Hybrid Brain-Tissue Based Model to Study the Role of Infiltrating Monocytes in Late-Stage Huntington's Disease

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

Huntington's Disease (HD) is an inherited, progressive, fatal, and late-onset neurodegenerative disorder. Its neuropathogenesis is characterized by selective loss of medium spiny neurons (MSNs) in the striatum and cortical neurons in the cortex. It is caused by an expansion of CAG repeats in the exon-1 of *IT-15* gene, which results in a mutated polyglutamine domain in the huntingtin protein (HTT) (Collaborative, 1993). The mutation that causes HD was identified almost thirty years ago, yet the ultimate cause of neuron death is still uncertain. This dissertation is based on the hypothesis that monocytes infiltrate the brain of late-stage HD patients and drive neuropathogenesis. To test this hypothesis, I developed a novel hybrid brain-slice experimental model in which human monocytes (MO) isolated from HD and non-disease control patients (CTRL) are engrafted into living postnatal striatal brain tissues prepared from neonatal rats.

In the first set of experiments, the inflammatory response of stimulated HD and CTRL MO were tested. HD MO exhibited an increased inflammatory response demonstrated by increased production of the cytokine IL-10. Furthermore, a late-stage HD patient exhibited an increased production of multiple cytokines, including IL-10.

The second set of experiments utilized a brain-tissue based model to measure the impact of engrafting HD MO on MSN health. In brain slices engrafted with HD MO, there was a decrease in the number of healthy MSNs. A further experiment revealed that stimulating MO prior to their engraftment differentially influenced the survival of MSNs depending on whether the MO came from HD or CTRL patients. Measurement of cytokine production from these unstimulated/stimulated engraftment experiments

revealed a trend for increased cytokine production in samples taken from a moderate-stage HD patient.

The third set of experiments assessed the ability of HD MO to influence microglia, the endogenous macrophages within the brain-slice tissue. MO engraftment, independent of MO genotype, altered microglia morphology within brain slices. Further results from these experiments demonstrated that the presence of HD MO increased microglial density and upregulated their phagocytosis of MSNs. While the mechanisms underlying these multicellular interactions remain to be determined, it is possible that such an increase in phagocytosis could lead to the observation of fewer healthy MSNs as quantified in the second set of experiments.

Together, these three sets of experiments support the application of a novel model to study neuropathogenesis and highlight that the genotype of infiltrating MO has the potential to quantifiably alter intercellular interactions and neuronal health in the context of HD.

Dedication

First and foremost, this work is dedicated to the Huntington's disease community: to the patients and their families who brave this devastating disease, to the clinicians and researchers who tirelessly search for treatments and the cure, and to those who live every day knowing that only hope can overcome that which we are otherwise powerless to prevent.

In addition, I would like to dedicate this work to the memory of Peggy Trice, a devoted phlebotomist, wife, mother, and daughter, whose humor and optimism brought joy to everyone that had the privilege of meeting her.

I couldn't have completed this journey without my family and friends. To my father, Ernesto, you taught me to pursue my aspirations with tenacity, a curious mind, and humor. You took time out of your busy career to coach me to be an athlete and a team player. You taught me to love nature and to appreciate things most of my peers took for granted. Thank you for continuing to remind me of the importance of honoring my commitments. To my mother, Hazel, your strength and determination inspire me to be a better person. You instilled within me the importance of unconditional love. You taught me that no matter where this life takes me, I'll always have my family to keep me grounded. I'm eternally grateful to you for giving me the confidence and courage to face all the seemingly insurmountable challenges that life has thrown my way. Through our family trips to El Salvador and Scotland, you both introduced me to vastly different cultures and you broadened my horizons to a world outside of the United States. To my brother, Ryan, thank you for tolerating me and allowing me to tag along with your older friends when I didn't have my own. Here's to our transatlantic adventures; one day I

hope to return the favor. To my sister, Eila, thank you for bringing out the silly side of me and reminding me to value the intangible aspects of life. To my friends Joshua and Brandon who traveled this state with me, thank you for demonstrating a different and healthier way of enjoying life along the way. Finally, to my best friend and partner, Lizzie, thank you for supporting me through the tough times, introducing me to my Durham family, and teaching me to find the joy.

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Finally, I am eternally grateful for my mentor Dr. Donald C. Lo. I immediately realized that Don is one of a kind. He's one of the most intelligent people I've ever worked with, yet he remains extraordinarily humble and down-to-earth. He cares deeply about the Huntington's family community. I distinctly remember the first time I met someone with Huntington's disease; Don introduced me to him in a way that was both respectful of the seriousness of the disease and yet also chiefly optimistic. I've attended several HD-related events, and Don is universally loved by the community and its researchers. He simply puts everyone around him at ease. He supported me through all the bumps in the road that came with graduate school by offering me invaluable advice on writing, speaking, teaching, collaborating, and navigating the politics of our field. His adventurous attitude and optimistic approach to science never cease to inspire me. In my eyes, Don is a model for what a scientist should be. He is a genuinely caring person who is devoted to furthering his field but also prioritizes helping people, his family, and those he encounters along the way. It has been an honor to learn from and work with him.

Chapter 1. Introduction

It is hard to imagine anything more terrifying for a family than a diagnosis of Huntington's disease (HD). HD is a progressive neurodegenerative disorder that usually appears between the third and fourth decades of life. It is caused by a genetic mutation in the *huntingtin* gene, also referred to as *HTT*. Although this gene encodes a protein that is expressed in nearly every cell in the human body, its exact function is not known. *HTT* was the first disease-associated gene to be mapped to a human chromosome; the disease is caused by a mutation that amplifies a naturally occurring CAG trinucleotide repeat expansion (Collaborative, 1993; Gusella et al., 1983).

HD is an autosomal dominant inherited disease that is clinically characterized by progressive movement dysfunction, cognitive decline culminating in dementia, and a range of behavioral disturbances that often precede diagnosis. Given its prevalence (estimated to be over 30,000 in the U.S.) and media attention, one can surely imagine the heartache of dementia, but the rest of the symptoms of HD are nearly impossible to comprehend without witnessing them. The combination of adult-onset and autosomal dominant inheritance is particularly cruel: it means that those who have it in their family are often adolescents when they are forced to watch one or both of their parents slowly be robbed their identities and physical faculties. To add insult to injury, children of parents with HD have a 50% chance of developing the disease themselves. The heroic efforts of clinicians and researchers led to the discovery of the mutation responsible for HD, and we can now determine with near 100% accuracy whether someone will develop

the disease during their lifetime. While increased CAG trinucleotide repeat expansions are associated with an earlier age of onset, the factors that drive disease progression after it starts are incompletely understood.

1.1 A Brief History of Huntington's Disease

Before the middle of the 19th century, Huntington's disease was often referred to as Huntington's chorea, a word derived from the Greed word for "dance," due to the abnormal involuntary movements that characterize the illness. There are historical descriptions of HD dating back as far as the Middle Ages, and a disease with similar features was known to many generations in families in colonial New England. At that time, this particular manifestation of movement dysfunction was interpreted as a sign of demonic possession, and those who were afflicted with it were executed (Bhattacharyya, 2011). While he wasn't the first to describe the disease, it was how he described it that made George Huntington famous and gave HD its name.

George Huntington was born in East Hampton, New York, USA, in 1850 to a family of physicians. Shortly after he finished medical school, he began reporting on cases of dementia and chorea in his hometown that he and his family observed (Bhattacharyya, 2016). A devoted doctor to his patients, he was described as a humorous and modest man who was endowed with a keen intellect (Bhattacharyya, 2011; Haymaker, 1970). It was his observational prowess that would make him famous. In his early twenties he published a concise description of the disease, "On Chorea" in which he not only vividly characterized the chorea inherent to the disease but he also made three observations, which he identified as "peculiarities," that would prove to be the jumping off point for worldwide recognition of HD: 1) its hereditary nature, 2) a

tendency towards insanity, and 3) its manifestation as a grave disease only in adult life (Bhattacharyya, 2016; Huntington, 1872).

Perhaps the most critical observation George Huntington made was his specific description of the hereditary nature of HD. He wrote that if either or both parents were afflicted, any children they had who lived to adulthood were likely to develop it as well. But if their child did not develop the disease “the thread was broken, and the grandchildren and great-grandchildren of the original shakers may rest assured they will be free from the disease.” This description identified for the first time that HD was a disease of autosomal dominant inheritance and started a medical odyssey that would significantly advance the field of genetics and culminate in the identification of the first human gene-linked disorder.

1.1.1 Contemporary history of HD

George Huntington’s work contributed to worldwide recognition of HD in the medical community, but, 100 years later, researchers hadn’t made much progress on the hereditary nature of the disease. Motor symptoms were attributed to neurodegeneration of the basal ganglia, and cognitive symptoms were attributed to the degeneration of neurons in the cortex, but why these cells died in the first place remained a mystery. The next breakthrough came at the 100-year commemoration ceremony in Columbus, Ohio. Ramon Ávila-Girón traveled from Venezuela to show video recordings of patients he took under the supervision of his mentor, Americo Negrette. Before the commemoration ceremony, Negrette had spent five years meticulously describing and tracing the family trees of the inhabitants of a small Venezuelan town called San Luis, located near Lake

Maracaibo. The disease was locally known as “el mal de San Vito,” but Negrette knew it to be HD based on his detailed observations of dementia, choreiform movements, and its conformation to an autosomal dominant pattern (Okun & Thommi, 2004). The community surrounding Lake Maracaibo would turn out to have the world’s highest prevalence of HD and would eventually provide the immense number of blood samples necessary to hunt down its genetic basis.

Nancy Wexler was one of the attendees of the centennial HD commemoration (Bhattacharyya, 2016). She was driven to attend not only by scientific inquiry but for personal reasons as well; her mother, Leonore Wexler, had recently been diagnosed with HD. Nancy Wexler’s father, Milton Wexler, responded to his wife’s diagnosis by forming a non-profit organization called the Hereditary Disease Foundation (HDF) whose purpose was to find the cure for HD. What Nancy Wexler saw on those videotapes left a lasting impression on her that would inspire her to become the executive director of the Congressional Commission for the Control of Huntington’s Disease and Its Consequences, and to procure the funding necessary to travel to the Lake Maracaibo region to learn what she could from its inhabitants.

In 1979, Nancy Wexler and a colleague, Tom Chase, traveled to Venezuela where they experienced firsthand the devastation Negrette described in his autobiography “Ciudad de Fuego” (Negrette, 1963). The community was ravaged by HD. Nancy Wexler witnessed children that were forced to take care of their choreatic parents and even children who were showing symptoms themselves. Together, Nancy Wexler and Tom Chase led a team that studied more than 18,000 individuals and collected over 4,000 blood samples over twenty years to work out a common pedigree (Goetz, 2013).

1.1.2 The search for genetic markers and identification of the huntingtin gene

With the basic question of how and why brain cells die in HD looming over the scientific community, Nancy Wexler and her team joined forces with David Housman, a molecular geneticist at MIT, to identify the genetic abnormality responsible for the disease. Since the human genome had not yet been sequenced, David Housman and his graduate student, James Gusella, developed markers that could be used to identify the location of genes. These markers were called restriction fragment length polymorphisms, or RFLPs. To find the HD gene, Housman's group needed to find an RFLP adjacent to the HD gene locus that had several different variants (e.g., A, B, C, D). Next, they needed samples from a large pedigree of HD patients to demonstrate that only one of the variants was inherited by those who developed HD while the other variants were only inherited by family members who were spared. Gusella eventually formed his own lab and collected samples from a large extended family in Iowa who suffered from HD to search for such a marker. He teamed up with Nancy Wexler and a population genetics analyst at Indiana University, Michael Conneally, who helped them mine their massive data set and identify the marker. Through this collaborative effort, the group discovered in November 1983 that the general location of the gene responsible for HD was on the short arm of chromosome 4. The identification of the gene itself, however, would require the formation of a scientific "supergroup" named the Huntington's Disease Collaborative Research Group and another 10 years of painstaking research.

The Huntington's Disease Collaborative Research group, or "Gene Hunters" as they called themselves, was made up of six international laboratories led by Hans Lehrach, Francis Collins, and Peter Harper. By 1989 approximately 50 researchers from 10 institutions/universities were members of the Gene Hunters group. Finally, in February of 1993, the group identified the *huntingtin* gene and decided to publish their findings without listing authors in recognition of the fact that their success came not from one individual or idea, but from the minds of all the devoted scientists involved (Collaborative, 1993). Along the way, the Gene Hunters invented 14 new technologies for the handling and manipulation of DNA to find genes. These breakthrough technologies were responsible for the identification of many other disease-related genes including those underlying or associated with familial forms of cystic fibrosis, amyotrophic lateral sclerosis, Alzheimer's disease, breast cancer, and numerous others (Glimm, 2005). Indeed, this group would eventually inspire the formation of the Human Genome Project that would sequence the human genome 10 years later.

1.1.3 The mutation that causes HD

The prevalence of juvenile HD is exceedingly rare; many clinicians who treat HD patients will not see a single case for the duration of their practice. However, the historically isolated nature of some HD-afflicted communities, such as those in the Lake Maracaibo region, has allowed the mutation to persist and foster the emergence of early-onset alleles in a disease that otherwise does typically not manifest until adulthood. One such case was vital for the discovery that HD is a trinucleotide repeat expansion disorder. Gusella was examining a DNA sample taken from a child named Angel who

developed HD at age 2 and died before reaching his 12th birthday. In analyzing Angel's DNA, Gusella noticed a sequence that turned out to be an exceedingly long CAG expansion. This discovery turned out to be instrumental both for the identification of the gene's location and for the recognition of the mutation as a trinucleotide expansion (Glimm, 2005).

1.1.4 The neuropathogenesis of HD

The neuropathogenesis of HD is primarily characterized by neurodegeneration in the central nervous system. In *post mortem* tissue of HD brains, the degeneration of cortical neurons and white matter is apparent, but the most striking atrophy occurs in the basal ganglia, especially the loss of medium spiny neurons (MSNs) in the striatum (Stoffers et al., 2010; Vonsattel et al., 1985). Indeed, MRI studies have revealed a loss of striatal volume that starts before the onset of symptoms and progressively worsens with disease advancement (Aylward, 2007; Aylward et al., 2000).

While the genetic cause of HD is now known, a repeat expansion in the polyglutamine-encoding CAG tract of *HTT,* the mechanisms behind such cell-specific death remain incompletely understood. Neither the class of mutation nor the protein *HTT* encodes for, offer clear explanations for the neuropathogenesis of HD. Polyglutamine, also known as polyQ, expansions are not unique to HD; 9 other inherited neurological disorders have been identified that are caused by polyQ expansions in separate and apparently unrelated genes. The protein *HTT* encodes for, huntingtin or htt, is not specifically expressed in MSNs or cortical neurons. In fact, htt is expressed in nearly every cell in the body, yet its normal function remains incompletely understood. While

studies in mice have shown that htt is critical for embryonic development, its absence in adulthood appears to be non-deleterious (Nasir et al., 1995; Wang, Liu, Gaertig, Li, & Li, 2016).

1.2 Treating HD

The success of The Huntington's Disease Collaborative Research Group in finding the gene responsible for HD was celebrated around the world not only for the landmark achievement itself but also because it was widely believed that identifying the gene would lead to a cure. Yet, nearly thirty years after the discovery of *HTT* no treatments are available to patients that are capable of altering the course of HD. However, treatments do exist to treat psychiatric and movement symptoms. Antidepressants, most commonly selective serotonin reuptake inhibitors, can be useful to treat depression. Additionally, anticonvulsants such as Valproic acid or Carbamazepine have been shown to reduce irritability and impulsive behaviors. For the treatment of chorea, Tetrabenazine, a drug that is said to "stabilize" dopaminergic signaling, has been shown to have a dose-dependent benefit and is one of two drugs specifically FDA approved for HD (Group, 2006). However, the side effects of Tetrabenazine, which include parkinsonism, gait disturbances, somnolence, and depression, make its use controversial. More recently, Deutetrabenazine, also known as DTBZ, was approved. It depletes monoamines at presynaptic nerve terminals and has demonstrated significant improvement of chorea compared to placebo. Importantly, while there have been no head-to-head studies comparing the safety of Tetrabenazine to DTBZ, an indirect comparative study suggests that DTBZ tolerability is similar to placebo (Richard & Frank, 2019).

While a cure for HD has not yet been identified, evidence from mouse studies and from MRI scans suggest that disease-modifying therapies are possible. The Rene Hen laboratory has used a conditional mouse model of HD to show that blocking mutant *HTT* expression in symptomatic mice led to an improvement of behavioral symptoms (Yamamoto, Lucas, & Hen, 2000). Furthermore, the direct relationship of striatal atrophy that begins before the onset of symptoms and worsens with disease progression implies that preventing such atrophy could delay and possibly prevent the onset of symptoms (Aylward, 2007).

1.3 The Role of the Immune System in the Neuropathogenesis of HD

As discussed in the preceding sections, the neuropathogenesis of HD is characterized by the selective death of MSNs in the striatum and of cortical neurons in the cortex, yet the cause of such cell-specific death remains unknown. Potential contributing factors of mutated *huntingtin* to HD include disrupted protein homeostasis, mitochondrial dysfunction, excessive or aberrant corticostriatal input, transcriptional dysregulation, loss of neuroprotective support, and impairments in axonal trafficking. Thus, the initial excitement that followed the discovery of HD's monogenetic origin and the hope that such a scientific breakthrough would lead to a cure, has been tempered by the realization that *HTT* encodes for a protein that is involved in an incredibly complex network of cellular and intercellular interactions that interfaces with nearly every aspect of bodily function. Indeed, the disease pathogenesis is not restricted to neurons in the CNS; notably, the final stages of HD are characterized by profound muscle wasting.

Another significant but less studied aspect of HD is inflammation, both in the CNS and in the periphery (Crotti & Glass, 2015; Silvestroni, Faull, Strand, & Moller, 2009). In the CNS, the brain has a unique population of resident immune cells called microglia, which make up 10-15% of all brain cells. Under non-diseased and/or homeostatic conditions in the adult brain, microglia have small cell bodies with long processes that survey the entire brain parenchyma once every few hours (Nimmerjahn, Kirchhoff, & Helmchen, 2005). This allows microglia to quickly respond to a wide array of CNS perturbations that include infection, injury, and disease. The detection of such disturbances causes microglia to take on an “activated” phenotype that is characterized by a shortening of their processes, an increase in cell body size, the upregulation of surface antigens, and the upregulation of phagocytosis. Importantly, this activated state is not permanent; microglia can revert to their homeostatic morphology once the activating agents have been resolved. Such dynamic reactivity creates a delicate balance between neuroprotective and neurotoxic microglial phenotypes whereby microglia can bolster neuronal health by secreting protective factors and removing inflammatory agents, or, under chronic inflammatory conditions, microglia can exacerbate inflammation resulting in an environment that is detrimental for neuron survival (Wyss-Coray & Mucke, 2002). In HD, imaging studies show that microglial activation can be detected early in the disease and that the percentage of activated microglia increases with disease progression (Sapp et al., 2001; Tai et al., 2007).

In the peripheral blood, both lymphocytes and monocytes have been found to be hyper-reactive in HD, even in pre-symptomatic patients. Therefore, the reduction of inflammation in the CNS and the periphery may offer an additional therapeutic strategy

for treating HD that has been much less pursued in previous research and clinical studies. Even if they are not directly involved in disease initiation, if peripheral inflammation and/or neuroinflammation are involved in driving disease pathogenesis, such as in the later stages of HD, then treating inflammation may reduce the rate of disease progression in symptomatic patients and delay the onset of disease in patients at risk for HD.

In this context, the core hypothesis of my dissertation research is that the infiltration of peripheral monocytes (MO) in the later stages of HD contributes to driving disease neuropathogenesis. To test this hypothesis, I pursued 3 sets of studies to ask the following experimental questions:

1) Are MO isolated from HD patients (HD MO) hyper-reactive *in vitro?*
2) How does the infiltration of HD MO into the brain affect the health of MSNs?
3) How does the infiltration of HD MO into a CNS environment alter endogenous intercellular interactions between resident microglia and MSNs?

The scientific rationales for each of these experimental questions and key supporting studies in the literature will be summarized in the following sections.

1.3.1 HD monocyte hyper-reactivity *in vitro*

Researchers studying HD have long reported that inflammation, both in the periphery and in the CNS, are associated with the disease (Crotti & Glass, 2015; Silvestroni et al., 2009). For example, although the HTT protein is constitutively and ubiquitously expressed, *HTT* mRNA expression is higher on average in immune cells (Soulet & Cicchetti, 2011; Strong et al., 1993). At the cellular level, peripheral immune

cells from HD patients were found to have aberrant receptor function (Varani et al., 2007). From a functional standpoint, HD MO have been found to be hyper-reactive to stimulation, as measured by increased production of inflammatory cytokines and chemokines, compared to MO isolated from non-disease controls (Bjorkqvist et al., 2008). The same group found that such hyper-reactivity is present in HD MO before patients exhibit symptoms, making MO viable candidates as contributing drivers of neuropathogenesis in later stages of the disease.

The first goal of my dissertation research was thus to determine whether MO isolated from HD patients demonstrate the similar hyper-reactivity in my hands as has been previously reported in the literature.

1.3.2 Consequences of monocyte infiltration for medium spiny neuron health

As discussed in previous sections, factors underlying the selective vulnerability of MSNs in HD remain largely unknown. Some hypothesize that MSNs are some of the first cells to succumb to the disorder because they are an intrinsically vulnerable neuronal cell type due to high metabolic demands and high levels of connectivity to neighboring neurons (Estrada-Sanchez et al., 2015; I. Han, You, Kordower, Brady, & Morfini, 2010; Rikani et al., 2014).

As such, MSNs may also be selectively vulnerable to the effects of neuroinflammation. A critical transition in HD is the infiltration of MO into CNS in the later stages of the disease. The CNS is separated from the periphery by a tightly regulated network of cells and membranes collectively termed the blood-brain barrier (BBB). The BBB is believed to be generally impermeable to peripheral immune cells, except in

cases of injury, disease, or pathogen infiltration into the brain parenchyma. It has also been thought that the presence of peripheral immune cells such as T cells, B cells, or monocytes under non-pathological circumstances would make the environment of the CNS hostile to neurons. This view is supported by disorders such as multiple sclerosis, in which monocytes infiltrate the brain and differentiate into macrophages that mediate myelin destruction (Heneka, 2014).

However, modern studies have revealed that peripheral immune cells may have far more interactions with the healthy CNS than previously believed. Recent discoveries have expanded upon and confirmed the existence of a lymphatic system in human dura mater and demonstrate that this lymphatic system regularly contains peripheral immune cells such as B cells, T cells, and monocytes (Absinta et al., 2017; Aspelund et al., 2015; Kipnis & Filiano, 2018; Lecco, 1953; Li, Zhao, Zhou, & Yu, 1996; Louveau et al., 2015).

The consequences of HD monocyte infiltration into the brain have been difficult to study directly due in large part to the lack of unambiguous markers that distinguish microglia (which are resident cells in the CNS) from infiltrating monocytes/macrophages from the periphery. The second goal of my dissertation research was thus to develop a novel platform where peripheral monocytes could be experimentally and directly introduced into CNS tissues. I sought to determine the impact of HD MO on MSN health using this new approach as a model for MO infiltration into the CNS.

1.3.3 Consequences of monocyte infiltration for endogenous microglia

Microglia are long-lived cells that serve as the resident and primary immunocompetent effectors of the CNS. They survey the brain and can become

activated when they encounter foreign antigens or detect inflammatory signals (Nayak, Zinselmeyer, Corps, & McGavern, 2012). Traditionally, microglial activation has been described as being either pro-inflammatory or anti-inflammatory, although this binary classification does not adequately describe the broad range of functional phenotypes that microglia can take on (Cherry, Olschowka, & O'Banion, 2014a, 2014b; Sierra, Abiega, Shahraz, & Neumann, 2013). What is clear is that activated microglia have the ability to both protect and phagocytose neurons. However, the factors that control these conflicting activities are not fully understood.

Emerging evidence suggests that direct contact between microglia and monocytes can significantly alter the function of both cell types (David, Greenhalgh, & Kroner, 2015; David, Kroner, Greenhalgh, Zarruk, & Lopez-Vales, 2018; Greenhalgh & David, 2014; Greenhalgh et al., 2016). The third goal of my dissertation was thus to determine whether MO interact with endogenous microglia as well as to determine whether such interactions have consequences for the health of MSNs in a manner that may be different for HD MO compared to those from non-disease controls.

1.4 Summary

In summary, the overall thesis of my dissertation is that infiltrating monocytes may interact with endogenous microglia and medium spiny neurons in the later stages of HD to drive neuropathogenesis. Accordingly, my dissertation research addressed the following 3 critical research questions:

1) Do monocytes isolated from patients with HD (HD MO) exhibit aberrant cytokine/chemokine production when stimulated *in vitro*?

2) Do HD MO drive neuropathogenesis of MSNs when engrafted into living organotypic brain tissue explants?
3) Do engrafted HD MO interact with endogenous microglia to negatively impact the health of MSNs in these brain tissue explants?

In the 3 research chapters that follow, I will describe my findings and experimental conclusions for each of these sets of studies, with the goal of contributing to the understanding of the role of inflammation in a central enigma of HD neuropathogenesis, the selective loss of MSNs in the striatum.

