan, J., Gowran, A., et al. (2012). The fatty acid amide hydrolase inhibitor URB597 exerts anti-inflammatory effects in hippocampus of aged rats and restores an age-related deficit in long-term potentiation. *J. Neuroinflammation* 9:79. doi: 10.1186/1742-2094-9-79
- Nakajima, K., Honda, S., Tohyama, Y., Imai, Y., Kohsaka, S., and Kurihara, T. (2001). Neurotrophin secretion from cultured microglia. *J. Neurosci. Res.* 65, 322–331. doi: 10.1002/jnr.1157
- Palazuelos, J., Aguado, T., Pazos, M. R., Julien, B., Carrasco, C., Resel, E., et al. (2009). Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain* 132, 3152–3164. doi: 10.1093/brain/awp239
- Panagis, G., Mackey, B., and Vlachou, S. (2014). Cannabinoid regulation of brain reward processing with an emphasis on the role of CB1 receptors: a step back into the future. *Front. Psychiatry* 5:92. doi: 10.3389/fpsy.2014.00092
- Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458. doi: 10.1126/science.1202529
- Park, H. R., Lee, J. M., Moon, H. E., Lee, D. S., Kim, B. N., Kim, J., et al. (2016). A short review on the current understanding of autism spectrum disorders. *Exp. Neurobiol.* 25, 1–13. doi: 10.5607/en.2016.25.1.1
- Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R. III, Lafaille, J. J., et al. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* 155, 1596–1609. doi: 10.1016/j.cell.2013.11.030
- Parsons, L. H., and Hurd, Y. L. (2015). Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neurosci.* 16, 579–594. doi: 10.1038/nrn4004
- Patterson, P. H. (2011). Maternal infection and immune involvement in autism. *Trends Mol. Med.* 17, 389–394. doi: 10.1016/j.molmed.2011.03.001
- Perez-Reyes, M., and Wall, M. E. (1982). Presence of delta-9-tetrahydrocannabinol in human milk. *N. Engl. J. Med.* 307, 819–820. doi: 10.1056/nejm198209233071311
- Postorino, V., Fatta, L. M., Sanges, V., Giovagnoli, G., De Peppo, L., Vicari, S., et al. (2016). Intellectual disability in autism spectrum disorder: investigation of prevalence in an Italian sample of children and adolescents. *Res. Dev. Disabil.* 48, 193–201. doi: 10.1016/j.ridd.2015.10.020
- Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., et al. (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* 33, 1113–1126. doi: 10.1038/sj.npp.1301475
- Rana, A., and Musto, A. E. (2018). The role of inflammation in the development of epilepsy. *J. Neuroinflammation* 15:144. doi: 10.1186/s12974-018-1192-7
- Ribeiro Xavier, A. L., Kress, B. T., Goldman, S. A., Lacerda de Menezes, J. R., and Nedergaard, M. (2015). A distinct population of microglia supports adult neurogenesis in the subventricular zone. *J. Neurosci.* 35, 11848–11861. doi: 10.1523/JNEUROSCI.1217-15.2015

- Richardson, G. A., Ryan, C., Willford, J., Day, N. L., and Goldschmidt, L. (2002). Prenatal alcohol and marijuana exposure: effects on neuropsychological outcomes at 10 years. *Neurotoxicol. Teratol.* 24, 309–320. doi: 10.1016/s0892-0362(02)00193-9
- Saijo, K., and Glass, C. K. (2011). Microglial cell origin and phenotypes in health and disease. *Nat. Rev. Immunol.* 11, 775–787. doi: 10.1038/nri3086
- Salgado, C. A., and Castellanos, D. (2018). Autism spectrum disorder and cannabidiol: have we seen this movie before? *Glob. Pediatr. Health* 5:2333794X18815412.