Chapter 2. Monocytes from Late-Stage Huntington's Disease Patients Exhibit Aberrant Cytokine/Chemokine Reactivity

2.1 Introduction

An individual carrying 40 or more CAG repeats in the HTT gene is highly likely to develop HD sometime between the third and fifth decades of life. While an increased number of CAG repeats is associated with earlier disease onset, the factors driving disease progression once it starts are incompletely understood (Langbehn, Hayden, Paulsen, & Group, 2010). One factor that may contribute to disease progression is inflammation, as researchers have identified aberrant inflammatory responses in both the brains and bodies of HD patients (Crotti & Glass, 2015; Silvestroni et al., 2009). Indeed, the Tabrizi laboratory found that HD MO are hyper-reactive when stimulated, and that such hyper-reactivity can even be detected in monocytes isolated from HD patients before the onset of symptoms (Bjorkqvist et al., 2008; Kwan et al., 2012; Trager et al., 2014; Trager et al., 2015; Weiss et al., 2012). Such early hyperreactivity could allow MO to drive neurodegeneration throughout disease progression and may be particularly harmful in the later stages of the disease. However, a causal relationship between hyper-reactive MO and disease stage has not yet been shown.

In the experiments described in this chapter, I sought to confirm that the HD patient MO I isolated for my dissertation studies are hyper-reactive *in vitro*. Blood samples were drawn from HD patients and non-disease controls (CTRL) at Duke's

Huntington's Disease Society of America (HDSA) Center of Excellence. MO from these whole blood samples were isolated and frozen until the day of experimentation. Experiments were conducted by stimulating MO *in vitro* following a previously published protocol (Bjorkqvist et al., 2008). I measured the resulting inflammatory MO response using a custom V-plex Meso Scale Discovery Assay (MesoScale Discovery) and quantified MO production of four cytokines and one chemokine that have been associated with HD. Based on the studies described above, I expected that such stimulation would result in increased production of the aforementioned cytokines/chemokine in HD compared to CTRL MO.

2.2 Materials and Methods

2.2.1 Collection of blood samples

Protocol and consent forms (Pro00078049) were created to collect and store blood samples from HD and CTRL patients. Samples from 29 HD patients at various disease stages and 30 CTRL age-matched patients were collected over 8 months at Duke's HDSA Center of Excellence [Table 1]. 30-40 mL of blood per patient was collected in BD Vacutainer® K2 EDTA blood collection tubes (VWR) at the clinic before being taken back to our home laboratory for further processing and isolation.

2.2.2 Isolation of monocytes from whole blood

Monocytes were isolated from whole blood samples using a combination of density centrifugation, antibody incubation, and magnetic separation. First, Histopaque® -10771 (Sigma-Aldrich), a medium adjusted to a specific density for the recovery of viable human lymphocytes and other mononuclear cells, was added to a 50 mL conical

Falcon tube (VWR). Then, 20-30 mL of whole blood from individual patients was carefully layered on top of Histopaque®-10771, maintaining separation between blood and Histopaque®-10771. These tubes were then centrifuged at 400 x *g* for 30 minutes at room temperature with the brake and acceleration settings set to 0. After centrifugation, the PBMC layer was aspirated, placed into a clean conical centrifuge tube, and washed with 20 mL of isotonic phosphate buffered saline before being centrifuged at 250 x *g* for 10 minutes. Pelleted PBMCs were then inspected for the presence of red blood cells (RBCs) as they can interfere with the isolation of monocytes. Samples that contained RBCs were washed with 1x RBC Lysis Buffer (Zen-Bio). Once free of RBCs, the cells were either frozen for later use or processed immediately to isolate monocytes. Cells that were frozen were diluted in 2 mL of Lymphocyte Cryopreservation Medium (Zen-Bio), pipetted into cryogenic storage vials (VWR), and then placed into a Nalgene® Mr. Frosty Freezing Container (Sigma-Aldrich) and stored at -80 ºC.

To isolate monocytes, frozen PBMCs were thawed using Lymphocyte Medium (Zen-Bio) to prevent clumping and maximize viability. Cells were then counted and incubated in a solution containing 80 µL of MACS buffer (Miltenyi Biotec) and 20 µL MACS CD14 Microbeads (Miltenyi Biotec) per 10^7 cells for 15 minutes at 4 ºC. Within the PBMC layer, CD14 is specific to monocytes, and the microbeads are tethered to magnetic particles which allows them to be magnetically separated after incubation. MACS CD14 Microbead tethered cells were then passed through a magnetic column, and CD14 cells were positively selected. These cells were then either frozen using the same methods detailed above or used immediately for experimentation.

2.2.3 Stimulation of monocytes *in vitro*

100,000 monocytes were seeded per well in 96 -well Corning™ Primaria™ plates in 150 µL of monocyte medium per well. This medium was comprised of RPMI (93%) (Thermo Fisher Scientific), fetal bovine serum (5%) (Thermo Fisher Scientific), L-glutamine 200 nM (1%) (Thermo Fisher Scientific), and penicillin 5000 U/mL/ streptomycin 5000µg/mL (1%) (Thermo Fisher Scientific). Cells recovered for 16 hours at 37 ºC with 5 % $C0^2$ prior to stimulation. After recovery, monocyte medium was removed and replaced either with new monocyte medium, for the unstimulated condition, or stimulation medium, for the stimulated condition. The stimulation medium was comprised of 2 µg/mL of LPS (Sigma-Aldrich) and 10 ng/mL human interferon-(IFNγ) (RD Systems) added to monocyte media. After 24 hours, supernatants were harvested and flash frozen.

2.2.4 Cytokine quantification *in vitro*

On the day of analysis, samples were taken to Duke's Molecular Physiology institute to be analyzed by Janet L. Huebner using MSD multiplex assays, according to the manufacturer's instructions (MesoScale Discovery). A custom MSD V-Plex human Pro-inflammatory cytokine panel, was used. The cytokines/chemokine were chosen based on a survey of the literature. A pilot experiment with CTRL MO was conducted to determine the appropriate sample dilutions. Unstimulated MO were diluted at 2x and stimulated MO were diluted at 100x to ensure measurements stayed within a quantifiable range.

2.2.5 Total protein analysis

A Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) was used for BCA analyses. Following the manufacturer's instructions, all standards and samples were created and diluted using the same buffer. For optimal signal detection, samples were diluted 5x prior to being added to the working reagent, which further diluted the samples 8x, making the total dilution for protein concentration calculations 40x. A SpectraMax M5^e (Molecular Devices) plate reader with SoftMax Pro 6.2.2 (Molecular Devices) software was used to read absorbance at 562nm and generate absorbance signals for protein concentration calculations.

2.2.6 Cell viability assay

A CyQuant™ XTT Cell Viability Assay (Thermo Fisher Scientific) was used to assess the viability of unstimulated and stimulated MO. The manufacturer's instructions were followed, and a SpectraMax M5^e (Molecular Devices) plate reader with SoftMax Pro 6.2.2 (Molecular Devices) software was used at 660 nm and 450 nm to generate absorbance signals and calculate cellular redox potential.

2.2.7 Data analysis and statistics

All data were analyzed using Sigmaplot 14 statistical software (SYSTAT). MSD cytokine data for every biological replicate were normalized to its total protein content as measured by BCA analysis. The fold change between unstimulated and stimulated MO was then calculated and used for analyses. For samples that had levels of detection that were below the lower limit of quantification, half the value of the lower limit of quantification for a given cytokine was used for statistical analyses (recommendation of

Ms. Huebner). A Student's *t*-test was used for data that passed normality tests, and Mann-Whitney Rank Sum tests were used for data that failed normality tests. When multiple groups were compared, one-way ANOVAs were used to analyze all data that passed the Shapiro-Wilk Normality Test followed up with *post hoc* comparisons (Tukey's HSD) to identify group differences, with significance assumed at $p<0.05$. If the normality test failed, a Kruskal-Wallis one-way ANOVA was used followed up with *post hoc* comparisons (Tukey's HSD) to identify group differences, with significance assumed at $p<0.05$. All reported p values are two-tailed. Finally, to control for individual patient variation in the MSD assay, three biological replicates per patient were used, and to control for pipetting errors, two technical replicates per patient were used for each sample.

2.3 Results

2.3.1 Sample collection from HD patients and non-disease controls at Duke's HDSA Center of Excellence

Blood samples were successfully collected from 29 HD patients and 30 age-matched controls at Duke's HDSA Center of Excellence. A majority of the collected HD samples came from Stage 1 and Stage 2 patients who had an average of 43 repeats [Table 1].

Table 1: Total number of HD patient samples collected.

Blood was drawn from 29 HD patients and 31 non-disease controls (not shown).

# of Patients	Age (average)	# CAG Repeats (average)	HD Stage
1	54.0	42.0	4
8	57.6	43.3	3
10	56.4	43.3	2
10	53.5	43.0	1

2.3.2 Increased PBMC yield over time and stable CD14 isolation

Next, clinic-laboratory procedures were standardized and optimized to improve PBMC isolation from whole blood. Monocyte isolation from isolated PBMCs remained consistently within the expected range of 1-10% of cells in human blood (Corkum et al., 2015) [Figure 1].

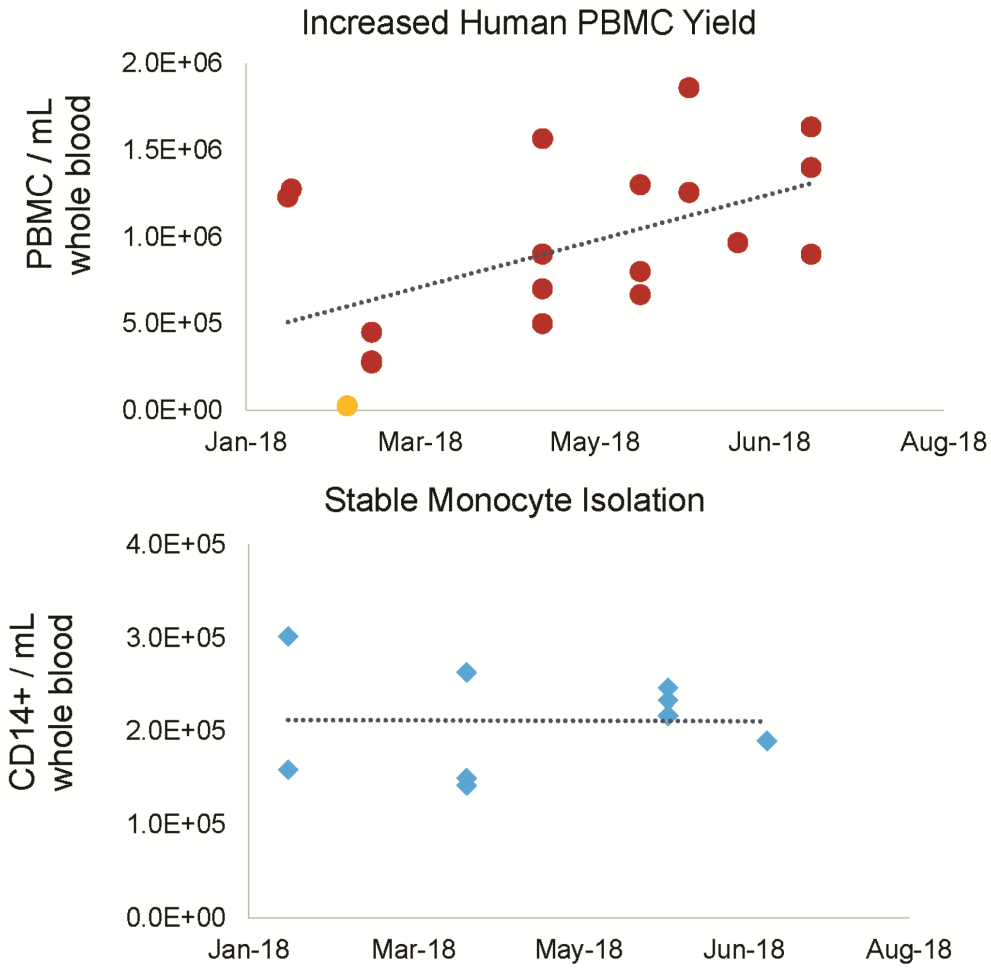

Figure 1: Optimization and standardization of isolation procedures for patient PBMCs and CD14+ monocytes.

Upper, the development of a standardized protocol improved PBMC yield over time. Lower, CD14+ isolation from PBMCs remained consistently within the expected range of monocytes per mL of whole blood. Note that the yellow point in the upper PBMC graph reflected poor yield due to a one-time blood clot complication that interfered with the density gradient, causing the loss of a large fraction of mononuclear cells.

2.3.3 PBMC viability is affected by the suspension medium

Monocyte isolation required a specific medium (MACS running buffer) to be run through the magnetic columns. To improve monocyte viability, the cell culture medium used to resuspend monocytes after isolation was optimized. The following media were compared to determine the optimal medium: 1) MACS running buffer, 2) a monocyte-specific buffer ("Lymphocyte," (Zen-Bio)), and 3) a medium used to culture brain slices, "NB." Both Lymphocyte and NB increased PBMC viability relative to MACS. To minimize complications of changing media and to minimize the metabolic transitions for monocytes once they were introduced into the brain-slice environment, NB was the chosen medium for experiments that involved brain slices [Figure 2]. For *in vitro* experiments, MO were suspended in MO medium or MO stimulation medium.

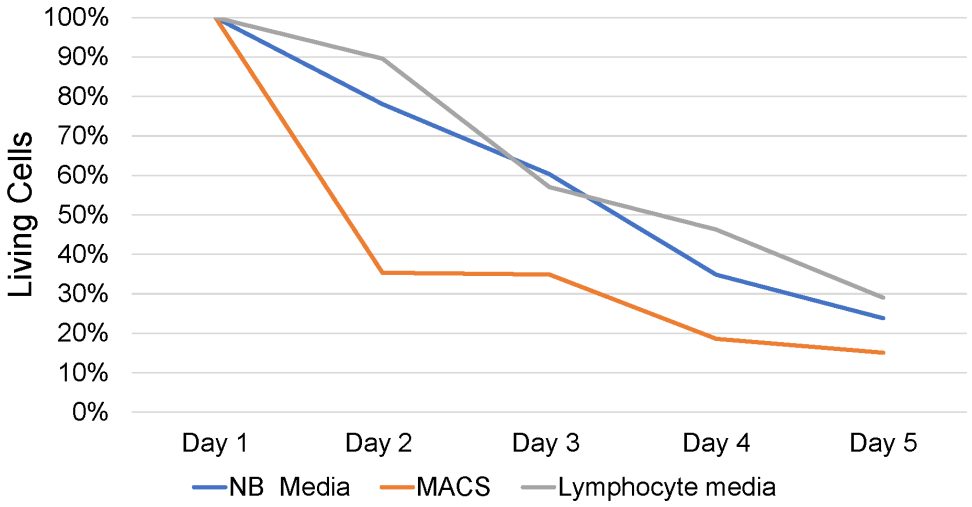

Figure 2: Optimization of MO cell culture medium.

After MO are isolated from PBMCs using MACS buffer, resuspending them in NB or Lymphocyte media improves their survival. NB is the medium used for brain-slice explant cultures, while Lymphocyte is a custom medium specific for lymphocytes.

2.3.4 Stimulated monocytes remain viable

The viability of stimulated MO was assessed, and it was determined that both HD and CTRL MO were viable as indicated by maintained/increased cellular redox potential after stimulation [Figure 3].

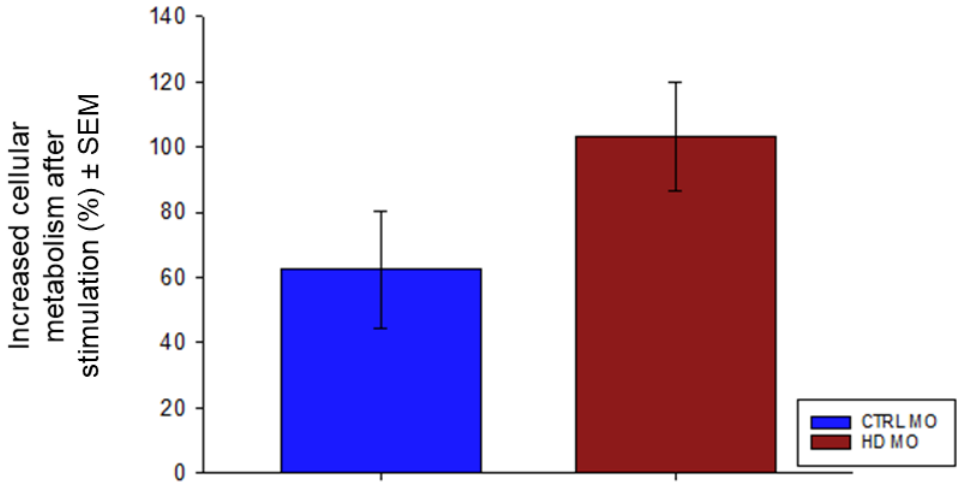

Figure 3: The cellular metabolism of both HD and CTRL MO sustains or increases after *in vitro* stimulation.

The apparent increase in metabolic activity indicates that both CTRL and HD MO survive after stimulation *in vitro*. Cellular metabolism was measured using a viability assay that quantifies the cellular redox potential of actively respiring cells. Data are expressed as means +/- SEM; n= 9/group (3 biological replicates from 3 HD and 3 CTRL patients).

2.3.5 HD MO exhibit hyper-reactivity of the cytokine IL-10

The effect of stimulation was assessed by calculating fold changes in cytokine/chemokine production between unstimulated and stimulated MO from HD and CTRL patients. In the case of IL-10, the fold change of HD MO in response to stimulation was significantly higher than that for CTRL MO. The median fold changes in HD MO and CTRL MO were 296.2 and 83.2. respectively (Man-Whitney U = 15, n1 = n2 = 9, p = 0.027) [Figure 4].

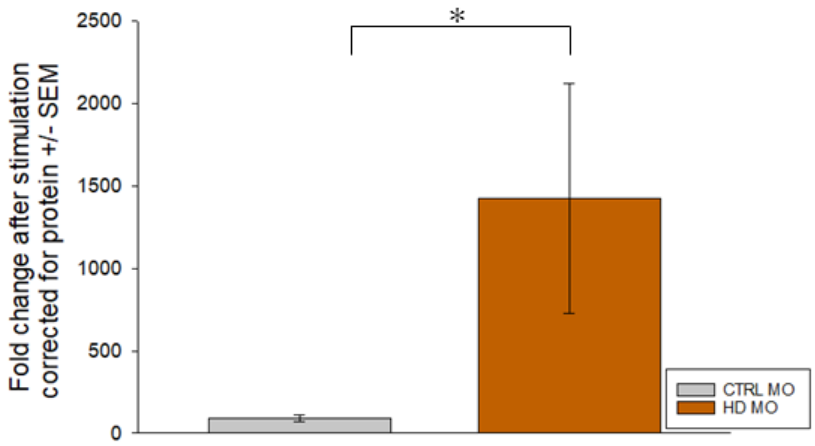

Figure 4: *In vitro* stimulation causes HD MO to produce significantly higher levels of IL-10 compared to CTRL MO.

CTRL and HD MO were stimulated for 24 hours with 2 µL/mL of LPS and 10 ng/mL of IFN-γ. Cytokine levels were measured using a customized V-plex human pro-inflammatory sandwich immunoassay run on an MSD plate reader. Data are expressed as ± SEM; n = 9 per group. *p = 0.03

Significant differences were not found for the cytokines IL-1β, IL-6, TNF-α, or for the chemokine IL-8 [Figure 5].

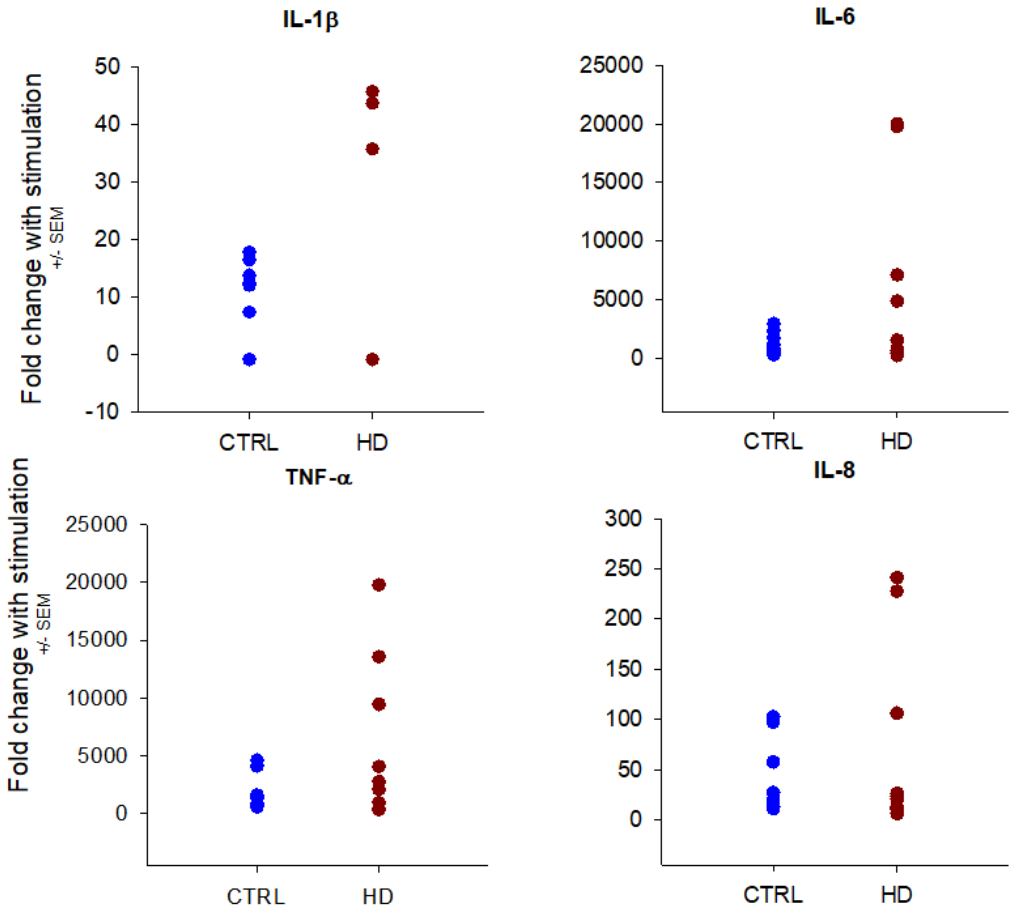

Figure 5: *In vitro* stimulation reveals a non-significant difference between HD MO and CTRL MO for 4 other cytokines/chemokine

CTRL and HD MO were stimulated for 24 hours with 2 µL/mL of LPS and 10 ng/mL of IFN-γ. Cytokine levels were measured using a customized V-plex human pro-inflammatory sandwich immunoassay run on an MSD plate reader. Data are expressed as ± SEM; n = 9 per group. Blue=CTRL; red=HD.

2.3.5.1 Late-Stage HD patient exhibits a trend for hyper-reactivity

The data for CTRL patients were tightly clustered across all measures. The HD patient data, however, exhibited an apparent bimodal spread, suggesting there may be

differences among the individual HD patients. To test this, CTRL patients were compared to HD patients separated by disease stage. This analysis revealed that the more advanced-stage HD patient had a significant fold increase in IL-10 production compared to CTRL patients [Kruskal-Wallis one-way ANOVA, $X^2(3) = 10.006$, $p = 0.019$, with mean IL-10-fold increases of 83.170 for CTRL, 72.340 for HD Stage 1, 244.540 for HD Stage 3, and 5253.50 for Stage 4; *post hoc* $p = 0.022$; Figure 6]. The advanced-stage HD patient also had a significant IL-1β fold increase compared to CTRL, the HD Stage 3 patient, and the HD Stage 1 patient [ANOVA main effect of group, $F(3,14) = 33.13$, $p < 0.001$; *post hoc* analyses Stage 4 vs. CTRL $p < 0.001$, Stage 4 vs. Stage 3 $p < 0.001$, Stage 4 vs. Stage 1 $p < 0.001$; Figure 6]. Finally, the Stage 4 HD patient also had a significant IL-8 fold increase compared the HD Stage 3 patient, and the HD Stage 1 patient [ANOVA main effect of group, $F(3,14) = 14.03$, $p < 0.001$; *post hoc* analyses Stage 4 vs. CTRL $p < 0.001$, Stage 4 vs. Stage 3 $p < 0.001$, Stage 4 vs. Stage 1 $p < 0.001$; Figure 6].

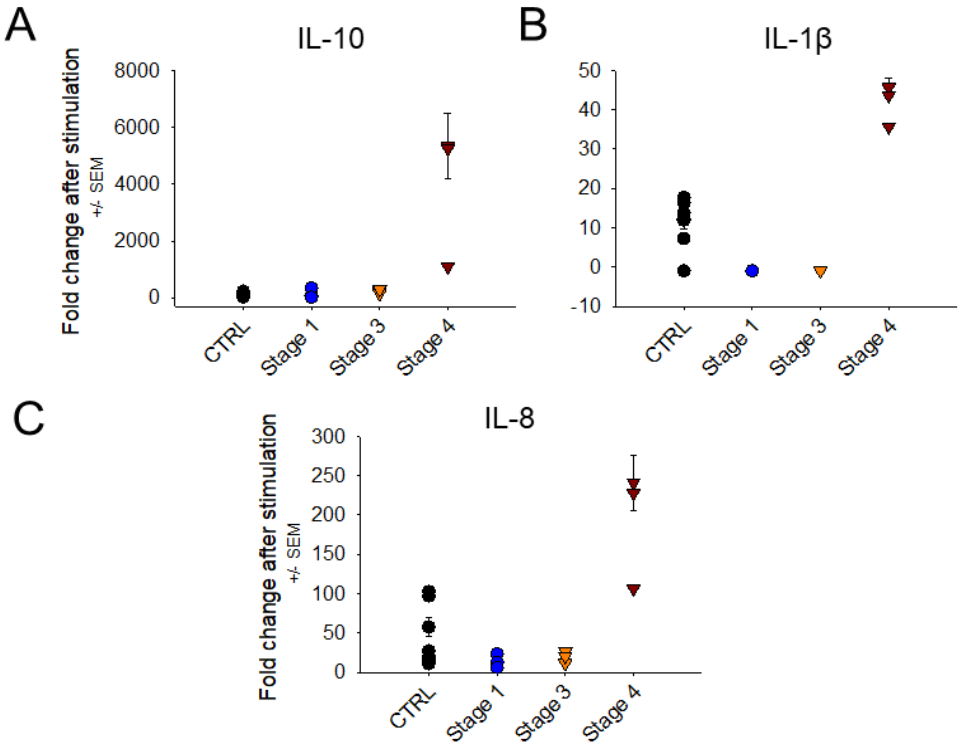

Figure 6: MO from a Stage 4 HD patient exhibit a trend for increased cytokines/chemokine production upon *in vitro* stimulation.

Note that while one-way ANOVA reached statistical significance, differences are referred to as trends because data for each HD stage came from a single patient. Cytokine levels were measured using a customized V-plex human pro-inflammatory sandwich immunoassay run on an MSD plate reader. Data are expressed as ± SEM; n = 3-9/group (3 biological replicates from 3 controls, 1 Stage 1, 1 Stage 3, and 1 Stage 4 HD patient).

2.4 Discussion

In summary, my findings were overall consistent with reports in the literature that *in vitro* stimulation of HD MO produces aberrant cytokine/chemokine reactivity. While there was only one late-stage HD patient in my data set, the data for this patient was also consistent with reports that MO from late-stage patients may have a heightened

response to inflammatory stimuli relative to earlier-stage patients. If so, this would support a role for MO in potentially exacerbating neuropathogenesis in the later stages of the disease. Further research is needed to define the mechanisms by which these differential responses occur. These findings may ultimately have implications for the treatment of late-stage patients through the targeted modulation of MO using therapeutic compounds or cytotherapies.

Chapter 3. Engraftment of Monocytes from Patients with HD Reduces the Survival of Healthy Medium Spiny Neurons in a Hybrid Brain Tissue-based Assay

3.1 Introduction

The progressive neurodegeneration and death of medium spiny neurons (MSNs) in the striatum is associated with and underlies functional deficits in HD. MSNs have been shown to be relatively fragile cells, which may explain why they are some of the first neurons to degenerate in HD (Estrada-Sanchez et al., 2015; I. Han et al., 2010; Vonsattel et al., 1985). In this context, I hypothesized that MSNs are vulnerable to the increasing levels of neuroinflammation associated with HD progression, as described in the previous chapter. In support of this hypothesis, mutant HTT levels in inflammatory cells like monocytes are significantly associated with disease burden score and caudate atrophy rates in HD patients (Weiss et al., 2012). Thus, the infiltration of peripheral monocytes into the CNS during the later stages of HD may be a significant contributing component to the neuropathogenesis of MSNs.

To access the CNS, peripheral monocytes would need to infiltrate the brain through the BBB. While one study found no evidence of MO infiltration in *postmortem* HD brain samples, such a negative finding cannot conclusively rule out the existence of monocytes in the HD brain, especially considering the following: a) there are a lack of cell-specific markers that can easily distinguish between monocytes, meningeal macrophages, and brain resident macrophages, and b) the BBB is compromised in the later stages of HD, which would allow MO access to the parenchyma (Di Pardo,

Castaldo, Capocci, Amico, & Vittorio, 2017; Islam, 2017; Lim et al., 2017; Montagne et al., 2015; Silvestroni et al., 2009).

The impact of infiltrating MO on MSN degeneration in HD has not been directly explored due in significant part to lack of experimentally accessible *in vivo* models. I believe this is a critical area of investigation due to the reported hyper-reactivity of HD MO and the compromised BBB in the later stages of HD (A. Di Pardo, E. Amico, et al., 2017; A. Di Pardo, S. Castaldo, et al., 2017; Islam, 2017; Lim et al., 2017; Trager et al., 2014). Thus, in the present chapter, I tested the hypothesis that HD MO will negatively impact MSN survival in a novel hybrid brain slice-based assay I developed containing living MSNs still resident in their local tissue environment. MO were isolated from HD and CTRL patients and engrafted directly into these cortico-striatal brain slice explants to mimic peripheral infiltration of MO in the later stages of HD. I predicted that such HD MO infiltration would exacerbate MSN cell death in the brain-slice model as measured by a decrease in the number of surviving and healthy MSNs.

3.2 Materials and Methods[1]

3.2.1 Development of a hybrid brain-slice model to study the impact of HD MO on neuropathology

3.2.1.1 Biolistic transfection and organotypic brain slice culture

All animal experiments and care complied with federal regulations and were reviewed and approved by the Duke University Medical Center Institutional Animal Care and Use Committee. Brain slice preparation and biolistic transfection were done as previously described (Reinhart et al., 2011). Briefly, brain tissue was dissected from CD Sprague Dawley rats killed on postnatal day 10 (P10) (Charles River Laboratory) and placed in ice-cold culture medium, referred to as “NB" medium, containing 15% heat-inactivated horse serum, 10 mM KCl, 10 mM HEPES, 100 U/ml penicillin/streptomycin, 1 mM sodium pyruvate, and 1 mM L-glutamine in Neurobasal-A (Themo Fisher). Brain tissue was cut into 250-μm thick coronal slices using a Vibratome and incubated at 37 °C under 5% CO_2 before biolistic transfection. Gold particles (1.6 μm gold microcarriers; Bio-Rad) were coated with expression constructs encoding yellow fluorescent protein (YFP) as per the manufacturer's instructions and loaded into Tefzel tubing (McMaster-Carr) for use with the Helios biolistic device (Bio-Rad), which was used at a delivery pressure of 95 psi. For each condition, transfections were done on 12 brain slices. Each set of 12 brain slices was taken from individual neonatal rats and were kept and tracked

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[1]Parts of these methods were originally published as: Koshnan, A., Sabbaugh, A., Callamini, B., Marinero, S.A., Dunn D.E., Yoo J.H., Ko, J., Lo, D.C., Patterson P.H. (2017). IKKβ and mutant huntington interactions regulate the expression of IL-34: implications for microglial-mediated neurodegeneration in HD. *Human Molecular Genetics.* 1;26(21): 4267-4277.

together in order to make intra-animal comparisons and thereby exclude potential variability among different littermates.

3.2.1.2 MO engraftment into brain slices

Frozen PBMCs were thawed using Lymphocyte Medium (Zen-Bio) to prevent clumping and maximize viability. Cells were counted and incubated in a solution containing 80 µL MACS buffer (Miltenyi Biotec) and 20 µL MACS CD14 Microbeads (Miltenyi Biotec) per 10^7 cells for 15 minutes at 4 ºC. MACS CD14 Microbead tethered cells were then passed through a magnetic column, and CD14 cells were positively selected. These cells were then resuspended in NB medium and allowed to recover for 2-4 hours before being engrafted onto brain slices.

3.2.1.3 Live cell tracking of engrafted MO

A variety of cell labeling techniques were tested and used including lentivirus, tracking dyes, and anti-human antibodies to detect human cells in rat brain slices. While lentiviral labeling has the benefit of permanently labeling cells, monocytes are reported to be inherently resistant to lentiviral transfection (M. Van Kanegan, personal communication). Tracking dyes transpired to be optimal for labeling living cells as they label for several days and are capable of tracking cells as they penetrated the slice. CellTracker dye (CT; Life Technologies) was compared to the longer lasting and more brightly fluorescent Q-Tracker dye (QDot; Life Technologies). For this experiment, 100,000 PBMCs were labeled with CT and QT and incubated according to the manufacturer's instructions. CT proved to be the superior marker of monocytes in brain slices and continues to label cells even after they have changed morphology [Figure 14].

However, the signal of CT degraded over time, and the manufacturer notes that at concentrations above 5 µL/mL CT may interfere with cellular function. Therefore, MO in all experiments conducted to study intercellular function were labeled post-fixation with the anti-human mitochondria antibody MAB1273.

3.2.1.4 Scoring healthy MSNs after MO engraftment

YFP-transfected neurons were visualized using fluorescence microscopy and counted manually by an experimenter blinded to treatment conditions. To be scored as healthy, MSNs had to meet the following criteria: 1) contain normal cell body diameters, 2) have even and/or continuous YFP expression within all compartments, and 3) have more than two primary dendrites that were a minimum of two cell body diameters long.

3.2.1.5 Brain slice immunohistochemistry

Brain slices were fixed in 4% paraformaldehyde followed by blocking with a permeability mixture containing 10% goat serum, 20% DMSO, and 2% Triton X- 100 in PBS (Dissing-Olesen & MacVicar, 2015). Brain slices were incubated with primary rabbit polyclonal anti-IBA1 antibody (1:1000; Wako) and primary mouse monoclonal anti-human mitochondrial antibody (1:100; Sigma-Aldrich) followed by an Alexa Fluor 594 secondary antibody to visualize resident microglia (1:500; Thermo Fisher) and a Pacific Blue secondary antibody to visualize engrafted MO (1:250; Thermo Fisher).

3.2.2 Measurement of cytokine production in the hybrid brain-slice model

Brain slices were incubated for 3 days after MO engraftment. Numbers of healthy living MSNs were scored for each experiment before the brain slices were lysed. In order

to prevent interference with the MSD assay, a non-ionic detergent (BD cell Lysis Buffer; Thermo Fisher) was used to lyse brain slices. First, slices were washed with PBS and placed into individual 1.5 mL microcentrifuge tubes (VWR) on ice. Then, slices were homogenized using 200 µL pipette tips before being pelleted at 7,000 x *g* for 30 seconds at room temperature. The supernatant was discarded and 120 µL of ice-cold BD lysate was added per slice. Samples were left for 30 minutes after which they were sonicated and then centrifuged at 23,000 x *g* for 20 minutes at 4 ºC. Supernatants were collected, flash frozen with liquid nitrogen, and stored at -80 ºC. On the day of analysis, samples were taken to Duke's Molecular Physiology institute to be analyzed by Janet L. Huebner using MSD multiplex assays, according to the manufacturer's instructions (MesoScale Discovery). A custom MSD V-Plex human Pro-inflammatory cytokine panel was used. A pilot experiment with CTRL MO engrafted into brain slices was conducted to determine the appropriate sample dilutions. This experiment determined that the appropriate sample dilution for all brain lysates was 2x. Please see Chapter 2 for additional details.

3.2.3 Data analysis and statistics

All data were analyzed using Sigmaplot 14 statistical software (SYSTAT). Raw MSD cytokine data were used for slices. For samples that had levels of detection that were below the lower limit of quantification, half the value of the lower limit of quantification for a given cytokine was used for statistical analyses, as recommended by Ms. Huebner. A Student's *t*-test was used for data that passed normality tests, and Mann-Whitney Rank Sum tests were used for data that failed normality tests. When multiple groups were being compared, one-way ANOVAs were used to analyze all data

that passed the Shapiro-Wilk Normality Test followed up with *post hoc* comparisons (Tukey's HSD) to identify group differences, with significance assumed at $p<0.05$. If the normality test failed, the Kruskal-Wallis one-way ANOVA was used followed up with *post hoc* comparisons (Tukey's HSD) to identify group differences, with significance assumed at $p<0.05$. All reported p values are two-tailed. For the MSD assay, three biological replicates per patient were used, and to control for pipetting errors, two technical replicates per patient were used for each sample.

3.3 Results

3.3.1 Brain-slice assay to monitor MSN health

For this study, a previously established brain-slice model was adapted for the study of MSN neurodegeneration (Lo, 2001). Cortico-striatal brain sections were transfected with DNA constructs encoding yellow fluorescent protein (YFP) to monitor the number and/or health of MSNs over the course of three days [Figure 7].

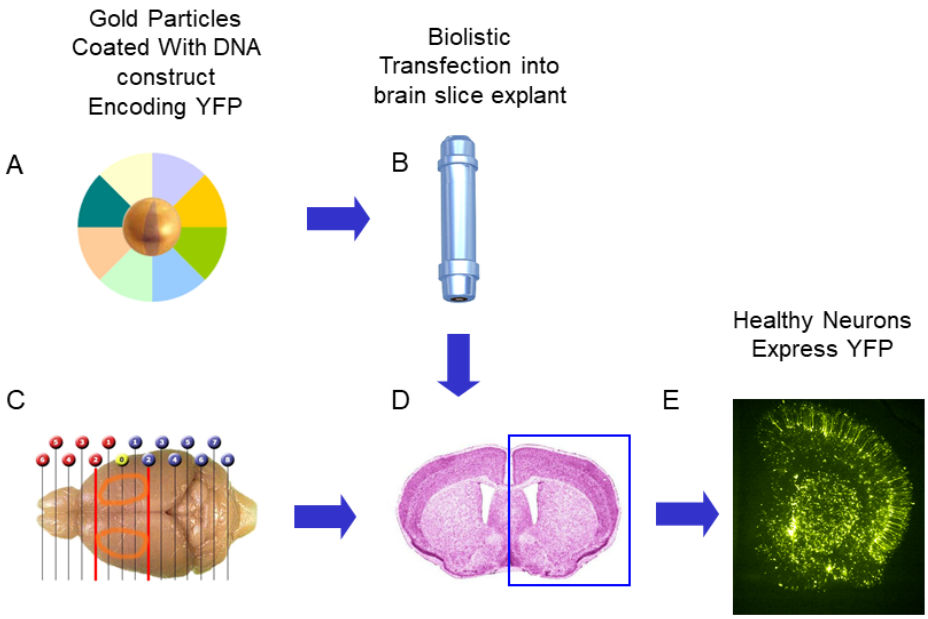

Figure 7[2]: Living MSNs are visualized using biolistic transfection of YFP.

A) Gold particles are coated with DNA constructs encoding YFP. B) Biolistic transfection is used to pneumatically shoot particles into cortico-striatal brain slice explants prepared from neonatal rats. C) Cortico-striatal brain sections are selected to model striatal neuropathogenesis in HD. D) Coronal view of a cortico-striatal brain section. E) Example fluorescence photomicrograph of brain slice explant expressing YFP.

In the brain-slice model, gold particles were coated with DNA constructs encoding YFP [Figure 7A]. Then, biolistic transfection was used to pneumatically shoot particles into cortico-striatal brain slice explants from neonatal rat pups [Figure 7B]. Cortico-striatal brain sections were selected for their relevance to striatal neuropathogenesis in HD [Figure 7C]. MSNs are located in the central portion of the slice, surrounded by cortical

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[2] Parts of this figure were originally published as: Hoffstrom, B.G., Kaplan, A., Letso, R., Schmid, R.S., Turmel, G.J., Lo, D.C., Stockwell, B.R. (2010). Inhibitors of protein disulfide isomerase suppress apoptosis induced by misfolded proteins. *Nature Chemical Biology,* 6, 900-906.

tissue regions. Such biolistic transfection labeled both cortical and striatal neurons [Figure 7E]. These live cells could then be visualized using fluorescence microscopy, and healthy MSNs were scored and compared among treatment groups [Figure 8].

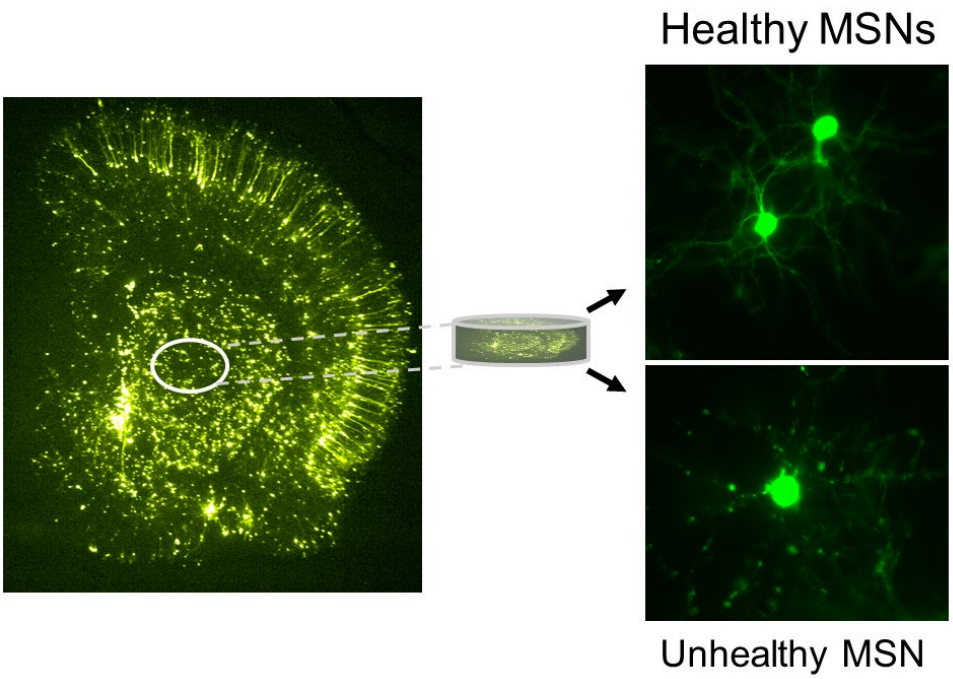

Figure 8: Scoring healthy MSNs in brain slices.

Left, whole view of a cortico-striatal brain slice explant expression YFP 24 hours after biolistic transfection. Middle, the striatum is visualized using fluorescence microscopy, and numbers of healthy MSNs are scored. Right, healthy MSNs meet the following criteria: normal cell body diameters, even and/or continuous YFP expression within all compartments, and two or more primary dendrites that are a minimum of two cell body diameters long.

3.3.2 CellTracker dye and 2 µL re-suspension are optimal for labeling and engrafting MO into brain slices

First, the ability of different tracking dyes to label MO in brain slices was assessed. CellTracker dye (CT; Life Technologies) labeled MO more effectively than Q-Tracker dye (QDot; Life Technologies) [Figure 9].

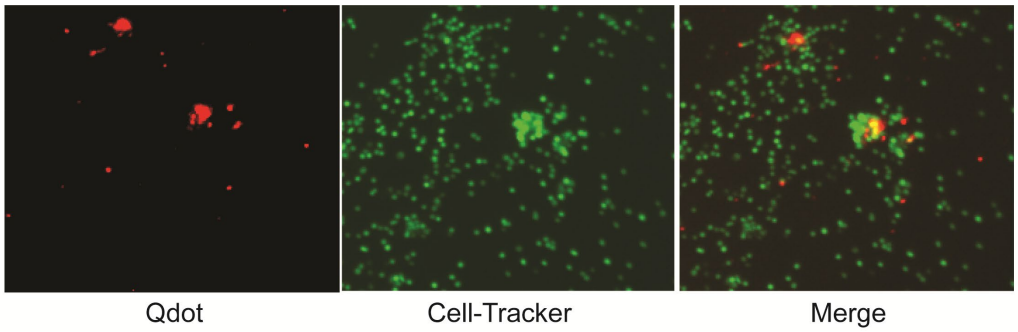

Figure 9: Direct comparison of MO doubled-labeled with Q-Tracker (Qdot) and CellTracker CMFDA (CT) demonstrates that CT is more effective at labeling MO.

100,000 MO were incubated with Qdot and CT following the manufacturer's instructions. They were then engrafted into brain slices and imaged after 1 hour at 4x using a fluorescence microscope.

Next, a protocol was developed to optimize MO engraftment into brain slices. Re-suspending MO in 2 µL of media was optimal for retention on and penetration into brain slices [Figure 10].

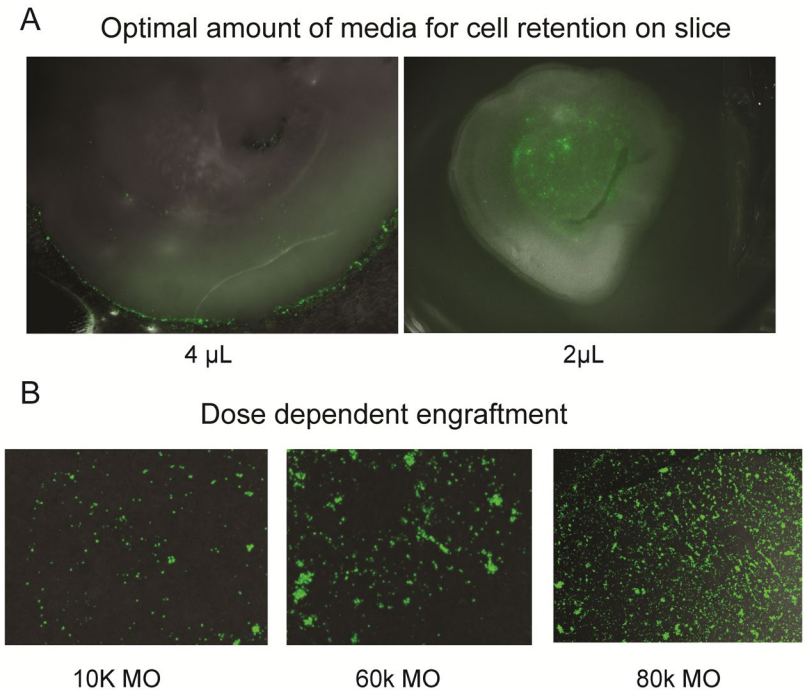

Figure 10: The optimal volume for effective engraftment of MO into brain slices is 2 µL.

A) Cells suspended in too much medium spill over the edges of brain slices. B) Concentration-dependent engraftment of MO into rat brain slices.

3.3.3 Engraftment of rat MO into the brain-slice model demonstrates that MO are viable and do not negatively impact the number of healthy MSNs in slice

Next, the survival of MO engrafted into the brain-slice assay was assessed.

Viability ranged between 12-100% with an average of 37% [Figure 11].

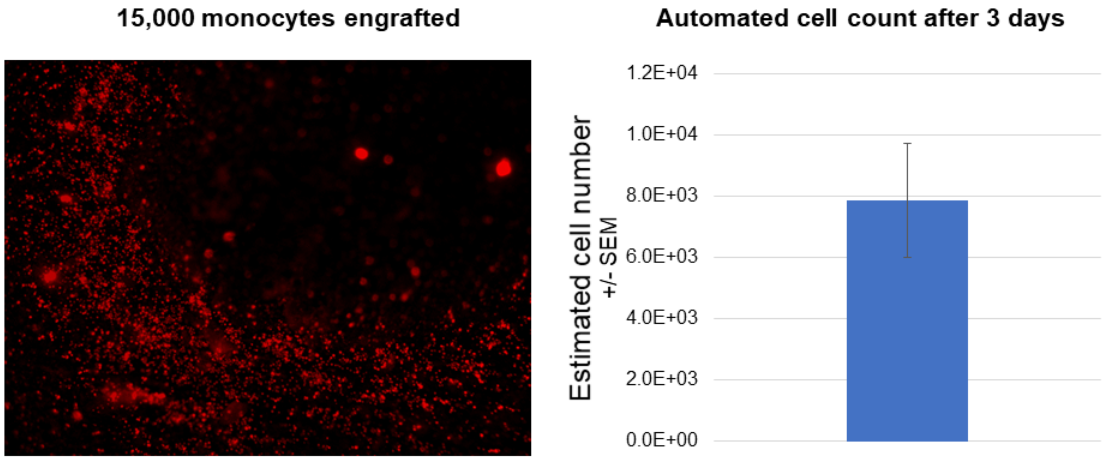

Figure 11: Rat MO are viable in brain slices 3 days after engraftment.

Left, 15,000 CD14+ MO isolated from a rat were labeled with CT prior to engraftment and imaged at 10x on a fluorescence microscope. This image shows viable cells immediately after engraftment (DIV0). Right, Image J was used to estimate the number of living cells imaged three days after engraftment. n= 8 brain slices. Data are mean ± SEM. Note that only living cells express the fluorescent dye.

Next, the number of healthy MSNs three days after rat MO engraftment was measured. Such engraftment did not negatively influence the number of healthy MSNs [Figure 12].

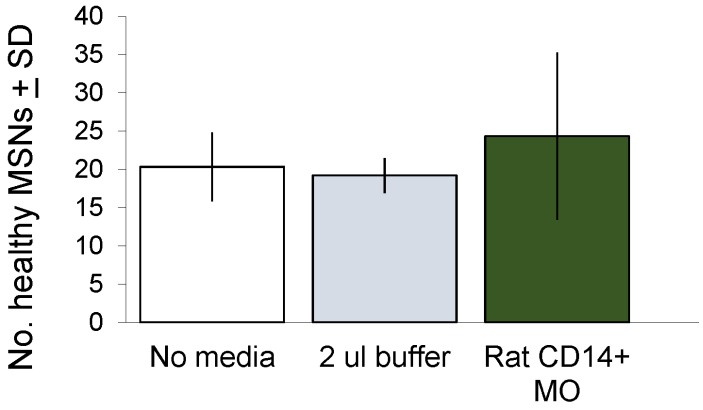

Figure 12: Engrafting rat MO does not negatively impact MSN health.