- Salter, M. W., and Stevens, B. (2017). Microglia emerge as central players in brain disease. *Nat. Med.* 23, 1018–1027. doi: 10.1038/nm.4397
- Sansa, G., Carlson, C., Doyle, W., Weiner, H. L., Bluvstein, J., Barr, W., et al. (2011). Medically refractory epilepsy in autism. *Epilepsia* 52, 1071–1075. doi: 10.1111/j.1528-1167.2011.03069.x
- Schafer, D. P., Heller, C. T., Gunner, G., Heller, M., Gordon, C., Hammond, T., et al. (2016). Microglia contribute to circuit defects in *Mecp2* null mice independent of microglia-specific loss of *Mecp2* expression. *eLife* 5:e15224. doi: 10.7554/eLife.15224
- Schneider, M., and Koch, M. (2007). The effect of chronic peripubertal cannabinoid treatment on deficient object recognition memory in rats after neonatal mPFC lesion. *Eur. Neuropsychopharmacol.* 17, 180–186. doi: 10.1016/j.euroneuro.2006.03.009
- Sekar, K., and Pack, A. (2019). Epidiolex as adjunct therapy for treatment of refractory epilepsy: a comprehensive review with a focus on adverse effects. *F1000Res* 8:234. doi: 10.12688/f1000research.16515.1
- Shi, L., Fatemi, S. H., Sidwell, R. W., and Patterson, P. H. (2003). Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J. Neurosci.* 23, 297–302. doi: 10.1523/jneurosci.23-01-00297.2003
- Shigemoto-Mogami, Y., Hoshikawa, K., Goldman, J. E., Sekino, Y., and Sato, K. (2014). Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. *J. Neurosci.* 34, 2231–2243. doi: 10.1523/JNEUROSCI.1619-13.2014
- Sierra, A., Encinas, J. M., Deudero, J. J., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., et al. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7, 483–495. doi: 10.1016/j.stem.2010.08.014
- Srinivasan, B., Roque, C. H., Hempstead, B. L., Al-Ubaidi, M. R., and Roque, R. S. (2004). Microglia-derived pronerve growth factor promotes photoreceptor cell death via p75 neurotrophin receptor. *J. Biol. Chem.* 279, 41839–41845. doi: 10.1074/jbc.m402872200
- Srivastava, A. K., and Schwartz, C. E. (2014). Intellectual disability and autism spectrum disorders: causal genes and molecular mechanisms. *Neurosci. Biobehav. Rev.* 46(Pt 2), 161–174. doi: 10.1016/j.neubiorev.2014.02.015
- Stella, N. (2009). Endocannabinoid signaling in microglial cells. *Neuropharmacology* 56(Suppl. 1), 244–253. doi: 10.1016/j.neuropharm.2008.07.037
- Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., et al. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell* 131, 1164–1178. doi: 10.1016/j.cell.2007.10.036
- Suzuki, K., Sugihara, G., Ouchi, Y., Nakamura, K., Futatsubashi, M., Takebayashi, K., et al. (2013). Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry* 70, 49–58.
- Tang, G., Gudsnuk, K., Kuo, S. H., Cotrina, M. L., Rosoklija, G., Sosunov, A., et al. (2014). Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron* 83, 1131–1143. doi: 10.1016/j.neuron.2014.07.040
- Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., and Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann. Neurol.* 57, 67–81. doi: 10.1002/ana.20315
- Voineagu, I., Wang, X., Johnston, P., Lowe, J. K., Tian, Y., Horvath, S., et al. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474, 380–384. doi: 10.1038/nature10110
- Wang, M., Li, H., Takumi, T., Qiu, Z., Xu, X., Yu, X., et al. (2017). Distinct defects in spine formation or pruning in two gene duplication mouse models of autism. *Neurosci. Bull.* 33, 143–152. doi: 10.1007/s12264-017-0111-8
- Wolf, Y., Boura-Halfon, S., Cortese, N., Haimon, Z., Sar Shalom, H., Kuperman, Y., et al. (2017). Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. *Nat. Immunol.* 18, 665–674. doi: 10.1038/ni.3746
- Wu, C. S., Jew, C. P., and Lu, H. C. (2011). Lasting impacts of prenatal cannabis exposure and the role of endogenous cannabinoids in the developing brain. *Future Neurol.* 6, 459–480. doi: 10.2217/fnl.11.27
- Yang, I., Han, S. J., Kaur, G., Crane, C., and Parsa, A. T. (2010). The role of microglia in central nervous system immunity and glioma immunology. *J. Clin. Neurosci.* 17, 6–10. doi: 10.1016/j.jocn.2009.05.006
- Zhan, Y., Paolicelli, R. C., Sforzini, F., Weinhard, L., Bolasco, G., Pagani, F., et al. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat. Neurosci.* 17, 400–406. doi: 10.1038/nn.3641
- Zhao, H., Zhu, C., and Huang, D. (2018). Microglial activation: an important process in the onset of epilepsy. *Am. J. Transl. Res.* 10, 2877–2889.
- Zuardi, A. W., Crippa, J. A., Hallak, J. E., Bhattacharyya, S., Atakan, Z., Martin-Santos, R., et al. (2012). A critical review of the antipsychotic effects of cannabidiol: 30 years of a translational investigation. *Curr. Pharm. Des.* 18, 5131–5140. doi: 10.2174/138161212802884681
- Zuckerman, B., Frank, D. A., Hingson, R., Amaro, H., Levenson, S. M., Kayne, H., et al. (1989). Effects of maternal marijuana and cocaine use on fetal growth. *N. Engl. J. Med.* 320, 762–768. doi: 10.1056/nejm198903233201203

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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