No significant differences were found between slices that were left untreated (white bar) vs. slices treated with 2 µL of cell suspension buffer (gray bar), vs. slices that were engrafted with 50,000 rat MO (green bar). n= 8-11 brain slices/group. p = 0.303. Data are mean ± SD.

3.3.4 Human MO are viable in rat brain-slice explants

Next, a hybrid species approach was developed in which human MO were engrafted into rat brain slices to determine the impact of human MO on MSN health [Figure 13].

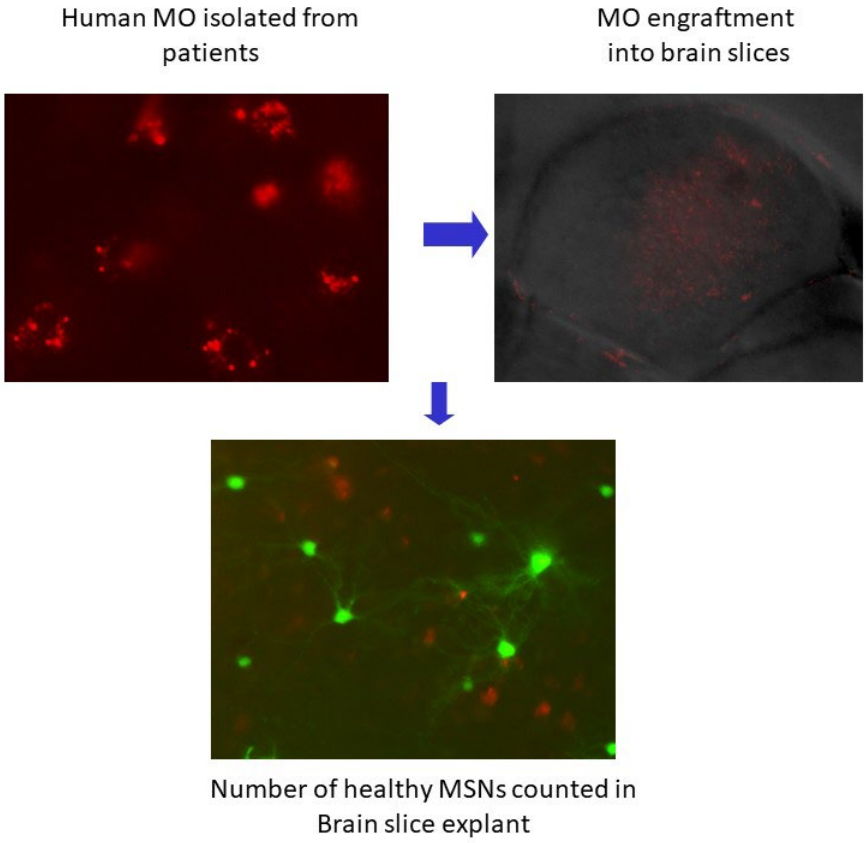

Figure 13: Hybrid model in which human MO are engrafted into rat brain slices.

Left, MO are isolated from whole blood taken from HD and non-disease controls using density gradient centrifugation and positive magnetic selection of CD14+ MO. Right, These human MO are engrafted into cortico-striatal brain slice explants from postnatal rats. Bottom, Visualization of healthy MSNs labeled via biolistic transfection of YFP and MO via staining with CT dye. Healthy MSNs are scored in brain slices to determine the effect of engrafting MO. Red, human MO labeled with CT dye; green, biolistically transfected MSNs.

Human MO are viable after engraftment into rat brain slices as determined by pre-labeling MO with CT dye and visualizing them at extended time points from the day

of engraftment. MO are not only viable, but they also appear to differentiate in the rat brain slice environment [Figure 14]

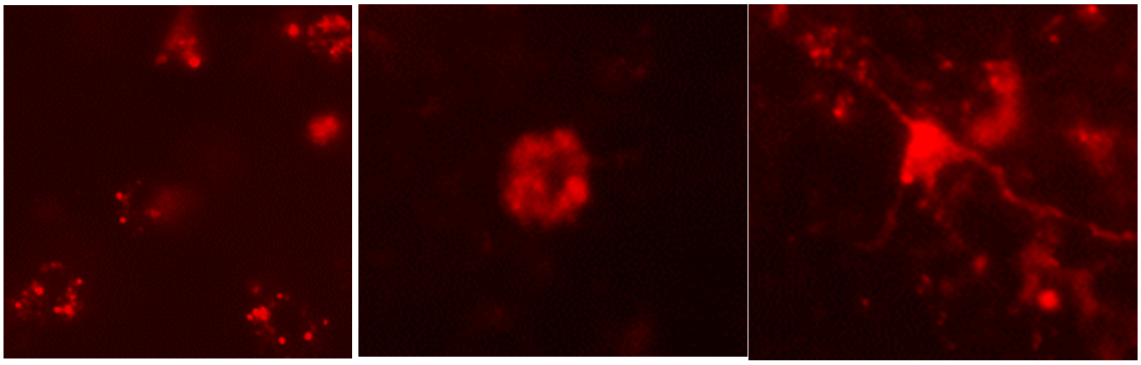

Figure 14: Human MO remain viable in rat brain slices for 6 days or more and appear to differentiate in the brain-slice environment.

MO were labeled with CT dye prior to engraftment. These images show that MO engrafted into brain slices continue to express the tracking dye and are capable of differentiation in the rat brain-slice environment.

Next, a pilot experiment was run to measure the number of healthy MSNs three days after human control MO engraftment. Engraftment did not influence the number of healthy MSNs in the brain-slice assay [Figure 15].

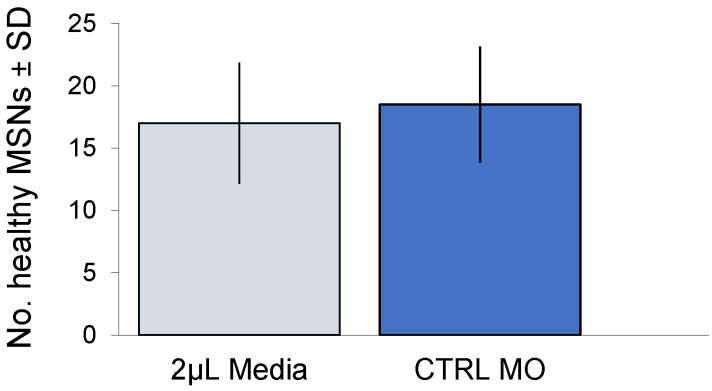

Figure 15: Pilot experiment showing that engrafting human CTRL MO into rat brain slices does not significantly impact the number of healthy MSNs.

N= 5 slices/group. p = 0.78. Gray, slices without MO; Blue, slices engrafted with CTRL human MO.

3.3.5 Engraftment of HD MO decreases the number of healthy MSNs

In contrast, a significant decrease was found in the number of healthy MSNs in slices engrafted with monocytes isolated from HD patients vs. those engrafted with monocytes isolated from age-matched non-disease controls [Student's *t*-test, $p < 0.015$) [Figure 16].

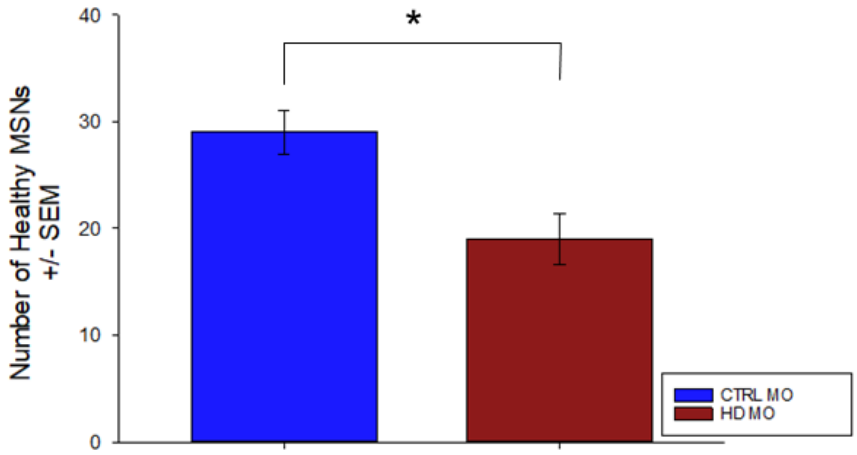

Figure 16: HD MO engraftment decreases the number of healthy MSNs in the hybrid brain-slice model.

Note that all brain slices per group were isolated from the same animals to exclude for potential variability between animals. Data are mean ± SEM, N=6 engrafted slices/group. *P=0.015.

3.3.5.1 Disease stage is associated with an increased negative impact of HD MO on numbers of healthy MSNs

Engraftment of HD MO from a Stage 2 patient into the hybrid brain-slice assay did not result in a significant difference in the number of healthy neurons compared to No MO control brain slices [Figure 17]. Next, the ability of MO from progressively later disease stages was tested to determine if they would exacerbate the loss of healthy neurons. The number of healthy neurons appeared to be significantly affected by disease stage [$F(3,30)=5.579$, P=0.005). *Post hoc* analyses revealed that slices engrafted with MO from a Stage 4 HD patient had significantly fewer healthy MSNs than Stage 1 patients (p=0.033), and Stage 2 patients (p=0.025). Additionally, the Stage 3 patient had significantly fewer MSNs than Stage 2 patients (p=0.035), and there was a

trend for the Stage 3 patient to have significantly fewer healthy MSNs than Stage 1 patients (p=0.051) [Figure 17].

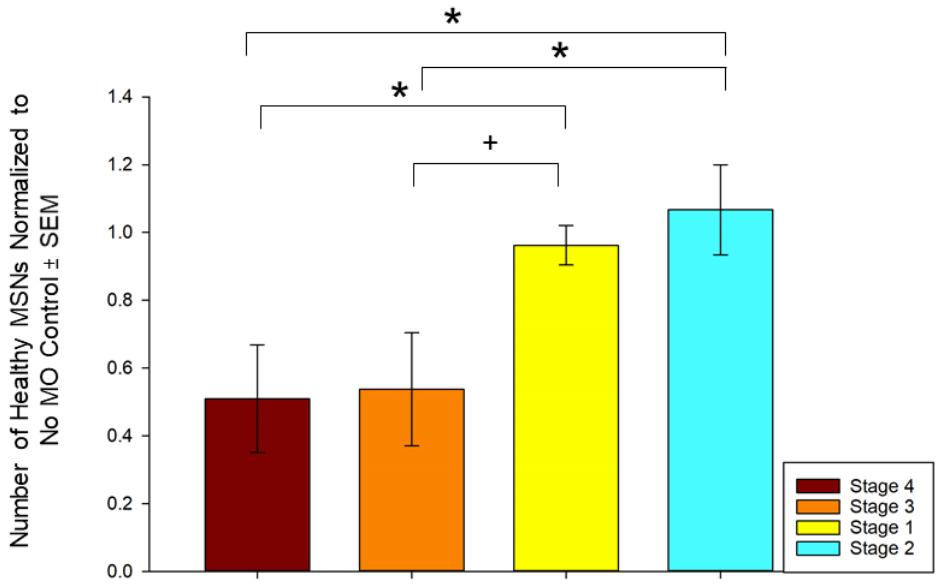

Figure 17: HD patient disease stage impacts the number of healthy MSNs in brain slices following engraftment of MO.

N = 4-20/group; data are means ± SEM. Data represent healthy MSN counts from 6 experiments. To combine experiments, HD MO counts were normalized to the No MO control for each experiment. Stage 1: n = 20 slices from 3 patients. Stage 2: n = 6 slices from 2 patients. Stage 3: n = 4 slices from 1 patient. Stage 4: n = 4 slices from 1 patient.

3.3.6 Pre-stimulation of MO differentially influences MSN health based on the genotype of engrafted MO

Previous research has shown that HD MO are hyper-reactive to stimulation as measured by increased production of the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α in a pro-inflammatory assay (Bjorkqvist et al., 2008; Trager et al., 2015). A previously published *in vitro* stimulation protocol was modified and optimized for the brain slice model [Figure 18].

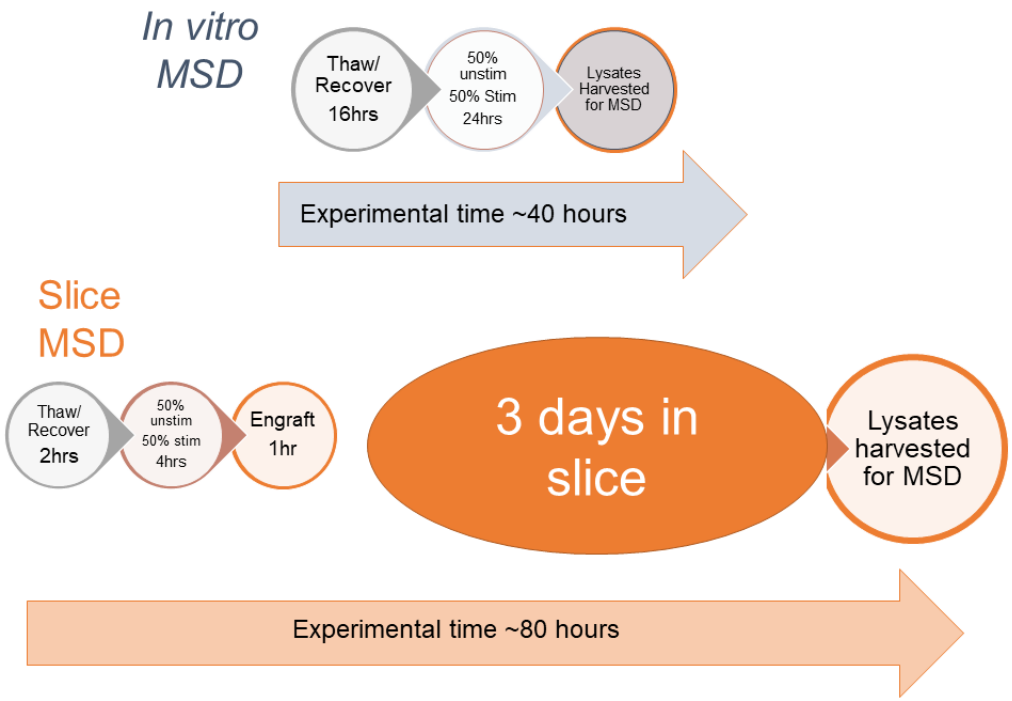

Figure 18: Schematic of experimental design for pre-stimulation of MO prior to engraftment into brain slices.

A previously published stimulation protocol was modified in the following ways: 1) After thawing, cells recovered in conical tubes at 4 ºC instead of in Primaria cell culture plates at 37 ºC. 2) Cells were stimulated for 4 hours instead of 24 hours. 3) Cells were engrafted into brain slices. 4) Engrafted MO were left in brain slices for 3 days, whereas *in vitro* MO were stimulated for 24 hours before the supernatant was harvested. 5) Brain slices were lysed using an MSD-compatible lysis buffer, and the supernatant was also harvested for the MSD assay.

To investigate the role of monocyte activation state in HD MO-induced MSN loss, MSN health outcomes were compared for HD and CTRL MO that were pre-stimulated prior to engraftment (HDϛ/ CTRLϛ) to HD and CTRL MO that were not stimulated prior to

engraftment (HDu/ CTRLu). A significant interaction was found between groups [F(4,43) = 5.117, p =0.002); Figure19]. Tukey *post hoc* comparison tests revealed a trend for HDu to decrease MSN number relative to No MO. While not significant at the P<0.05 level (p=0.13), data from this independent experiment is consistent with the earlier finding that HD MO decrease MSN health in the hybrid brain-slice assay [Figure 16]. Additionally, CTRLς had significantly more healthy MSNs than HDς (p=0.015) and HDu (p=0.002). [Figure 19].

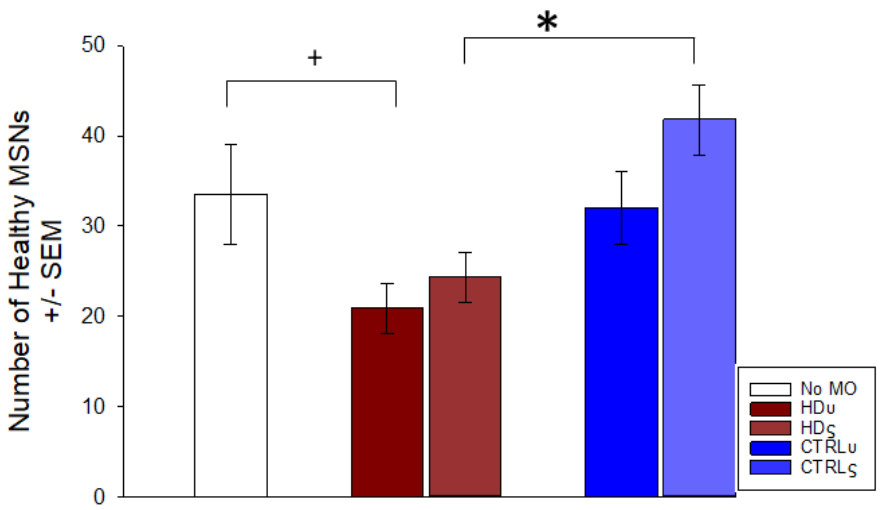

Figure 19: Pre-stimulating MO prior to engraftment does not alter the impact of HD MO on numbers of healthy MSNs but may be protective for CTRL MO.

Data are mean ± SEM, N=8-12 slices/group. *p<0.05 CTRLς vs. HDς; + Trend for HDu vs. No MO.

3.3.7 The brain-slice environment differentially affects HD and CTRL MO reactivity as measured by cytokine production

3.3.7.1 HD MO engrafted into brain slices do not significantly differ from CTRL MO in their production of cytokines/chemokine

Finally, whether the brain-slice environment itself would aberrantly stimulate HDu vs. CTRLu in the absence of stimulation [Figure 18] was investigated using the same human-specific V-plex pro-inflammatory MSD assay used to test cytokine/chemokine production in HD vs. CTRL MO *in vitro.* No significant differences were observed between the groups for any of the measured cytokines or the chemokine IL-8 [Figure 20].

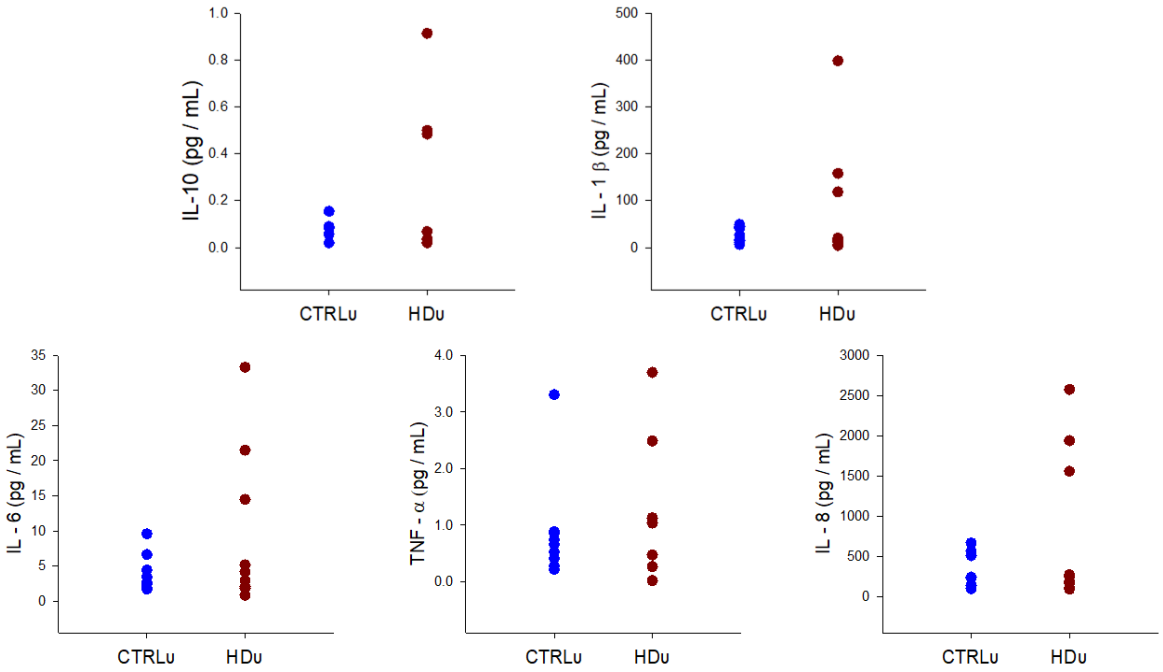

Figure 20: No difference in cytokine/chemokine production between HDu and CTRLu after engraftment into rat brain slices.

HDu and CTRLu were not stimulated prior to being engrafted into brain slices, where they remained for 3 days. Cytokine/chemokine levels were then measured using a customized V-plex human-specific pro-inflammatory sandwich immunoassay. N = 9/group. Note the contrast between the tight clustering of CTRLu data and the apparent bimodal spread of HDu data prompted further analyses to investigate if disease stage may be a relevant factor.

3.3.7.2 Trend for unstimulated moderate-stage patient MO engrafted into brain slices to have increased IL-6 production

While there were no significant differences in the production of cytokines or the chemokine IL-8 when HDu were compared to CTRLu, the data for the CTRLu was tightly clustered together while the HDu data appeared to be split into two clusters; one cluster with low secretion levels and another with high levels suggesting there may be differences among the individual HDu patients. To test this, CTRLu patients were

compared to HDu patients separated by disease stage. The moderate (Stage 3) patient had significantly increased levels of IL-6 production [Kruskal-Wallis one-way ANOVA, $X^2(3) = 10.092$, $p = 0.018$, with mean IL-6 pg/mL 2.615 for CTRL, 1.802 for HD Stage 1, 21.497 for HD Stage 3, and 4.174 for Stage 4; *post hoc* analyses revealed a significant difference between Stage 3 and Stage 1, $p = 0.013$, and a trend for Stage 3 > Control $p = 0.097$; Figure 21]. The Stage 3 patient also exhibited a trend to have increased IL-10 production [Kruskal-Wallis one-way ANOVA, $X^2(3) = 8.373$, $p = 0.039$, with mean IL-10 pg/mL 0.02 for CTRL, 0.02 for HD Stage 1, 0.50 for HD Stage 3, and 0.0365 for Stage 4; *post hoc* analyses revealed a trend for Stage 3 > Stage 1 $p = 0.072$, and Stage 3 > Control $p = 0.093$; Figure 21].

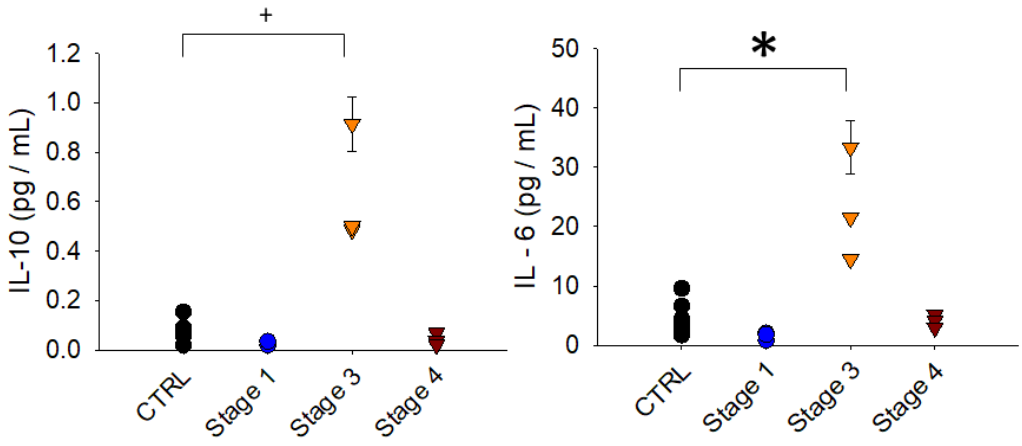

Figure 21: Unstimulated MO from a Stage 3 patient appeares to exhibit increased IL-10 and IL-6 production after 3 days in slice.

Cytokine/chemokine levels were measured using a customized V-plex human-specific pro-inflammatory sandwich immunoassay. n= 9 CTRL from 3 CTRL patients, 3 biological replicates from one Stage 1 patient, 3 biological replicates from one Stage 3 patient, and 3 biological replicates from one Stage 4 patient. +$p<0.10$; *$p<0.05$.

3.3.7.3 Pre-stimulated HD MO engrafted into brain slices do not significantly differ from CTRL MO in their production of cytokines/chemokine

Next, the impact of pre-stimulating MO prior to engrafting them into brain slices on cytokine/chemokine production was assessed. Similar to the unstimulated group, there were no significant differences between CTRLς vs. HDς for any of the measured cytokines or the chemokine IL-8 [Figure 22].

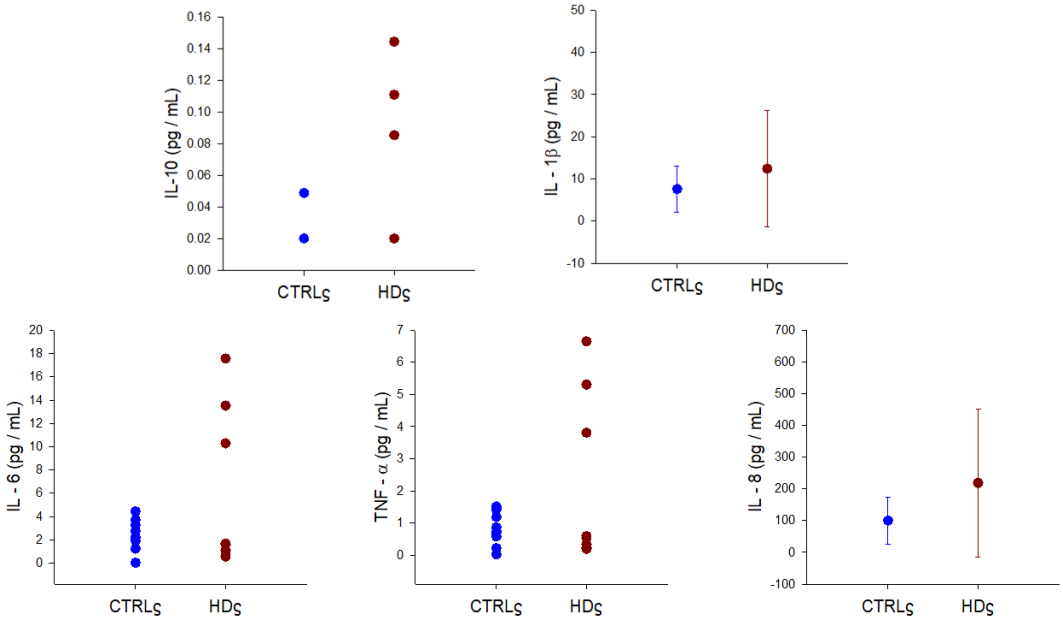

Figure 22: No difference in cytokine/chemokine production between HDς and CTRLς following engraftment into brain slices.

HDς and CTRLς were stimulated prior to being engrafted into brain slices, where they remained for 3 days. Cytokine/chemokine levels were then measured using a customized V-plex human-specific pro-inflammatory sandwich immunoassay. N = 9/group. The contrast between the tight clustering of CTRLς data and the apparent bimodal spread of HDς data prompted further analyses to investigate if disease stage was a contributing factor to the observed variation.

3.3.7.4 Pre-stimulated moderate-stage patient MO engrafted into brain slices exhibit a trend for increased production of IL-1β, IL-6, IL-8, and TNF-α

Because the cytokine/chemokine data for pre-stimulated MO exhibited a tight clustering of CTRL data but much greater spread of HD data, whether the stage of HD patient may be a factor in the production of cytokines/chemokine in pre-stimulated MO engrafted into slices was investigated. The Stage 3 patient was found to have significantly increased IL-1β production [$F(3,14)=15.411$, $p<0.001$; Figure 23A]. *Post hoc* analyses suggested that the IL-1β levels for the Stage 3 patient may have been significantly higher than all other groups: Stage 3>CTRL $p<0.001$; Stage 3>Stage 4 $p<0.001$; and Stage 3>Stage 1 $p=0.001$ [Figure 23]. The Stage 3 patient also appeared to have significantly increased IL-6 production [Kruskal-Wallis one-way ANOVA, $X^2(3) = 10.474$, $p = 0.015$, with mean IL-6 pg/mL 13.528 for Stage 3, 2.223 for CTRL, 1.110 for HD Stage 1, and 1.664 for HD Stage 4; *post hoc* analyses showed a significant difference between Stage 3 and Stage 1, $p=0.017$, and a trend for Stage 3 > Stage 4 $p = 0.070$, with no significant difference between Stage 3 and CTRL $p=0.295$; Figure 23B]. The Stage 3 patient also had significantly increased IL-8 production [$F(3,14)=31.910$, $p<0.001$; Figure 23C]. *Post hoc* analyses showed that the IL-8 levels for the Stage 3 patient were significantly higher than all of the other groups: Stage 3>Stage1>CTRL>Stage3 $p<0.001$; [Figure 23C]. The Stage 3 patient also appeared to have significantly increased TNF-α production [Kruskal-Wallis one-way ANOVA, $X^2(3) = 10.442$, $p = 0.015$, with mean TNF-α 5.310 for Stage 3, 0.731 for CTRL, 0.199 for HD Stage 1, and 0.533 for HD Stage 4; *post hoc* analyses showed a significant difference between Stage 3 and Stage 1, $p=0.010$; Figure 23D].

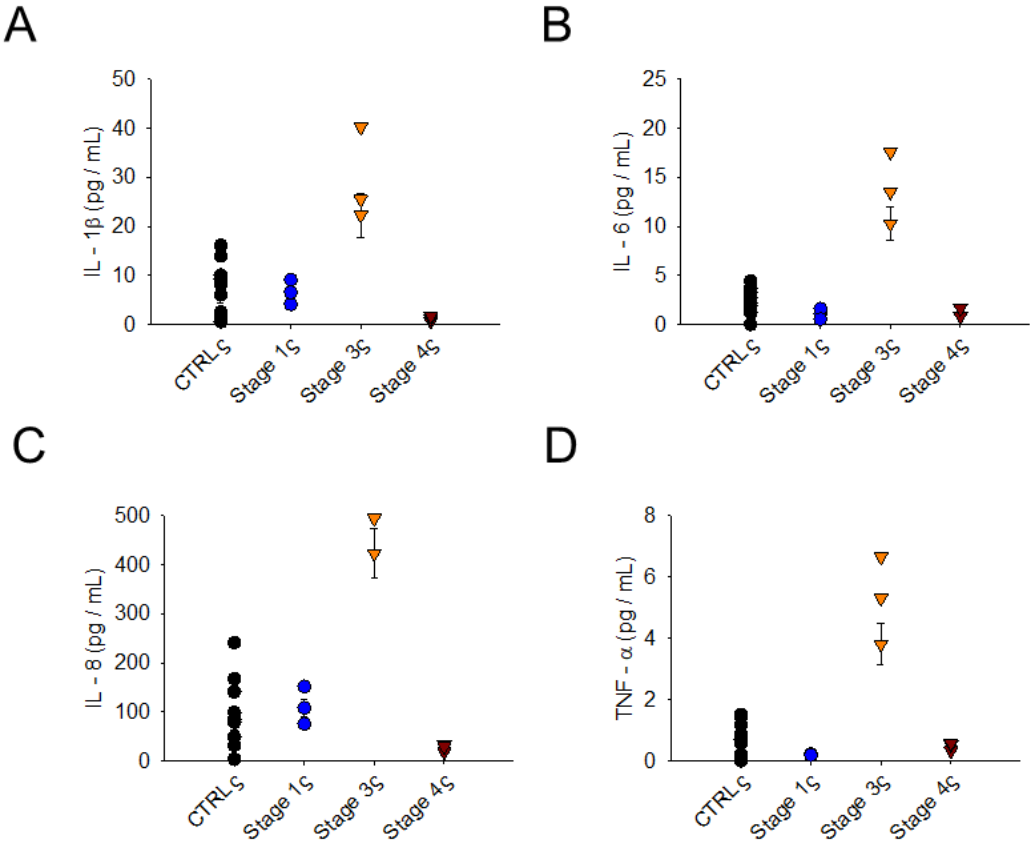

Figure 23: Pre-stimulated MO isolated from a Stage 3 HD patient (Stage 3ς) exhibit increased production of IL-1β, IL-6, IL-8, and TNF-α production after 3 days in slice.

Cytokine/chemokine levels were measured using a customized V-plex human-specific pro-inflammatory sandwich immunoassay. n= 9 CTRL from 3 CTRL patients, 3 biological replicates from 1 Stage 1 patient, 3 biological replicates from 1 Stage 3 patient, and 3 biological replicates from 1 Stage 4 patient.

3.4 Discussion

For my dissertation research, I developed a hybrid brain-slice model to study the ability of MO isolated from HD patients to effect MSN health in their native local brain-tissue environment. I experimented with a variety of engraftment techniques and found that the best method was to concentrate MO into a 2 µL volume of media before adding them to the top of biolistically transfected slices. Using this technique, I found that MO, both conspecific and from humans (hybrid), are viable in organotypic rat brain slices. This is consistent with other findings reported in the literature (Anselmo et al., 2015; Chung, Zelivyanskaya, & Gendelman, 2002; Girard et al., 2013; Hohsfield & Humpel, 2015a, 2015b). Using this model, I found that the engraftment of HD MO results in a significant decrease in the numbers of healthy MSNs, whereas CTRL MO had no effect. These main findings support my central hypothesis that HD MO, due to their disease genotype/phenotype, may be a contributing factor to driving neuropathogenesis in later stages of HD in which there is CNS infiltration of peripheral MO.

Furthermore, I compared multiple engraftment studies and found that MO isolated from patients who are in the later stages of the disease may be significantly more harmful to MSNs. However, these last experiments are suggestive rather than conclusive because of the limited amount of data available from patients in each stage of HD.

In conclusion, I have provided evidence that the hybrid brain-slice approach can be used to model neuropathogenesis driven by monocyte infiltration into the CNS and that the brain-slice environment itself is a significant contributing factor to neuropathogenesis. As will be described in the following chapter, I would expect that

upon infiltration into the brain tissues, MO can interact not only with neurons such as MSNs but also with the resident macrophages of the brain, namely microglial cells.

Chapter 4. Engraftment of MO from Patients with HD Alters Endogenous Microglia Morphology and Promotes Phagocytosis of MSNs in a Hybrid Brain-Tissue Model

4.1 Introduction

The overall goal of my dissertation is to investigate the potential role of peripheral monocytes as drivers of later stages of HD progression. For these studies, I have developed a novel hybrid brain-tissue based assay approach in which I can directly introduce human MO, isolated from HD and non-disease control patients, into living rat brain-tissue slice explants. In this chapter, I will report my findings on the consequences of such MO engraftments on the interactions between resident MSNs and microglial cells.

Microglia are the resident immune cells of the brain and in this model exist as endogenous cells within the brain-slice tissue. Microglia survey the parenchyma and maintain CNS homeostasis; they are highly dynamic cells that are capable of both ameliorating and exacerbating neuropathogenesis (B. M. Davis, Salinas-Navarro, Cordeiro, Moons, & De Groef, 2017; E. J. Davis, Foster, & Thomas, 1994; Nayak et al., 2012). For example, in a model of spinal cord injury, microglia and infiltrating monocytes were found to directly communicate and modulate each other's functions whereby monocytes suppressed microglia-mediated phagocytosis and inflammation (Zarruk, Greenhalgh, & David, 2018).

However, whether the same interactions occur when infiltrating monocytes enter the brain, and whether such interactions are beneficial or harmful for neurons remains understudied. This knowledge gap exists, in part, because of a lack of markers that are easily able to distinguish between microglia and monocytes once monocytes have

infiltrated into the CNS. Additionally, for experiments done *in vivo,* the numbers of MO infiltrating and exiting the CNS cannot be controlled.

Here, the organotypic brain-slice approach offers an experimental setting addressing both of these problems as it enables me to mark MO either by pre-labeling them prior to engraftment or by using immunohistochemistry for human cell markers. It also allows me to control the numbers of cells that are engrafted into brain slice explants and to track where they migrate within the slice. Some investigators have used similar approaches to study the effects of infiltrating monocytes but their results have been conflicting: some have found the infiltration of MO to be neuroprotective, while others have found that such infiltration exacerbates neurodegeneration (Girard et al., 2013; Hohsfield & Humpel, 2015a, 2015b; Jiang et al., 2017; Pavese et al., 2006).

My goal was to use the novel hybrid brain slice platform described in the previous chapters to directly study the impact of infiltrating human MO taken from patients on endogenous neurons and microglial cells native to the brain slice tissues. In particular, I believe that the question of whether infiltrating monocytes can influence endogenous microglia is essential for understanding neuroinflammatory processes in later stages of Huntington's disease. Indeed, both because of the peripheral inflammation inherent to HD and because of degeneration of the blood-brain barrier, it would seem likely that microglia and infiltrating monocytes would interact in later stages of the disease and that such interactions may be important in driving the striking neurodegeneration observed over the course of HD.

In this context, I developed assays to study microglial/MO interactions with healthy striatal medium spiny neurons (MSNs), including methods to quantify such

intercellular interactions using Imaris software (Bitplane Inc.) to create 3D reconstructions of cellular morphologies.

I tested the hypothesis that engrafted/infiltrating monocytes would directly affect microglia-MSN interactions in the brain-slice model, and that the genotype of the engrafted monocyte would influence whether such interactions would be neurotrophic or neurodegenerative. MO were isolated from HD and non-disease control patients and engrafted into organotypic brain slices that had been biolistically transfected with YFP to label MSNs. I found that engrafted MO survive well in brain slices, penetrate slices to the depths where transfected and healthy MSNs reside, and interact with both native MSNs and microglia. The interactions between monocytes, MSNs and microglia were imaged and quantified using immunohistochemistry, confocal imaging, and 3D rendering of intercellular interactions. Based on my central hypothesis, I predicted that engraftment of HD MO would aberrantly influence endogenous microglia and negatively impact MSN health.

4.2 Materials and Methods[1]

4.2.1 Confocal imaging and 3D reconstructions of intercellular interactions

Confocal z-scans of IBA1-positive microglia, YFP+ MSNs, and MAB1273+ MO were acquired through a 40× objective (oil DIC; NA 1.4) in 0.33-µm *z*-steps. Image series were saved in a .lif format, and ImageJ was used to perform background

[1] Parts of these methods were originally published as: Bolton, J.L., Marinero, S., Hassanzadeh, T., Natesan, D., Le, D., Belliveau, C., Manson, S.N., Auten, R.L., Bilbo, S.D. (2017). Gestational Exposure to Air Pollution Alters Cortical Volume, Microglial Morphology, and Microglia-Neuron Interactions in a Sex-Specific Manner. *Frontiers in Synaptic Neuroscience.* 9: 10

subtraction, smoothing, and thresholding. Imaris software (Bitplane) was used to perform 3D reconstruction and surface renderings of microglia, neurons, MO, and the overlap between them (Dorothy P. Schafer, Lehrman, Heller, & Stevens, 2014; D. P. Schafer et al., 2012). The 3D renderings of the microglia-neuron interactions were then inspected to verify that they met criteria to be included for analysis (detailed below). Those that passed inspection were digitally cleared of extraneous microglia and neurons so that only the microglia and neurons directly involved in intercellular interactions remained. These surface renderings were then used to quantify microglial metrics including volume, surface area, surface area to volume ratio, and density. Surface renderings were also used to quantify the volume of MSNs phagocytosed by microglia.

4.2.2 Selection of regions of interest for 3D reconstruction

Sections containing monocytes, microglia, and neurons were chosen from the striatal regions of organotypic brain slice explants (3-6 slices/group; 3–5 monocyte-microglia-neuron interaction renderings per prenatal rat). Using confocal microscopy, z-stacks of approximately 40 μm were collected in 0.33-μm *z*-steps. From these *z*-stacks, intercellular interactions were chosen for analysis based on the following criteria: 1) the presence of MO, microglia, and MSNs, 2) only MSNs (identified by their size, dendritic branching, and lack of apical dendrite) were included, 3) only full microglial and MSN cell bodies that did not touch the *z*-stack perimeter were included, and 4) processes were only included if they were clearly attached to the MSN and/or microglia of interest.

4.2.3 Data analysis and statistics

All data were analyzed using Sigmaplot 14 statistical software (SYSTAT). Student's *t-test*s were used for data that passed normality tests, and Mann-Whitney

Rank Sum tests were used for data that failed normality tests. When multiple groups were being compared, one-way ANOVAs were used to analyze all data that passed the Shapiro-Wilk Normality Test followed up with *post hoc* comparisons (Tukey's HSD) to identify group differences, with significance assumed at $p<0.05$. If the normality test failed, a Kruskal-Wallis one-way ANOVA was used followed up with *post hoc* comparisons (Tukey's HSD) to identify group differences, with significance assumed at $p<0.05$. All reported p values were two-tailed.

4.3 Results

4.3.1 Post-experimental immunohistochemistry enables the accurate reconstruction of intercellular interactions

Initial experiments suggested that CT dye may be leaking from labeled monocytes in some slices and may impede accurate image analysis and reconstruction of intercellular interactions [Figure 24 A/B]. Therefore, the anti-human antibody MAB1273, which detects the surface of human mitochondria, was used to label human MO in organotypic slices without distorting the MO signal [Figure 24 C]. However, because MAB1273 is an intracellular antibody that does not label the cell's cytoplasm, it could not be used to measure MO volume [Figure 24 D]. Therefore, all experiments conducted to determine the impact of human MO on MSN neurodegeneration were completed prior to MO labeling. Visualization of cells was accomplished by fixing the tissue and using anti-IBA-1 antibodies to detect microglia and the human-specific anti-mitochondrial antibody MAB1273 to detect engrafted MO. Neurons labeled using biolistic transfection maintained YFP expression with fixation and did not need to be secondarily labeled with antibodies. Volumetric and morphological analyses were only conducted on microglia because, as noted above, MAB1273 is an intracellular antibody and does not

allow accurate quantification of MO volume or morphological reconstruction [Figure 24]. Depending on their activation state, monocyte volumes have been reported to range between 150-480 μm^3 (Arenson, Epstein, & Seeger, 1980).

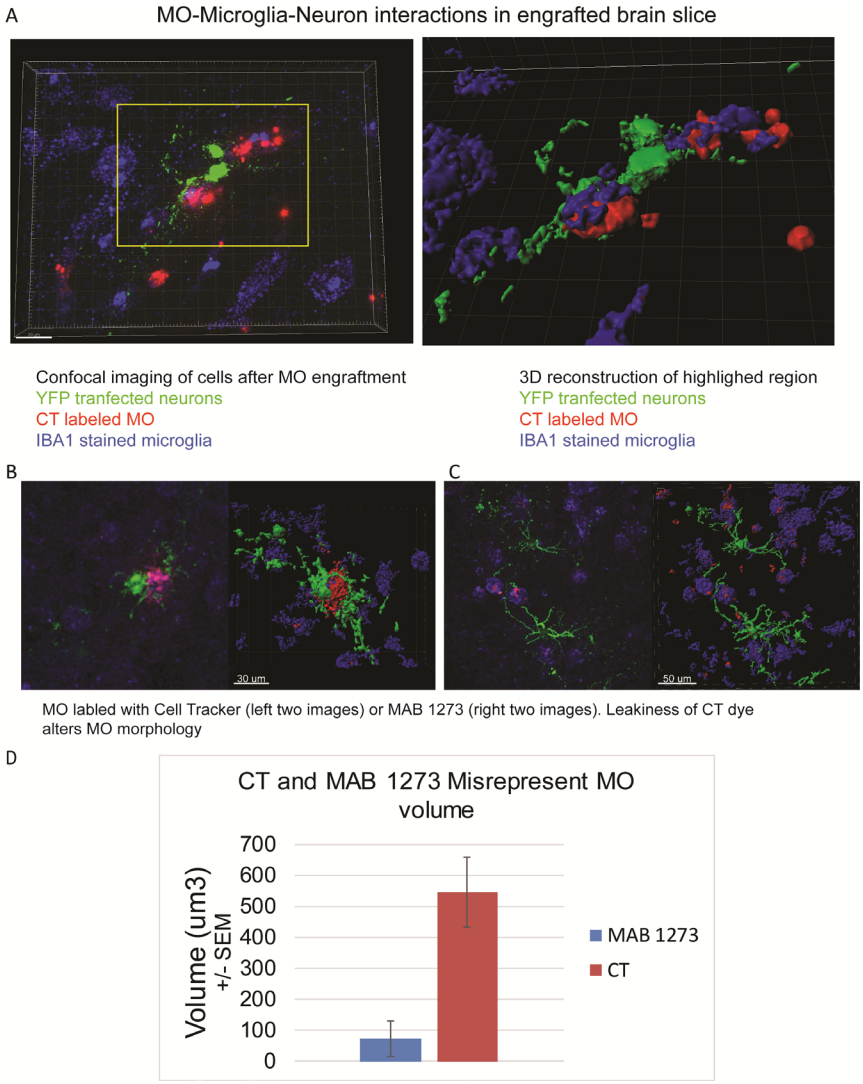

Figure 24: Leakage of CellTracker dye (CT) from MO compromises 3D reconstruction of MO morphologies.

A, left, confocal imaging of organotypic brain slice biolistically transfected with YFP to label neurons (green), stained with anti-IBA1 to label microglia (blue), and dyed with CT to label engrafted MO (red). Right, confocal 3D reconstruction of the area highlighted by the yellow box. Note the apparent leakage of CT from MO that can be seen in the

fluorescence image results in 3D reconstructions of MO that are well beyond the expected volume of MO. B, confocal images and 3D reconstructions of MO labeled with CT vs. MAB1273 two days after engraftment. C, bar graph showing the average volume of MO measured after 3D reconstruction and volumetric analyses using CT vs. MAB1273. Data are means ± SEM. Average MO volume was calculated using 3 slices used per condition (n=6 slices/ 43 MO labeled with MAB1273 and 31 labeled with CT). Although not an accurate label for volumetric analysis, MAB1273 was chosen for subsequent experiments as a reliable label for the presence and location of human MO.

4.3.2 Microglial morphology in organotypic brain slice cultures prior to MO engraftment

First, changes in microglia morphology, in the absence of engraftment, were measured over the three-day time course of brain-slice organotypic culture. Microglia in slices had small cell bodies with ramified processes immediately after brain slices were cut. Thereafter, these processes progressively retracted and, by 48 hours after slicing, most microglia were amoeboid and contained fewer processes. By day three, microglia were less amoeboid, and their processes started to elongate [Figure 25]

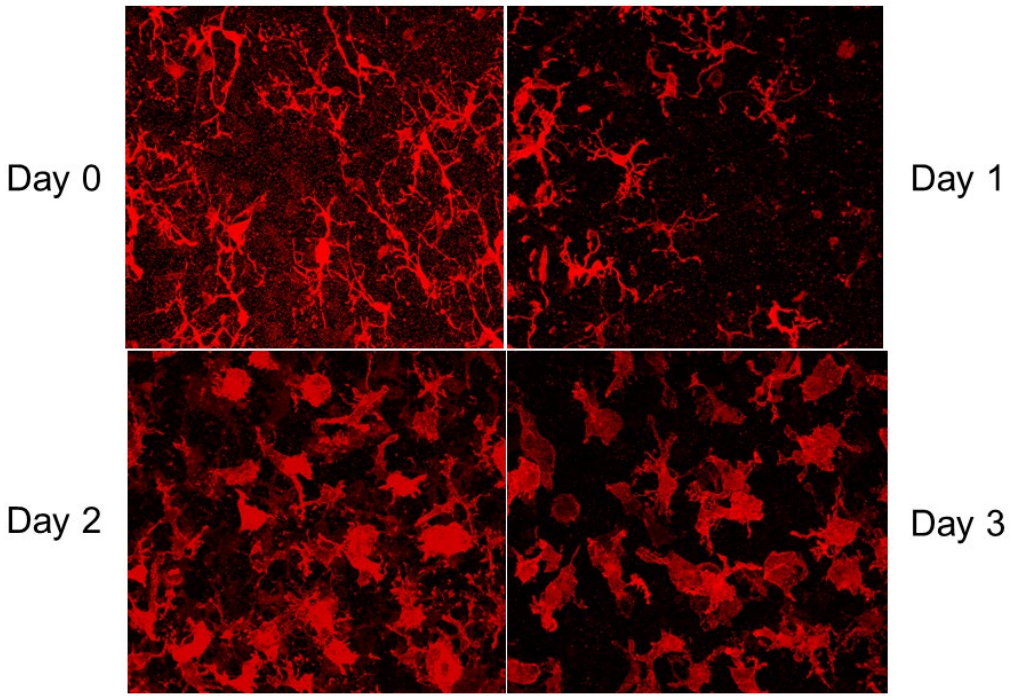

Figure 25: Microglia change their morphology in brain slices over the course of 3 days in organotypic culture.

Immediately after brain slices are cut, resident microglia show ramified morphology with small cell bodies. Between days 1 and 2, their processes retract, and microglia take on a more “activated” amoeboid morphology. By day 3, microglia are still amoeboid, but their processes appear to be elongating. Microglia in brain slices were immunoassayed with anti-IBA1 and imaged under confocal microscopy.

4.3.3 Microglial interactions with neurons in brain slice cultures prior to MO engraftment

Next, immunohistochemistry combined with confocal microscopy and 3D reconstructions using Imaris 9.2 software (Bitplane Inc.) were used to verify that interactions between microglia and neurons could be reconstructed in brain slices. Microglia surrounded MSNs and appeared to interact with both MSN dendrites and cell bodies [Figure 26].

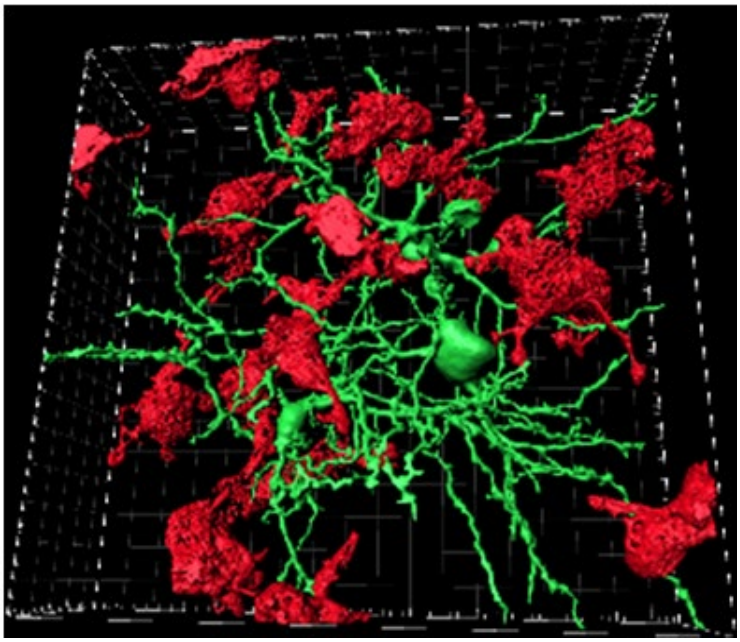

Figure 26: Microglia surround MSNs and extend their processes to interact with MSNs.

Digital reconstructions of confocally imaged microglia (labeled with anti-IBA1; red) and MSNs (labeled with biolistic transfection of a YFP reporter; green).

4.3.4 Engrafted MO penetrate brain slices to the depths of transfected and healthy MSNs

The MSNs transfected in the brain-slice assay are approximately 70 μm below the surface of the brain slice. Using CT dye to label MO and immunohistochemistry to label microglia, engrafted MO were identified as they penetrated slices to the depths of transfected and healthy MSNs [Figure 27A]. A control experiment revealed that MO must be alive to penetrate slices, as MO that were pre-fixed prior to engraftment remained at the surface [Figure 27B].

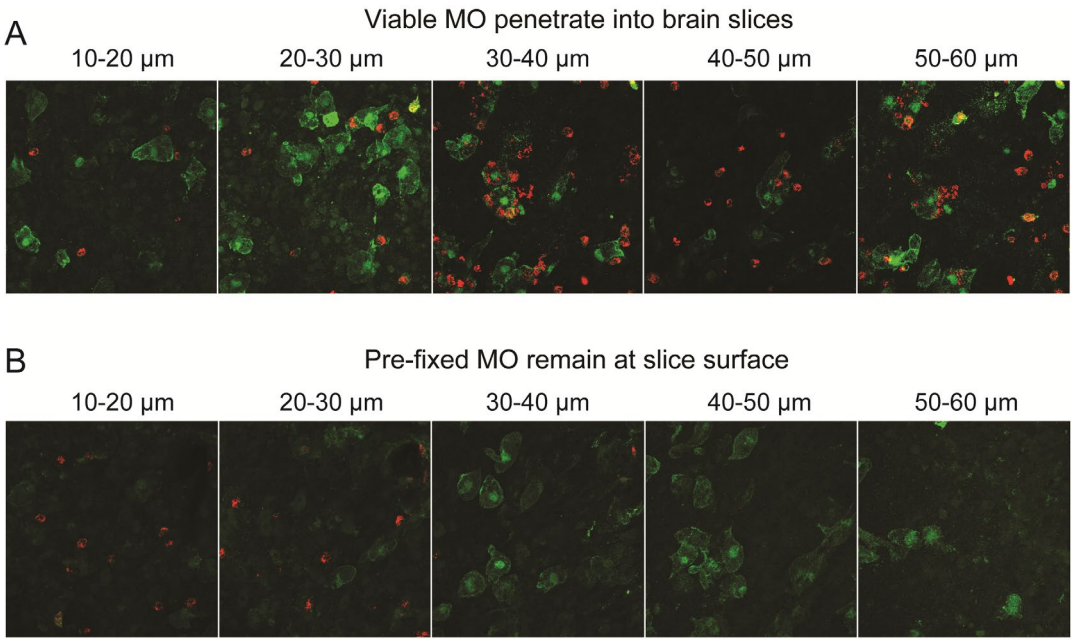

Figure 27: Engrafted MO penetrate brain slices to the depths of transfected and healthy MSNs within 48 hours.

Human MO and microglia were imaged using confocal microscopy in organotypic brain slice cultures. A, living MO penetrate brain slices to depths of 60 µm or more within 48 hours, which is the same approximate depth as transfected and healthy MSNs in the hybrid brain-slice assay. B, as a control, MO were pre-fixed prior to engraftment. These cells remained near the surface of the slices. Human MO were dyed with CT; red. Endogenous microglia were labeled with anti-IBA1; green.

4.3.5 Quantification of microglial morphology and phagocytosis of MSNs

Imaris 9.2 software (Bitplane Inc.) was used to collect data on microglia number as well as morphological characteristics of microglia including surface area, volume, surface area to volume ratio, and the sphericity of microglia [Figure 28A]. The volume of MSNs phagocytosed by endogenous microglia was calculated by reconstructing areas of IBA1-YFP overlap within a given ROI.

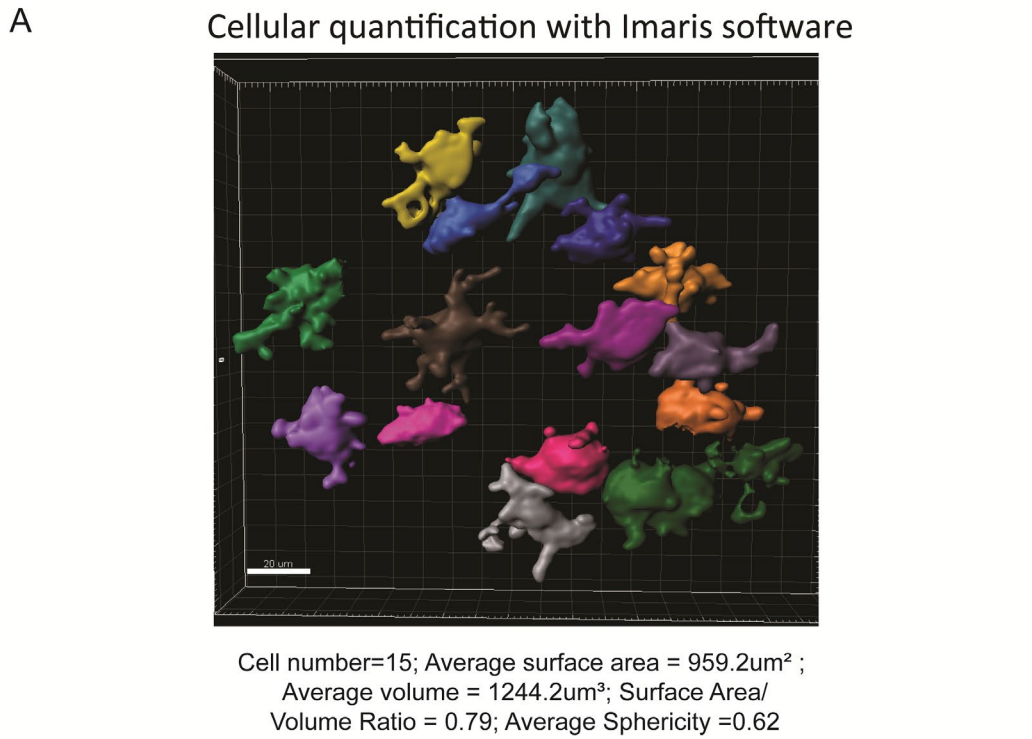

Figure 28: Quantification of cellular and intercellular interactions in organotypic brain slices using confocal imaging and Imaris software analysis.

A, reconstructed individual microglia within a region of interest (ROI) within the striatal region of an organotypic brain slice. Numbers below the image are example metrics for the microglia displayed in the image. B, Left, fluorescence image of a MSN (biolistically

transfected with YFP; green), microglia (labeled with Anti-IBA1; red), and human MOs (labeled Anti-MAB1273; cyan) within an organotypic brain slice. Right, confocal imaging and Imaris software were used to render morphologies and intercellular interactions in 3D. Z-stacks were collected in 0.33 µm steps, enabling the reconstruction of the volume of MSN phagocytosed by microglia (orange).

4.3.6 Microglial density and phagocytic activity are unchanged by rat MO engraftment

First, pilot experiments were conducted to determine whether the species of the engrafted MO, conspecific rat MO vs. human MO, would significantly alter microglial metrics. No differences in microglial density (Student's *t*-test, p=0.42) or volume of phagocytosed MSN material were found (Student's *t*-test, p=0.39) [Figure 29].

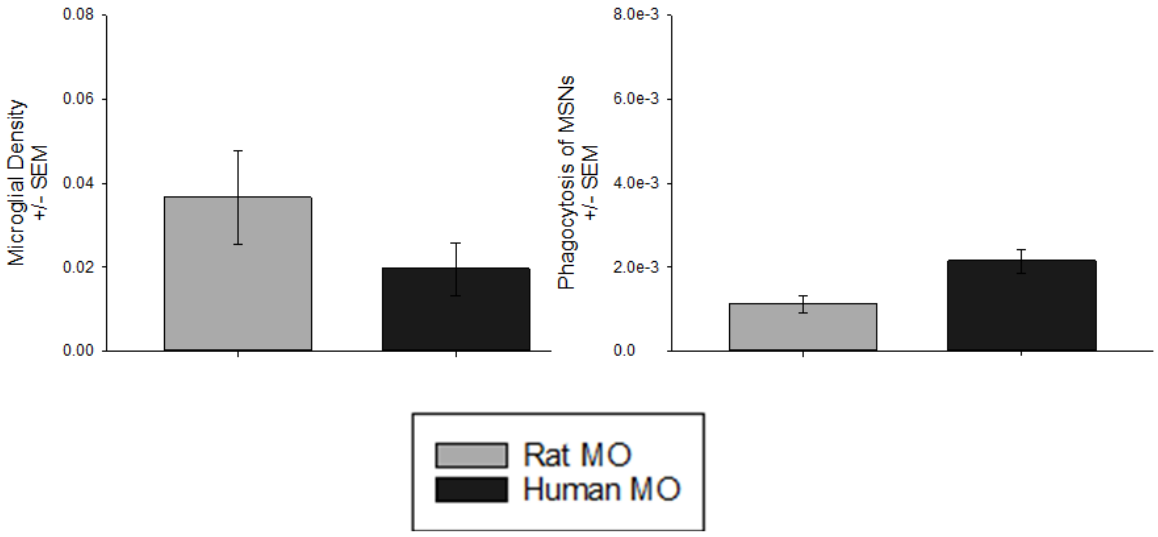

Figure 29: Engraftment of human MO does not significantly alter microglia density or MSN engulfment compared to engraftment of conspecific rat MO.

Data are means +/- SEM, n = 4-6 slices/group.

4.3.7 MO engraftment alters microglial sphericity and surface area to volume ratio

Next, whether the engraftment of HD MO would alter the morphology of endogenous microglia as measured by sphericity and surface area to volume ratio was tested. The engraftment of MO significantly reduced microglial sphericity relative to slices in which No MO had been engrafted [$F(2,18)=7.303, p=0.005$; Figure 30]. *Post hoc* analyses showed that the No MO condition was significantly more spherical than CTRL MO (Tukey Test, $p=0.007$) and more than HD MO (Tukey Test, $p=0.024$). There was no significant difference between CTRL and HD MO ($p=0.772$) [Figure 30]. Engraftment of MO also increased microglial surface area to volume ratio relative to No MO [$F(2,18)=10.974, p<0.001$; Figure 30]. *Post hoc* tests showed a significant difference between CTRL MO and No MO ($p=0.002$) and between CTRL MO and HD MO ($p=0.002$), but no difference between No MO and HD MO ($p=0.955$) [Figure 30].

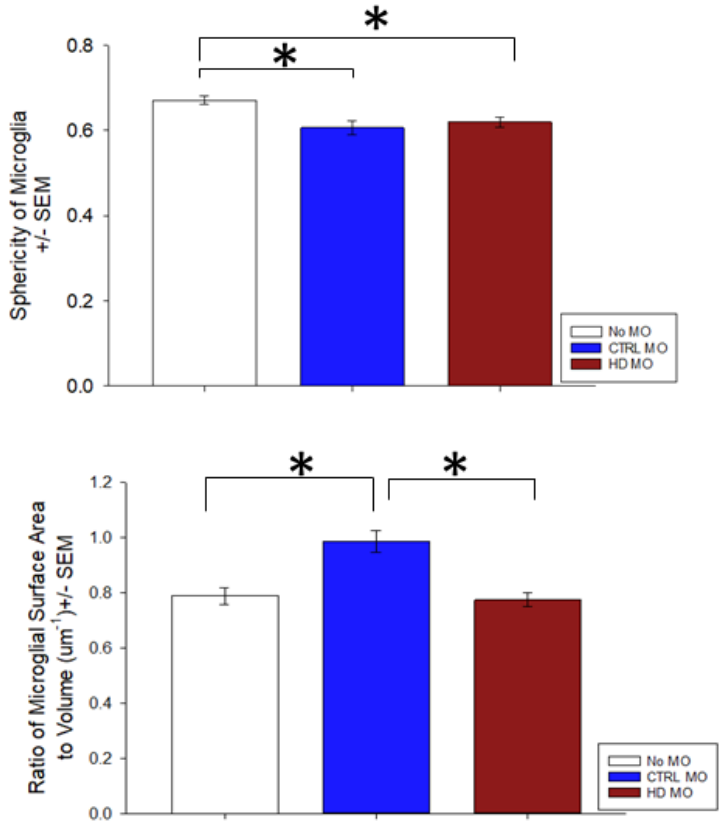

Figure 30: MO engraftment alters microglial morphology.

Upper, engraftment of MO, independent of genotype, decreases microglial sphericity. Lower, engraftment of CTRL MO increases the surface area to volume ratio of endogenous microglia. Engraftment of HD MO appears to have no effect, or alternatively, to lack the effect of CTRL MO engraftment. Data are means ± SEM, n=6-8/group from two separate experiments. *p<0.05.

4.3.8 HD MO engraftment increases microglial density

Next, the density of endogenous microglia within slices was measured. Engraftment of HD MO, but not that of CTRL MO, significantly increased microglial density [Kruskal-Wallis one-way ANOVA, $X^2(2) = 8.356$, $p = 0.015$]. *Post hoc* analyses

showed a significant difference between HD MO and No MO (p=0.013) with no significant differences between the other groups [Figure 31].

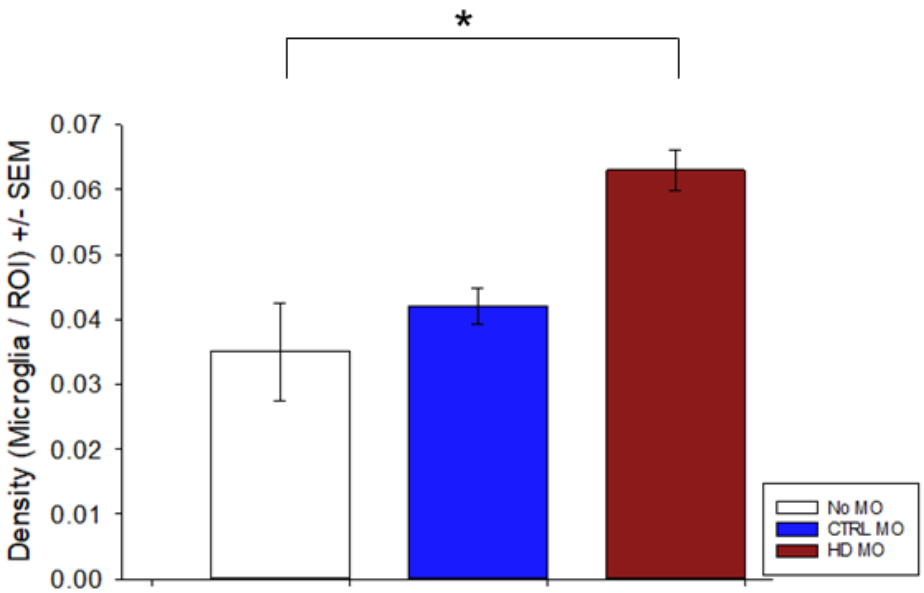

Figure 31: Engraftment of HD MO, but not that of CTRL MO, increases microglial cell density in brain-tissue slice explants.

Data are means ± SEM, n=6-8/group from two separate experiments. *p<0.05.

4.3.9 HD MO engraftment increases microglial phagocytosis of MSNs

Finally, the apparent microglial phagocytosis of MSNs (i.e., overlapping volumes of microglial and MSN reconstructions) was quantified. These experiments revealed that the engraftment of HD MO significantly increased microglial phagocytosis of MSNs. [Kruskal-Wallis one-way ANOVA, $X^2(2) = 9.372$, $p = 0.009$]. *Post hoc* analyses revealed a significant difference between HD MO and No MO (p=0.022) and a significant difference between HD MO and CTRL MO (p=0.027) [Figure 32]. In contrast, engraftment of CTRL MO did not increase microglial phagocytosis of MSNs and, in fact, may have reduced this measure of microglial-MSN intercellular interaction.

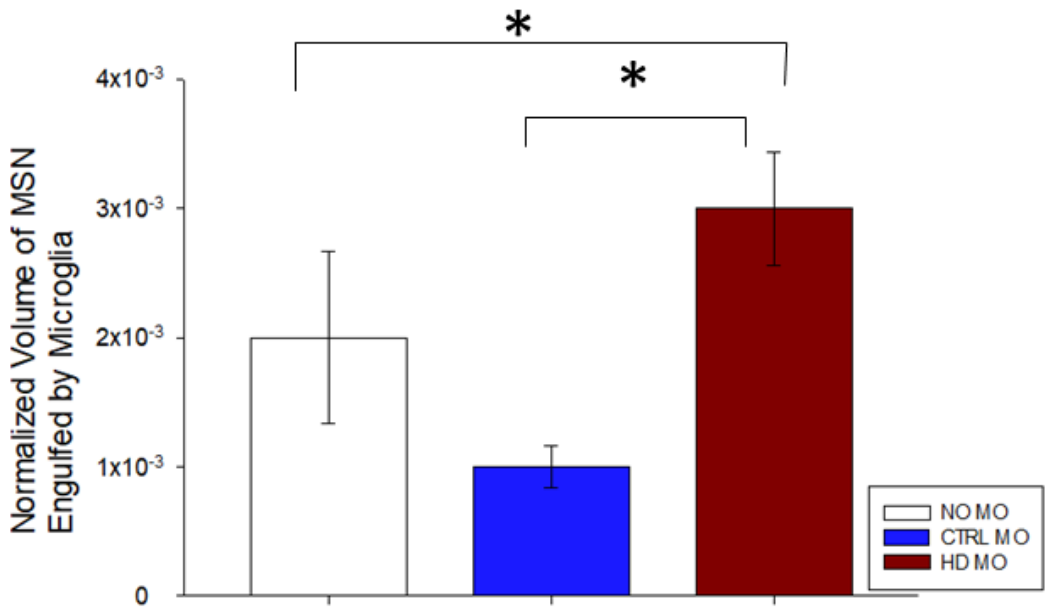

Figure 32: Engraftment of HD MO increases microglial phagocytosis of MSNs, while CTRL MO may have decreased microglial phagocytosis compared to No MO controls.

Data are means ± SEM, n=6-8/group from two separate experiments. *p<0.05.

4.4 Discussion

To address my core hypothesis, I have developed a hybrid brain-slice approach to study how the infiltration of peripheral MO may alter the morphological and functional characteristics of endogenous microglia, including microglial-neuronal interactions. An exciting finding is that HD MO engraftment—but not that of CTRL MO—appears to increase microglial density as well as microglial phagocytosis of MSNs.

Together, my studies support that a hybrid human-monocyte rat-brain slice approach can be used to model the influence peripheral MO have on endogenous microglia and/or MSNs native to the brain slice tissue, and to model interactions

between these critical CNS cell populations. A key advantage of this approach is that the effects of MO from HD patients vs. non-disease controls can be directly compared.

Future studies aimed at uncovering the importance of HD disease stage and elucidating the mechanisms behind the observed intercellular interactions have the potential to identify novel drug pathways and drug candidates to target infiltrating peripheral monocytes for neuroprotective benefit in HD. Importantly, this could include the repurposing of drugs that are already FDA approved or under clinical development for other disease indications. Effective targeting of neuroinflammation could substantially delay or slow HD progression, providing the equivalent of a cure for some or many patients at risk for HD.

5. Conclusions

5.1 Differential Roles of Monocytes and Microglia in Neuroinflammation and Neurodegeneration

The central focus of my dissertation research was on interactions between microglial cells resident in the brain and peripheral monocytes that infiltrate the brain parenchyma under conditions of disease such as the in late stages of HD. Monocytes are peripheral immune cells that are important for normal immune function. Under non-pathological conditions, monocytes are present in the meninges and endovascular space within the brain but do not infiltrate the brain parenchyma. However, they are still able to influence processes and functions within the CNS through indirect mechanisms, such as cytokine secretion, or through minimal passage through the choroid plexus (Baruch et al., 2015; Möhle et al., 2016).

Monocyte infiltration into the CNS observed in neurodegenerative disorders may not depend solely on such indirect mechanisms as the BBB has been shown to be compromised due to the disruption of endothelial cell-to-cell connections (Maiuolo et al., 2018). The BBB is likely to be compromised in HD, especially in the later stages, as studies have shown that the BBB is compromised even in the course of healthy aging (Montagne et al., 2015). Studies more directly related to HD demonstrate disruption of the BBB in mouse models and that activated microglia, a pre-symptomatic feature of HD, can, in turn, disrupt the BBB (Alba Di Pardo et al., 2017; Shigemoto-Mogami, Hoshikawa, & Sato, 2018). Such BBB disruption supports the hypothesis that monocyte-microglia interactions may occur in HD, and indeed may be a critical interaction and process underlying neuroinflammation in later stages of the disease. This important

possibility was the rationale for developing the novel experimental platform I used to study monocyte-microglia interactions directly, and prompted a series of experiments such as those described in Chapter 4 to investigate the consequences of such interactions for the health of MSNs in the striatum, the part of the brain that is first and most susceptible to neurodegeneration in HD.

Microglia and monocytes were once considered to be nearly identical cell types, and monocyte-derived macrophages were thought to have identical origins, gene expression, and function to microglia. This was based on shared morphological characteristics and expression of cellular markers such as F4/80, CD11b, CD45, CD68, CSF1R, CD200R, Cx3Cr1, and IBA1 (Greter, Lelios, & Croxford, 2015). Additionally, monocytes and microglia are both “professional” phagocytes that also have the potential to excrete both anti-inflammatory and pro-inflammatory cytokines. Until recently, it was thought that monocytes helped to maintain the population of microglia in the brains of mature animals by infiltrating into the CNS and transforming into microglia (Lassmann & Hickey, 1993; Lassmann, Schmied, Vass, & Hickey, 1993). However, recent studies using fate mapping have revealed that microglia and monocytes have distinct origins; microglia are generated from Myb-independent myeloid progenitors originating from the yolk sac and seed the CNS during the first two trimesters of gestation, while monocyte progenitors are generated later in development and are derived from Myb-dependent definitive hematopoiesis (Ginhoux, Lim, Hoeffel, Low, & Huber, 2013; Goldmann et al., 2013; Prinz & Priller, 2014; Schulz et al., 2012).

Moreover, it is clear that monocytes are not a homogeneous cell type. Cell surface markers have been used to identify at least 3 separate cell subtypes within the monocyte category: classical monocytes, intermediate monocytes, and non-classical

monocytes (Boyette et al., 2017; Franca et al., 2017). While these subtypes have been assigned separate functions (non-classical monocytes, for example, are also called patrolling monocytes and are thought to be involved in tissue repair), all monocytes are capable of transforming into macrophages in response to their environment (Boyette et al., 2017).

The studies that previously demonstrated monocytes were capable of replenishing microglial populations were found to have a critical flaw: namely, that the lethal irradiation used to deplete endogenous monocytes perturbed the BBB and allowed for monocyte infiltration that would not otherwise occur (Mildner et al., 2007). It has since been established that microglia are long-lived cells that can replenish themselves, while monocytes are short-lived cells that are continually being regenerated in the bone marrow.

As such, distinct roles for infiltrating monocytes and resident microglia are emerging in neuroinflammation (Ajami, Bennett, Krieger, McNagny, & Rossi, 2011; Ajami, Bennett, Krieger, Tetzlaff, & Rossi, 2007). However, the roles of microglia and monocytes are complex, and may even be variable depending on the exact situation. For example, as both cell types are capable of adapting to their environment, monocytes that do find their way into the CNS are capable of developing into macrophages with microglia-like properties (Lassmann & Hickey, 1993; Rocca et al., 2017). Furthermore, recent studies have shown that monocyte-derived macrophages from the periphery are capable of infiltrating the CNS in the absence of irradiation when injured, upon infection, or when microglia are pharmacologically depleted (Cronk et al., 2018; Karlen et al., 2018). Thus, while there are increasing reports of monocytes identified in the brain parenchyma in multiple neurodegenerative disorders, their contributions to CNS

pathology remain highly debated (Oleg Butovsky et al., 2012; Chiu et al., 2013; Jay et al., 2015; Prinz & Priller, 2014; Thériault, ElAli, & Rivest, 2015; Y. Wang et al., 2016; Yamasaki et al., 2014)).

While the origins of microglia vs. monocytes have clearly been established to be distinct, the ability of monocytes to influence microglia and to modify their phenotypes in the CNS is still a topic of debate. Together, the data that I have collected in my dissertation research support that the presence of HD MO, but not CTRL MO, alters microglial density, and increases their phagocytosis of healthy MSNs.

Other researchers have also found that monocytes can influence microglia in the CNS. One laboratory found that the infiltration of MO into the injured spinal cord altered microglial phagocytosis, whereby microglia stopped phagocytosing injured dendrites and monocytes became the primary phagocytic cells (Anderson, Greenhalgh, Takwale, David, & Vadigepalli, 2017). Another laboratory found that monocyte-derived macrophages release IGF-1 and that such secretion redirected the phagocytic capacity of “non-professional” phagocytes (C. Z. Han et al., 2016).

In my studies, I found that cytokine secretion was altered in HD MO, particularly in MO isolated from later stage patients. Others have shown that cytokine secretion, namely IL-1β, TNF-α, IL-6, and IL-10, are key nodes in the inflammatory network (Anderson et al., 2017). Therefore, I speculated that the differences I observed in cytokine production between HD MO and CTRL MO could be responsible, at least in part, for the differential microglial phenotypes and functional responses I observed, notably in microglial morphology/density and in the numbers of surviving and healthy MSNs in the presence of engrafted HD vs. CTRL MO.

In particular, I found that MO isolated from a Stage 3 HD patient exhibited a trend for increased IL-10 and IL-6 production relative to MO collected from CTRL patients. IL-10 in the CNS has been associated with the upregulation of phagocytosis which could explain my finding of increased MSN phagocytosis in slices engrafted with HD MO. My findings using pre-stimulated MO, however, implied that MO from the same patient not only had increased IL-10 and IL-6 production, but also increased IL-1β, and TNFα in brain slices relative to pre-stimulated controls. Such increased cytokine production was not associated with a decrease in healthy MSNs but, surprisingly, the pre-stimulated CTRL MO group appeared to take on a neuroprotective role as indicated by a trend for increased healthy MSN numbers.

I speculated that these differences arise due to aberrant HD MO reactivity. While the pre-stimulation of CTRL MO caused them to take on a protective role, the pre-stimulation of HD MO seemed to have no effect on MSNs. This could be because HD MO are already maximally stimulated in and/or by their engraftment into brain tissue slices. Alternatively, HD MO may be incapable or less capable of responding to the stimulation protocol used because of their genotype and consequent phenotype. While beyond the scope of my dissertation, an interesting and informative future study would be to investigate how pre-stimulation of HD MO and CTRL MO affects their phagocytic function. I would predict that phagocytosis of MSNs would be unchanged in unstimulated vs. pre-stimulated HD MO, but that MSN phagocytosis would be decreased in pre-stimulated CTRL MO relative to unstimulated CTRL MO.

One of the main findings of my dissertation research was that engraftment of CTRL MO into brain slice explants, as an experimental model for infiltration of peripheral monocytes into the CNS, did not appear to negatively affect MSN health. There are

conflicting results on this topic in the literature, as some find that the infusion of MO can be neuroprotective while others find that MO in the CNS exacerbate neuronal damage (Hohsfield & Humpel, 2015a, 2015b; Varvel et al., 2016). One explanation for these differences may be that the MO used were in different states of activation. The monocytes from the Varvel study infiltrated the CNS in an *in vivo* experiment in response to *status epilepticus*. Cellular markers indicated that these MO were inflammatory, and it is likely that the parenchymal environment was severely affected by the seizures. The combination of these two factors may have prevented MO in this situation from adopting a protective phenotype. In the Hohsfield study, on the other hand, MO were taken from young animals and infused into the tail vein of an Alzheimer's disease mouse model. The mice in this study exhibited a decreased β-amyloid load and temporary improvement of memory function. These MO were isolated from non-inflamed, young mice, which could explain their protective capacity in this very different experimental setting.

5.2 The Importance of Developing a Tissue-Based Experimental System

While *in vivo* studies are ultimately required for the elucidation of disease mechanisms and for the validation of new drug and drug target candidates, current genetic mouse and rat models of HD models have significant limitations including restricted experimental access, incomplete *HTT* gene constructs, genomic instability, slow onset of pathology, and a lack of degeneration of the striatum and cortex (Menalled & Chesselet, 2002). Moreover, in these immunocompetent animal models, it would not be possible to introduce non-syngeneic cells such as monocytes from HD patients.

Thus, the goal of my dissertation research was to develop a hybrid brain-tissue based assay in which I could directly evaluate the ability of monocytes isolated from HD patients to affect MSN survival and health, as an experimental model mimicking the infiltration of peripheral monocytes into the brain parenchyma in neurodegenerative diseases such as HD. As the adaptive immune system is no longer present or operant in brain slice explants, cells from other species including human can be introduced without producing an immune reaction.

Another critical reason for using brain tissue explants (compared to conventional cell cultures, for example) is because monocytes and microglia have been shown to be highly sensitive to their environment. In fact, tissue environment has been shown to influence macrophage differentiation, transcription, and function (Beattie et al., 2016; Gibbings et al., 2015; Lavin et al., 2014; Sichien et al., 2016; van de Laar et al., 2016). Indeed, it has been shown that even within the CNS, the environment of different anatomical regions can alter phagocyte function (Molteni et al., 2018). These changes have been reported to be driven, at least in part, by changes in the use and function of enhancers controlling tissue-specific macrophage identities (Gosselin et al., 2014). For these reasons, to best investigate the functions of and interactions between microglia and MO in HD, human brain tissue should be used. Since this was not possible, I instead chose to adapt a well-established rat organotypic brain slice experimental platform to investigate both microglia and MSNs in their native brain tissue environment and to maintain their local intercellular interactions.

5.3 The Importance of using Peripheral Monocytes Collected from HD Patients

The use of MO isolated from HD patients was also a critical aspect of my studies. By using monocytes from HD patients, I guaranteed that my experiments were relevant to HD. In contrast, monocytes isolated from mouse or rat models of HD would share the intrinsic limitations of genetic models as outlined above and have uncertain relation to human MO in phenotype and functional states. Finally, by directly engrafting MO into organotypic brain-slice explants, I was able to control their numbers in each study, which would be very difficult to achieve in any *in vivo* study, especially with the recent discovery of lymphatic systems within the CNS as discussed previously (Absinta et al., 2017; Aspelund et al., 2015; Lecco, 1953; Li et al., 1996; Louveau et al., 2015).

However, a limitation of using patient samples is their intrinsic and unavoidable variability that reduces the statistical power of a given study. While this made the interpretation of some of my results more difficult, the use of patient samples allowed me to potentially capture another aspect of HD that is lost in animal models; namely that patients may vary in their presentation of “classical” clinical features of HD even when bearing seemingly identical mutations in their *HTT* genes. Thus, my data were not conclusive as to the question of whether disease stage was positively correlated with aberrant MO activity that I observed for the HD MO samples as a whole. However, my finding, using two different experimental techniques, that samples from patients in different disease stages appeared to have altered cytokine production was suggestive that disease stage, as well as tissue environment, may have a significant impact on MO function in HD. If these findings can be verified with larger sample sizes this would support a role for MO in exacerbating neuropathogenesis in later stages of the disease.

5.4 Translation of Findings to other CNS Diseases

Neuroinflammation is associated with nearly every neurodegenerative disorder and has also been observed in neuropsychiatric disorders such as schizophrenia as well as CNS developmental disorders such as autism (Barichello, Simoes, Quevedo, & Zhang, 2019; Felger, 2018; Garay, Hsiao, Patterson, & McAllister, 2013; Hsiao, McBride, Chow, Mazmanian, & Patterson, 2012; Malkova, Yu, Hsiao, Moore, & Patterson, 2012). I hope that the new experimental approach I have developed for my dissertation research could also be useful in research in these other disease states in which the roles of infiltrating monocytes are also not fully understood. For example, monocytes from amyotrophic lateral sclerosis (ALS) patients have been shown to exhibit functional alterations and the degree to which they invade the CNS remains controversial (O. Butovsky et al., 2015; Nardo et al., 2011; Zhang, Hang, Yao, & Lim, 2015; Zhao et al., 2017; Zondler et al., 2017). The involvement of the peripheral immune system in Parkinson's disease is similarly likely as elevated pro-inflammatory mediators have been found in the periphery and monocytes have been shown to express distinct transcriptome signatures and hyper-reactivity to LPS stimulation (Grozdanov et al., 2014). For both Parkinson's and Alzheimer's disease, infiltration of inflammatory monocytes into the CNS has also been observed, although the degree to which this happens still being debated (Depboylu et al., 2012; Kalkonde et al., 2007; Parillaud et al., 2017; Rodriguez, Fernandez, Haq, & Okun, 2007). For other disorders in which the BBB is disrupted and MO infiltration is a known part of disease progression, such as multiple sclerosis, an analogous experimental approach could perhaps be used to track the progression of MO-mediated pathology and determine whether MO-microglial intercellular interactions are involved.

5.5 Translation of Findings to Therapeutic Development

In the context of developing new drug strategies for HD and other CNS neurodegenerative diseases involving neuroinflammation and monocyte infiltration as discussed above, the new assay approach I have developed could potentially be used to test the impact of modulating MO prior to engraftment, to determine how compounds affect microglia-monocyte-neuron interactions after engraftment, and to study mechanisms through which cytotherapies could alter disease course. Given the prevalence of neuroinflammation in neurodegenerative disorders, there has been considerable interest in the development of new anti-inflammatory drugs that would provide neuroprotective benefits. Yet, to date, most of the anti-inflammatory compounds that have been researched using more conventional experimental methods and taken into clinical trials, such as NSAIDs, have been unsuccessful. The hybrid organotypic brain-slice approach could provide an important additional assay platform for the direct evaluation of drug effects on human immune cells such as monocytes, including those isolated directly from patients, on ameliorating cellular and tissue-relevant mechanisms and processes associated with disease and disease progression.

Beyond CNS neurodegenerative diseases, macrophage engraftment has been shown to ameliorate symptoms related to lysosomal storage diseases. However, the mechanisms through which symptoms are ameliorated are not fully understood (Krivit, Aubourg, Shapiro, & Peters, 1999; Krivit, Lockman, Watkins, Hirsch, & Shapiro, 1995; Krivit, Peters, & Shapiro, 1999; Krivit, Sung, Shapiro, & Lockman, 1995; Platt & Lachmann, 2009; Walkley et al., 1994). Using the hybrid brain-slice model, macrophages could be engrafted into slices taken from lysosomal disease models to determine how they affect disease mechanisms that could confer a benefit to patients.

This could potentially elucidate therapeutic mechanisms and lead to the development of improved and/or less invasive therapies.

5.6 Summary and Future Directions

In summary, my dissertation research focused on the core hypothesis that the neuropathogenesis of HD is driven in significant part by the infiltration of MO into the CNS in the later stages of HD, and by the consequences such MO infiltration has on microglial function and microglia-neuron interactions, notably striatal MSNs. I used a range of experimental measures to characterize cellular neuropathology in the hybrid brain-slice model and observed decreased numbers of healthy MSNs together with altered morphological/functional characteristics of endogenous microglia. The observation of apparent increased phagocytosis of MSNs by microglia after HD MO engraftment adds to the existing literature that suggests glial cell types contribute to MSN death in HD (Lobsiger & Cleveland, 2007; Strong et al., 1993). As such, my dissertation research supports the hypothesis that the genetic mutation of *HTT* in human monocytes alters their function that ultimately may lead to detrimental effects on CNS neurons including striatal MSNs known to be selectively vulnerable in HD. Further research aimed at elucidating whether infiltrating HD MO also have direct effects on MSNs health and survival in addition to modulating endogenous intercellular interactions involving microglia, astroglia, and other cell types—or a combination thereof—will be critical for fully understanding the role of neuroinflammation in neurodegenerative diseases such as HD, and in identifying effective interventions that are capable of altering the course of HD.

While beyond the scope of my dissertation, the results of my experiments would be bolstered by the addition of a larger number of HD patients and from a broader range

of the HD spectrum, including pre-symptomatic patients and end-stage patients. Additionally, the use of MO from another neurodegenerative disease, such as Alzheimer's disease, would be informative to determine whether my findings are specific for HD, or may be more general to a range of neurodegenerative disorders. For my dissertation, I used a human-specific cytokine panel to measure the production of cytokines by human MO, but it would be informative to also know how the endogenous brain slice environment was altered by MO engraftment. To test this, a rat-specific cytokine panel could be used to determine whether the engraftment of MO increased inflammatory cytokines and chemokines produced by the CNS tissues.

In addition, further experimental perturbations could be made in the hybrid brain-slice platform. For example, activated microglia are associated with almost every neurodegenerative disease studied to date. Microglia could be depleted using pharmacological compounds, or entirely ablated by using brain tissue from mouse models that lack microglia (Beers et al., 2006; Gowing, Vallieres, & Julien, 2006; J. Han, Harris, & Zhang, 2017; J. Han, Zhu, Zhang, & Harris, 2019; X. Han et al., 2019; Heppner et al., 2005; McKercher et al., 1996). Additionally, due to the low biolistic transfection rate that comes with using brain tissue from older animals, I was restricted to the use of brain-tissue from neonatal pups. However, transgenic mouse models in which neurons express reporters could be used to bypass the need for biolistic transfection. Slices taken from these adult animals could be used to study how age influences neuropathogenesis in this model.

My brain slice cultures utilized a medium containing serum to optimize MSN survival. Other groups have found that microglia morphology is significantly changed with the use of a serum-free medium (Czapiga & Colton, 1999). Given the activated

morphology of microglia I observed independent of MO engraftment, starting with microglia that are less activated prior to engraftment could be informative.

Additionally, this model could be used to determine the effects of mutant *HTT* expression in other cell types. Biolistic transfection could be used to transfect MSNs with mutant gene constructs to determine how the expression of mutant HTT in neurons affects intercellular interactions. Similarly, astrocytes, microglia, and other cell types could be isolated from HD mouse and rat models and engrafted into brain slices.

Finally, recent advances in human-induced pluripotent stem cells are making this technology more readily accessible for human disease models. Such technology could be incorporated into the brain-slice assay to test how human MSNs are affected by different molecular and cellular perturbations. The use of HD MSNs would undoubtedly advance the applications of the brain slice approach and has the potential to lead to improved therapies to slow or halt disease progression in HD.

References

Absinta, M., Ha, S. K., Nair, G., Sati, P., Luciano, N. J., Palisoc, M., . . . Reich, D. S. (2017). Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI. *Elife, 6*. doi:10.7554/eLife.29738

Ajami, B., Bennett, J. L., Krieger, C., McNagny, K. M., & Rossi, F. M. (2011). Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nature Neuroscience, 14*(9), 1142-1149. doi:10.1038/nn.2887

Ajami, B., Bennett, J. L., Krieger, C., Tetzlaff, W., & Rossi, F. M. (2007). Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nature Neuroscience, 10*(12), 1538-1543. doi:10.1038/nn2014

Anderson, W. D., Greenhalgh, A. D., Takwale, A., David, S., & Vadigepalli, R. (2017). Novel Influences of IL-10 on CNS Inflammation Revealed by Integrated Analyses of Cytokine Networks and Microglial Morphology. *Frontiers in Cellular Neuroscience, 11*, 233. doi:10.3389/fncel.2017.00233

Anselmo, A. C., Gilbert, J. B., Kumar, S., Gupta, V., Cohen, R. E., Rubner, M. F., & Mitragotri, S. (2015). Monocyte-mediated delivery of polymeric backpacks to inflamed tissues: a generalized strategy to deliver drugs to treat inflammation. *Journal of Controlled Release, 199*, 29-36. doi:10.1016/j.jconrel.2014.11.027

Arenson, E. B., Jr., Epstein, M. B., & Seeger, R. C. (1980). Volumetric and functional heterogeneity of human monocytes. *Journal of Clinical Investigation, 65*(3), 613-618. doi:10.1172/jci109706

Aspelund, A., Antila, S., Proulx, S. T., Karlsen, T. V., Karaman, S., Detmar, M., . . . Alitalo, K. (2015). A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *Journal of Experimental Medicine, 212*(7), 991-999. doi:10.1084/jem.20142290

Aylward, E. H. (2007). Change in MRI striatal volumes as a biomarker in preclinical Huntington's disease. *Brain Research Bulletin, 72*(2-3), 152-158. doi:10.1016/j.brainresbull.2006.10.028

Aylward, E. H., Codori, A. M., Rosenblatt, A., Sherr, M., Brandt, J., Stine, O. C., . . . Ross, C. A. (2000). Rate of caudate atrophy in presymptomatic and symptomatic

stages of Huntington's disease. *Movement Disorders, 15*(3), 552-560. doi:10.1002/1531-8257(200005)15:3<552::AID-MDS1020>3.0.CO;2-P

Barichello, T., Simoes, L. R., Quevedo, J., & Zhang, X. Y. (2019). Microglial Activation and Psychotic Disorders: Evidence from Pre-clinical and Clinical Studies. *Current Topics in Behavioral Neurosciences*. doi:10.1007/7854_2018_81

Baruch, K., Rosenzweig, N., Kertser, A., Deczkowska, A., Sharif, A. M., Spinrad, A., . . . Schwartz, M. (2015). Breaking immune tolerance by targeting Foxp3(+) regulatory T cells mitigates Alzheimer's disease pathology. *Nat Commun, 6*, 7967. doi:10.1038/ncomms8967

Beattie, L., Sawtell, A., Mann, J., Frame, T. C. M., Teal, B., de Labastida Rivera, F., . . . Kaye, P. M. (2016). Bone marrow-derived and resident liver macrophages display unique transcriptomic signatures but similar biological functions. *Journal of Hepatology, 65*(4), 758-768. doi:10.1016/j.jhep.2016.05.037

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., . . . Appel, S. H. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America, 103*(43), 16021-16026. doi:10.1073/pnas.0607423103

Bhattacharyya, K. B. (2011). *Eminent Neuroscientists: Their Lives and Works* (1st ed.): Kolkata: Academic Publishers.

Bhattacharyya, K. B. (2016). The story of George Huntington and his disease. *Annals of the Indian Academy of Neurology, 19*(1), 25-28. doi:10.4103/0972-2327.175425

Bjorkqvist, M., Wild, E. J., Thiele, J., Silvestroni, A., Andre, R., Lahiri, N., . . . Tabrizi, S. J. (2008). A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. *Journal of Experimental Medicine, 205*(8), 1869-1877. doi:10.1084/jem.20080178

Boyette, L. B., Macedo, C., Hadi, K., Elinoff, B. D., Walters, J. T., Ramaswami, B., . . . Metes, D. M. (2017). Phenotype, function, and differentiation potential of human monocyte subsets. *PloS One, 12*(4), e0176460. doi:10.1371/journal.pone.0176460

Butovsky, O., Jedrychowski, M. P., Cialic, R., Krasemann, S., Murugaiyan, G., Fanek, Z., . . . Weiner, H. L. (2015). Targeting miR-155 restores abnormal microglia and attenuates disease in SOD1 mice. *Annals of Neurology, 77*(1), 75-99. doi:10.1002/ana.24304

Butovsky, O., Siddiqui, S., Gabriely, G., Lanser, A. J., Dake, B., Murugaiyan, G., . . . Weiner, H. L. (2012). Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. *The Journal of clinical investigation, 122*(9), 3063-3087. doi:10.1172/JCI62636

Cherry, J. D., Olschowka, J. A., & O'Banion, M. K. (2014a). Are "resting" microglia more "m2"? *Frontiers in Immunology, 5*, 594. doi:10.3389/fimmu.2014.00594

Cherry, J. D., Olschowka, J. A., & O'Banion, M. K. (2014b). Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *Journal of Neuroinflammation, 11*, 98. doi:10.1186/1742-2094-11-98

Chiu, Isaac M., Morimoto, Emiko T. A., Goodarzi, H., Liao, Jennifer T., O'Keeffe, S., Phatnani, Hemali P., . . . Maniatis, T. (2013). A Neurodegeneration-Specific Gene-Expression Signature of Acutely Isolated Microglia from an Amyotrophic Lateral Sclerosis Mouse Model. *Cell Reports, 4*(2), 385-401. doi:https://doi.org/10.1016/j.celrep.2013.06.018

Chung, I., Zelivyanskaya, M., & Gendelman, H. E. (2002). Mononuclear phagocyte biophysiology influences brain transendothelial and tissue migration: implication for HIV-1-associated dementia. *Journal of Neuroimmunology, 122*(1-2), 40-54.

Collaborative, H. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell, 72*(6), 971-983.

Corkum, C. P., Ings, D. P., Burgess, C., Karwowska, S., Kroll, W., & Michalak, T. I. (2015). Immune cell subsets and their gene expression profiles from human PBMC isolated by Vacutainer Cell Preparation Tube (CPT) and standard density gradient. *BMC Immunology, 16*, 48. doi:10.1186/s12865-015-0113-0

Cronk, J. C., Filiano, A. J., Louveau, A., Marin, I., Marsh, R., Ji, E., . . . Kipnis, J. (2018). Peripherally derived macrophages can engraft the brain independent of irradiation and maintain an identity distinct from microglia. *The Journal of experimental medicine, 215*(6), 1627-1647. doi:10.1084/jem.20180247

Crotti, A., & Glass, C. K. (2015). The choreography of neuroinflammation in Huntington's disease. *Trends in Immunology, 36*(6), 364-373. doi:10.1016/j.it.2015.04.007

Czapiga, M., & Colton, C. A. (1999). Function of microglia in organotypic slice cultures. *Journal of Neuroscience Research, 56*(6), 644-651. doi:10.1002/(sici)1097-4547(19990615)56:6<644::Aid-jnr10>3.0.Co;2-9

David, S., Greenhalgh, A. D., & Kroner, A. (2015). Macrophage and microglial plasticity in the injured spinal cord. *Neuroscience, 307*, 311-318. doi:10.1016/j.neuroscience.2015.08.064

David, S., Kroner, A., Greenhalgh, A. D., Zarruk, J. G., & Lopez-Vales, R. (2018). Myeloid cell responses after spinal cord injury. *Journal of Neuroimmunology, 321*, 97-108. doi:10.1016/j.jneuroim.2018.06.003

Davis, B. M., Salinas-Navarro, M., Cordeiro, M. F., Moons, L., & De Groef, L. (2017). Characterizing microglia activation: a spatial statistics approach to maximize information extraction. *Scientific Reports, 7*(1), 1576. doi:10.1038/s41598-017-01747-8

Davis, E. J., Foster, T. D., & Thomas, W. E. (1994). Cellular forms and functions of brain microglia. *Brain Research Bulletin, 34*(1), 73-78.

Depboylu, C., Stricker, S., Ghobril, J. P., Oertel, W. H., Priller, J., & Hoglinger, G. U. (2012). Brain-resident microglia predominate over infiltrating myeloid cells in activation, phagocytosis and interaction with T-lymphocytes in the MPTP mouse model of Parkinson disease. *Experimental Neurology, 238*(2), 183-191. doi:10.1016/j.expneurol.2012.08.020

Di Pardo, A., Amico, E., Scalabri, F., Pepe, G., Castaldo, S., Elifani, F., . . . Maglione, V. (2017). Impairment of blood-brain barrier is an early event in R6/2 mouse model of Huntington Disease. *Scientific Reports, 7*, 41316. doi:10.1038/srep41316

Di Pardo, A., Amico, E., Scalabrì, F., Pepe, G., Castaldo, S., Elifani, F., . . . Maglione, V. (2017). Impairment of blood-brain barrier is an early event in R6/2 mouse model of Huntington Disease. *Scientific Reports, 7*, 41316. doi:10.1038/srep41316

Di Pardo, A., Castaldo, S., Capocci, L., Amico, E., & Vittorio, M. (2017). Assessment of Blood-brain Barrier Permeability by Intravenous Infusion of FITC-labeled Albumin

in a Mouse Model of Neurodegenerative Disease. *J Vis Exp*(129). doi:10.3791/56389

Dissing-Olesen, L., & MacVicar, B. A. (2015). Fixation and Immunolabeling of Brain Slices: SNAPSHOT Method. *Current Protocols in Neuroscience, 71*, 1 23 21-12. doi:10.1002/0471142301.ns0123s71

Estrada-Sanchez, A. M., Burroughs, C. L., Cavaliere, S., Barton, S. J., Chen, S., Yang, X. W., & Rebec, G. V. (2015). Cortical efferents lacking mutant huntingtin improve striatal neuronal activity and behavior in a conditional mouse model of Huntington's disease. *Journal of Neuroscience, 35*(10), 4440-4451. doi:10.1523/jneurosci.2812-14.2015

Felger, J. C. (2018). Role of Inflammation in Depression and Treatment Implications. *Handbook of Experimental Pharmacology*. doi:10.1007/164_2018_166

Franca, C. N., Izar, M. C. O., Hortencio, M. N. S., do Amaral, J. B., Ferreira, C. E. S., Tuleta, I. D., & Fonseca, F. A. H. (2017). Monocyte subtypes and the CCR2 chemokine receptor in cardiovascular disease. *Clinical Science (London, England: 1979), 131*(12), 1215-1224. doi:10.1042/cs20170009

Garay, P. A., Hsiao, E. Y., Patterson, P. H., & McAllister, A. K. (2013). Maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. *Brain, Behavior, and Immunity, 31*, 54-68. doi:10.1016/j.bbi.2012.07.008

Gibbings, S. L., Goyal, R., Desch, A. N., Leach, S. M., Prabagar, M., Atif, S. M., . . . Jakubzick, C. V. (2015). Transcriptome analysis highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. *Blood, 126*(11), 1357-1366. doi:10.1182/blood-2015-01-624809

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., & Huber, T. (2013). Origin and differentiation of microglia. *Frontiers in Cellular Neuroscience, 7*, 45-45. doi:10.3389/fncel.2013.00045

Girard, S., Brough, D., Lopez-Castejon, G., Giles, J., Rothwell, N. J., & Allan, S. M. (2013). Microglia and macrophages differentially modulate cell death after brain injury caused by oxygen-glucose deprivation in organotypic brain slices. *Glia, 61*(5), 813-824. doi:10.1002/glia.22478

Glimm, A. (2005). *Gene hunter : the story of neuropsychologist Nancy Wexler*. New York Washington, D.C.: Franklin Watts ;
Joseph Henry Press.

Goetz, C. G. (2013). Eminent Neuroscientists: Their Lives and Works by Kalyan B. Bhattacharyya Academic Publishers. 2012. *Movement Disorders, 28*(3), 409-409. doi:10.1002/mds.25393

Goldmann, T., Wieghofer, P., Muller, P. F., Wolf, Y., Varol, D., Yona, S., . . . Prinz, M. (2013). A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nature Neuroscience, 16*(11), 1618-1626. doi:10.1038/nn.3531

Gosselin, D., Link, V. M., Romanoski, C. E., Fonseca, G. J., Eichenfield, D. Z., Spann, N. J., . . . Glass, C. K. (2014). Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell, 159*(6), 1327-1340. doi:10.1016/j.cell.2014.11.023

Gowing, G., Vallieres, L., & Julien, J. P. (2006). Mouse model for ablation of proliferating microglia in acute CNS injuries. *Glia, 53*(3), 331-337. doi:10.1002/glia.20288

Greenhalgh, A. D., & David, S. (2014). Differences in the phagocytic response of microglia and peripheral macrophages after spinal cord injury and its effects on cell death. *Journal of Neuroscience, 34*(18), 6316-6322. doi:10.1523/jneurosci.4912-13.2014

Greenhalgh, A. D., Passos Dos Santos, R., Zarruk, J. G., Salmon, C. K., Kroner, A., & David, S. (2016). Arginase-1 is expressed exclusively by infiltrating myeloid cells in CNS injury and disease. *Brain, Behavior, and Immunity, 56*, 61-67. doi:10.1016/j.bbi.2016.04.013

Greter, M., Lelios, I., & Croxford, A. L. (2015). Microglia Versus Myeloid Cell Nomenclature during Brain Inflammation. *Frontiers in Immunology, 6*, 249. doi:10.3389/fimmu.2015.00249

Group, H. S. (2006). Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. *Neurology, 66*(3), 366-372. doi:10.1212/01.wnl.0000198586.85250.13

Grozdanov, V., Bliederhaeuser, C., Ruf, W. P., Roth, V., Fundel-Clemens, K., Zondler, L., . . . Danzer, K. M. (2014). Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. *Acta Neuropathologica, 128*(5), 651-663. doi:10.1007/s00401-014-1345-4

Gusella, J. F., Wexler, N. S., Conneally, P. M., Naylor, S. L., Anderson, M. A., Tanzi, R. E., . . . Martin, J. B. (1983). A polymorphic DNA marker genetically linked to Huntington's disease. *Nature, 306*(5940), 234-238. doi:10.1038/306234a0

Han, C. Z., Juncadella, I. J., Kinchen, J. M., Buckley, M. W., Klibanov, A. L., Dryden, K., . . . Ravichandran, K. S. (2016). Macrophages redirect phagocytosis by non-professional phagocytes and influence inflammation. *Nature, 539*(7630), 570-574. doi:10.1038/nature20141

Han, I., You, Y., Kordower, J. H., Brady, S. T., & Morfini, G. A. (2010). Differential vulnerability of neurons in Huntington's disease: the role of cell type-specific features. *Journal of Neurochemistry, 113*(5), 1073-1091. doi:10.1111/j.1471-4159.2010.06672.x

Han, J., Harris, R. A., & Zhang, X. M. (2017). An updated assessment of microglia depletion: current concepts and future directions. *Molecular Brain, 10*(1), 25. doi:10.1186/s13041-017-0307-x

Han, J., Zhu, K., Zhang, X. M., & Harris, R. A. (2019). Enforced microglial depletion and repopulation as a promising strategy for the treatment of neurological disorders. *Glia, 67*(2), 217-231. doi:10.1002/glia.23529

Han, X., Li, Q., Lan, X., El-Mufti, L., Ren, H., & Wang, J. (2019). Microglial Depletion with Clodronate Liposomes Increases Proinflammatory Cytokine Levels, Induces Astrocyte Activation, and Damages Blood Vessel Integrity. *Molecular Neurobiology*. doi:10.1007/s12035-019-1502-9

Haymaker, W., Schiller, F. (1970). *Founders of Neurology*: Springfield.

Heneka, M. T. (2014). Macrophages derived from infiltrating monocytes mediate autoimmune myelin destruction. *The Journal of experimental medicine, 211*(8), 1500-1500. doi:10.1084/jem.2118insight1

Heppner, F. L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hovelmeyer, N., . . . Aguzzi, A. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. *Nature Medicine, 11*(2), 146-152. doi:10.1038/nm1177

Hohsfield, L. A., & Humpel, C. (2015a). Intravenous infusion of monocytes isolated from 2-week-old mice enhances clearance of Beta-amyloid plaques in an Alzheimer mouse model. *PloS One, 10*(4), e0121930. doi:10.1371/journal.pone.0121930

Hohsfield, L. A., & Humpel, C. (2015b). Migration of blood cells to beta-amyloid plaques in Alzheimer's disease. *Experimental Gerontology, 65*, 8-15. doi:10.1016/j.exger.2015.03.002

Hsiao, E. Y., McBride, S. W., Chow, J., Mazmanian, S. K., & Patterson, P. H. (2012). Modeling an autism risk factor in mice leads to permanent immune dysregulation. *Proceedings of the National Academy of Sciences of the United States of America, 109*(31), 12776-12781. doi:10.1073/pnas.1202556109

Huntington, G. (1872). On Chorea. *The Medical and Surgical Reporter: A Weekly Journal (Philadelphia: S.W. Butler), Vol. 26*(no. 15), 317-321.

Islam, M. T. (2017). Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurological Research, 39*(1), 73-82. doi:10.1080/01616412.2016.1251711

Jay, T. R., Miller, C. M., Cheng, P. J., Graham, L. C., Bemiller, S., Broihier, M. L., . . . Lamb, B. T. (2015). TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer's disease mouse models. *The Journal of Experimental Medicine, 212*(3), 287. doi:10.1084/jem.20142322

Jiang, W., Li, D., Han, R., Zhang, C., Jin, W. N., Wood, K., . . . Hao, J. (2017). Acetylcholine-producing NK cells attenuate CNS inflammation via modulation of infiltrating monocytes/macrophages. *Proceedings of the National Academy of Sciences of the United States of America, 114*(30), E6202-e6211. doi:10.1073/pnas.1705491114

Kalkonde, Y. V., Morgan, W. W., Sigala, J., Maffi, S. K., Condello, C., Kuziel, W., . . . Ahuja, S. K. (2007). Chemokines in the MPTP model of Parkinson's disease: absence of CCL2 and its receptor CCR2 does not protect against striatal neurodegeneration. *Brain Research, 1128*(1), 1-11. doi:10.1016/j.brainres.2006.08.041

Karlen, S. J., Miller, E. B., Wang, X., Levine, E. S., Zawadzki, R. J., & Burns, M. E. (2018). Monocyte infiltration rather than microglia proliferation dominates the early immune response to rapid photoreceptor degeneration. *Journal of Neuroinflammation, 15*(1), 344. doi:10.1186/s12974-018-1365-4

Kipnis, J., & Filiano, A. J. (2018). Neuroimmunology in 2017: The central nervous system: privileged by immune connections. *Nature Reviews: Immunology, 18*(2), 83-84. doi:10.1038/nri.2017.152

Krivit, W., Aubourg, P., Shapiro, E., & Peters, C. (1999). Bone marrow transplantation for globoid cell leukodystrophy, adrenoleukodystrophy, metachromatic leukodystrophy, and Hurler syndrome. *Current Opinion in Hematology, 6*(6), 377-382.

Krivit, W., Lockman, L. A., Watkins, P. A., Hirsch, J., & Shapiro, E. G. (1995). The future for treatment by bone marrow transplantation for adrenoleukodystrophy, metachromatic leukodystrophy, globoid cell leukodystrophy and Hurler syndrome. *Journal of Inherited Metabolic Disease, 18*(4), 398-412.

Krivit, W., Peters, C., & Shapiro, E. G. (1999). Bone marrow transplantation as effective treatment of central nervous system disease in globoid cell leukodystrophy, metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis, fucosidosis, aspartylglucosaminuria, Hurler, Maroteaux-Lamy, and Sly syndromes, and Gaucher disease type III. *Current Opinion in Neurology, 12*(2), 167-176.

Krivit, W., Sung, J. H., Shapiro, E. G., & Lockman, L. A. (1995). Microglia: the effector cell for reconstitution of the central nervous system following bone marrow transplantation for lysosomal and peroxisomal storage diseases. *Cell Transplantation, 4*(4), 385-392.

Kwan, W., Trager, U., Davalos, D., Chou, A., Bouchard, J., Andre, R., . . . Muchowski, P. J. (2012). Mutant huntingtin impairs immune cell migration in Huntington disease. *Journal of Clinical Investigation, 122*(12), 4737-4747. doi:10.1172/jci64484

Langbehn, D. R., Hayden, M. R., Paulsen, J. S., & Group, a. t. P.-H. D. I. o. t. H. S. (2010). CAG-repeat length and the age of onset in Huntington disease (HD): a review and validation study of statistical approaches. *American Journal of Medical Genetics. Part B: Neuropsychiatric Genetics, 153B*(2), 397-408. doi:10.1002/ajmg.b.30992

Lassmann, H., & Hickey, W. F. (1993). Radiation bone marrow chimeras as a tool to study microglia turnover in normal brain and inflammation. *Clinical Neuropathology, 12*(5), 284-285.

Lassmann, H., Schmied, M., Vass, K., & Hickey, W. F. (1993). Bone marrow derived elements and resident microglia in brain inflammation. *Glia, 7*(1), 19-24. doi:10.1002/glia.440070106

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., . . . Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell, 159*(6), 1312-1326. doi:10.1016/j.cell.2014.11.018

Lecco, V. (1953). Probable modification of the lymphatic fissures of the walls of the venous sinuses of the dura mater. *Archivio Italiano di Otologia, Rinologia e Laringologia, 64*(3), 287-296.

Li, J., Zhao, Z., Zhou, J., & Yu, S. (1996). A study of the three-dimensional organization of the human diaphragmatic lymphatic lacunae and lymphatic drainage units. *Ann Anat, 178*(6), 537-544. doi:10.1016/s0940-9602(96)80113-0

Lim, R. G., Quan, C., Reyes-Ortiz, A. M., Lutz, S. E., Kedaigle, A. J., Gipson, T. A., . . . Thompson, L. M. (2017). Huntington's Disease iPSC-Derived Brain Microvascular Endothelial Cells Reveal WNT-Mediated Angiogenic and Blood-Brain Barrier Deficits. *Cell Reports, 19*(7), 1365-1377. doi:10.1016/j.celrep.2017.04.021

Lo, D. C. (2001). Neuronal transfection using particle-mediated gene transfer. *Current Protocols in Neuroscience, Chapter 3*, Unit 3.15. doi:10.1002/0471142301.ns0315s05

Lobsiger, C. S., & Cleveland, D. W. (2007). Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nature Neuroscience, 10*(11), 1355-1360. doi:10.1038/nn1988

Louveau, A., Smirnov, I., Keyes, T. J., Eccles, J. D., Rouhani, S. J., Peske, J. D., . . . Kipnis, J. (2015). Structural and functional features of central nervous system lymphatic vessels. *Nature, 523*(7560), 337-341. doi:10.1038/nature14432

Maiuolo, J., Gliozzi, M., Musolino, V., Scicchitano, M., Carresi, C., Scarano, F., . . . Mollace, V. (2018). The "Frail" Brain Blood Barrier in Neurodegenerative Diseases: Role of Early Disruption of Endothelial Cell-to-Cell Connections. *International Journal of Molecular Sciences, 19*(9). doi:10.3390/ijms19092693

Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J., & Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain, Behavior, and Immunity, 26*(4), 607-616. doi:10.1016/j.bbi.2012.01.011

McKercher, S. R., Torbett, B. E., Anderson, K. L., Henkel, G. W., Vestal, D. J., Baribault, H., . . . Maki, R. A. (1996). Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO Journal, 15*(20), 5647-5658.

Menalled, L. B., & Chesselet, M. F. (2002). Mouse models of Huntington's disease. *Trends in Pharmacological Sciences, 23*(1), 32-39.

Mildner, A., Schmidt, H., Nitsche, M., Merkler, D., Hanisch, U. K., Mack, M., . . . Prinz, M. (2007). Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nature Neuroscience, 10*(12), 1544-1553. doi:10.1038/nn2015

Möhle, L., Mattei, D., Heimesaat, Markus M., Bereswill, S., Fischer, A., Alutis, M., . . . Wolf, Susanne A. (2016). Ly6Chi Monocytes Provide a Link between Antibiotic-Induced Changes in Gut Microbiota and Adult Hippocampal Neurogenesis. *Cell Reports, 15*(9), 1945-1956. doi:https://doi.org/10.1016/j.celrep.2016.04.074

Molteni, L., Rizzi, L., Bresciani, E., Meanti, R., Fehrentz, J. A., Verdie, P., . . . Torsello, A. (2018). STIM Proteins and Orai Ca(2+) Channels Are Involved in the Intracellular Pathways Activated by TLQP-21 in RAW264.7 Macrophages. *Frontiers in Pharmacology, 9*, 1386. doi:10.3389/fphar.2018.01386

Montagne, A., Barnes, S. R., Sweeney, M. D., Halliday, M. R., Sagare, A. P., Zhao, Z., . . . Zlokovic, B. V. (2015). Blood-brain barrier breakdown in the aging human hippocampus. *Neuron, 85*(2), 296-302. doi:10.1016/j.neuron.2014.12.032

Nardo, G., Pozzi, S., Pignataro, M., Lauranzano, E., Spano, G., Garbelli, S., . . . Bonetto, V. (2011). Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. *PloS One, 6*(10), e25545. doi:10.1371/journal.pone.0025545

Nasir, J., Floresco, S. B., O'Kusky, J. R., Diewert, V. M., Richman, J. M., Zeisler, J., . . . Hayden, M. R. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell, 81*(5), 811-823.

Nayak, D., Zinselmeyer, B. H., Corps, K. N., & McGavern, D. B. (2012). In vivo dynamics of innate immune sentinels in the CNS. *Intravital, 1*(2), 95-106. doi:10.4161/intv.22823

Negrette, A. (1963). *Corea de Huntington* (2nd ed.). Maracaibo: Talleres Graficos de la Universidad del Zulia.

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science, 308*(5726), 1314-1318. doi:10.1126/science.1110647

Okun, M. S., & Thommi, N. (2004). Americo Negrette (1924 to 2003): diagnosing Huntington disease in Venezuela. *Neurology, 63*(2), 340-343.

Parillaud, V. R., Lornet, G., Monnet, Y., Privat, A. L., Haddad, A. T., Brochard, V., . . . Lobsiger, C. S. (2017). Analysis of monocyte infiltration in MPTP mice reveals that microglial CX3CR1 protects against neurotoxic over-induction of monocyte-attracting CCL2 by astrocytes. *Journal of Neuroinflammation, 14*(1), 60. doi:10.1186/s12974-017-0830-9

Pavese, N., Gerhard, A., Tai, Y. F., Ho, A. K., Turkheimer, F., Barker, R. A., . . . Piccini, P. (2006). Microglial activation correlates with severity in Huntington disease: a clinical and PET study. *Neurology, 66*(11), 1638-1643. doi:10.1212/01.wnl.0000222734.56412.17

Platt, F. M., & Lachmann, R. H. (2009). Treating lysosomal storage disorders: current practice and future prospects. *Biochimica et Biophysica Acta, 1793*(4), 737-745. doi:10.1016/j.bbamcr.2008.08.009

Prinz, M., & Priller, J. (2014). Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nature Reviews: Neuroscience, 15*(5), 300-312. doi:10.1038/nrn3722

Reinhart, P. H., Kaltenbach, L. S., Essrich, C., Dunn, D. E., Eudailey, J. A., DeMarco, C. T., . . . Lo, D. C. (2011). Identification of anti-inflammatory targets for Huntington's disease using a brain slice-based screening assay. *Neurobiology of Disease, 43*(1), 248-256. doi:10.1016/j.nbd.2011.03.017

Richard, A., & Frank, S. (2019). Deutetrabenazine in the treatment of Huntington's disease. *Neurodegenerative Disease Management, 9*(1), 31-37. doi:10.2217/nmt-2018-0040

Rikani, A. A., Choudhry, Z., Choudhry, A. M., Rizvi, N., Ikram, H., Mobassarah, N. J., & Tulli, S. (2014). The mechanism of degeneration of striatal neuronal subtypes in Huntington disease. *Annals of neurosciences, 21*(3), 112-114. doi:10.5214/ans.0972.7531.210308

Rocca, C. J., Goodman, S. M., Dulin, J. N., Haquang, J. H., Gertsman, I., Blondelle, J., . . . Cherqui, S. (2017). Transplantation of wild-type mouse hematopoietic stem and progenitor cells ameliorates deficits in a mouse model of Friedreich's ataxia. *Science Translational Medicine, 9*(413). doi:10.1126/scitranslmed.aaj2347

Rodriguez, R. L., Fernandez, H. H., Haq, I., & Okun, M. S. (2007). Pearls in patient selection for deep brain stimulation. *Neurologist, 13*(5), 253-260. doi:10.1097/NRL.0b013e318095a4d5

Sapp, E., Kegel, K. B., Aronin, N., Hashikawa, T., Uchiyama, Y., Tohyama, K., . . . DiFiglia, M. (2001). Early and progressive accumulation of reactive microglia in the Huntington disease brain. *Journal of Neuropathology and Experimental Neurology, 60*(2), 161-172.

Schafer, D. P., Lehrman, E. K., Heller, C. T., & Stevens, B. (2014). An engulfment assay: a protocol to assess interactions between CNS phagocytes and neurons. *Journal of visualized experiments : JoVE*(88), 51482. doi:10.3791/51482

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., . . . Stevens, B. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron, 74*(4), 691-705. doi:10.1016/j.neuron.2012.03.026

Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., . . . Geissmann, F. (2012). A lineage of myeloid cells independent of Myb and

hematopoietic stem cells. *Science, 336*(6077), 86-90. doi:10.1126/science.1219179

Shigemoto-Mogami, Y., Hoshikawa, K., & Sato, K. (2018). Activated Microglia Disrupt the Blood-Brain Barrier and Induce Chemokines and Cytokines in a Rat in vitro Model. *Frontiers in Cellular Neuroscience, 12*, 494. doi:10.3389/fncel.2018.00494

Sichien, D., Scott, C. L., Martens, L., Vanderkerken, M., Van Gassen, S., Plantinga, M., . . . Guilliams, M. (2016). IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional and Plasmacytoid Dendritic Cells, Respectively. *Immunity, 45*(3), 626-640. doi:10.1016/j.immuni.2016.08.013

Sierra, A., Abiega, O., Shahraz, A., & Neumann, H. (2013). Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. *Frontiers in Cellular Neuroscience, 7*, 6. doi:10.3389/fncel.2013.00006

Silvestroni, A., Faull, R. L., Strand, A. D., & Moller, T. (2009). Distinct neuroinflammatory profile in post-mortem human Huntington's disease. *Neuroreport, 20*(12), 1098-1103. doi:10.1097/WNR.0b013e32832e34ee

Soulet, D., & Cicchetti, F. (2011). The role of immunity in Huntington's disease. *Molecular Psychiatry, 16*(9), 889-902. doi:10.1038/mp.2011.28

Stoffers, D., Sheldon, S., Kuperman, J. M., Goldstein, J., Corey-Bloom, J., & Aron, A. R. (2010). Contrasting gray and white matter changes in preclinical Huntington disease. *Neurology, 74*(15), 1208. doi:10.1212/WNL.0b013e3181d8c20a

Strong, T. V., Tagle, D. A., Valdes, J. M., Elmer, L. W., Boehm, K., Swaroop, M., . . . Albin, R. L. (1993). Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nature Genetics, 5*(3), 259-265. doi:10.1038/ng1193-259

Tai, Y. F., Pavese, N., Gerhard, A., Tabrizi, S. J., Barker, R. A., Brooks, D. J., & Piccini, P. (2007). Imaging microglial activation in Huntington's disease. *Brain Research Bulletin, 72*(2-3), 148-151. doi:10.1016/j.brainresbull.2006.10.029

Thériault, P., ElAli, A., & Rivest, S. (2015). The dynamics of monocytes and microglia in Alzheimer’s disease. *Alzheimer's Research & Therapy, 7*(1), 41. doi:10.1186/s13195-015-0125-2

Trager, U., Andre, R., Lahiri, N., Magnusson-Lind, A., Weiss, A., Grueninger, S., . . . Tabrizi, S. J. (2014). HTT-lowering reverses Huntington's disease immune dysfunction caused by NFkappaB pathway dysregulation. *Brain, 137*(Pt 3), 819-833. doi:10.1093/brain/awt355

Trager, U., Andre, R., Magnusson-Lind, A., Miller, J. R., Connolly, C., Weiss, A., . . . Tabrizi, S. J. (2015). Characterisation of immune cell function in fragment and full-length Huntington's disease mouse models. *Neurobiology of Disease, 73*, 388-398. doi:10.1016/j.nbd.2014.10.012

van de Laar, L., Saelens, W., De Prijck, S., Martens, L., Scott, C. L., Van Isterdael, G., . . . Guilliams, M. (2016). Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity, 44*(4), 755-768. doi:10.1016/j.immuni.2016.02.017

Varani, K., Bachoud-Levi, A. C., Mariotti, C., Tarditi, A., Abbracchio, M. P., Gasperi, V., . . . Cattaneo, E. (2007). Biological abnormalities of peripheral A(2A) receptors in a large representation of polyglutamine disorders and Huntington's disease stages. *Neurobiology of Disease, 27*(1), 36-43. doi:10.1016/j.nbd.2007.03.011

Varvel, N. H., Neher, J. J., Bosch, A., Wang, W., Ransohoff, R. M., Miller, R. J., & Dingledine, R. (2016). Infiltrating monocytes promote brain inflammation and exacerbate neuronal damage after status epilepticus. *Proceedings of the National Academy of Sciences of the United States of America, 113*(38), E5665-5674. doi:10.1073/pnas.1604263113

Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D., & Richardson, E. P., Jr. (1985). Neuropathological classification of Huntington's disease. *Journal of Neuropathology and Experimental Neurology, 44*(6), 559-577.

Walkley, S. U., Thrall, M. A., Dobrenis, K., Huang, M., March, P. A., Siegel, D. A., & Wurzelmann, S. (1994). Bone marrow transplantation corrects the enzyme defect in neurons of the central nervous system in a lysosomal storage disease. *Proceedings of the National Academy of Sciences of the United States of America, 91*(8), 2970-2974.

Wang, G., Liu, X., Gaertig, M. A., Li, S., & Li, X.-J. (2016). Ablation of huntingtin in adult neurons is nondeleterious but its depletion in young mice causes acute pancreatitis. *Proceedings of the National Academy of Sciences, 113*(12), 3359. doi:10.1073/pnas.1524575113

Wang, Y., Ulland, T. K., Ulrich, J. D., Song, W., Tzaferis, J. A., Hole, J. T., . . . Colonna, M. (2016). TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. *The Journal of Experimental Medicine, 213*(5), 667. doi:10.1084/jem.20151948

Weiss, A., Trager, U., Wild, E. J., Grueninger, S., Farmer, R., Landles, C., . . . Tabrizi, S. J. (2012). Mutant huntingtin fragmentation in immune cells tracks Huntington's disease progression. *Journal of Clinical Investigation, 122*(10), 3731-3736. doi:10.1172/jci64565

Wyss-Coray, T., & Mucke, L. (2002). Inflammation in neurodegenerative disease--a double-edged sword. *Neuron, 35*(3), 419-432.

Yamamoto, A., Lucas, J. J., & Hen, R. (2000). Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell, 101*(1), 57-66. doi:10.1016/s0092-8674(00)80623-6

Yamasaki, R., Lu, H., Butovsky, O., Ohno, N., Rietsch, A. M., Cialic, R., . . . Ransohoff, R. M. (2014). Differential roles of microglia and monocytes in the inflamed central nervous system. *The Journal of Experimental Medicine, 211*(8), 1533. doi:10.1084/jem.20132477

Zarruk, J. G., Greenhalgh, A. D., & David, S. (2018). Microglia and macrophages differ in their inflammatory profile after permanent brain ischemia. *Experimental Neurology, 301*(Pt B), 120-132. doi:10.1016/j.expneurol.2017.08.011

Zhang, C. W., Hang, L., Yao, T. P., & Lim, K. L. (2015). Parkin Regulation and Neurodegenerative Disorders. *Frontiers in Aging Neuroscience, 7*, 248. doi:10.3389/fnagi.2015.00248

Zhao, W., Beers, D. R., Hooten, K. G., Sieglaff, D. H., Zhang, A., Kalyana-Sundaram, S., . . . Appel, S. H. (2017). Characterization of Gene Expression Phenotype in Amyotrophic Lateral Sclerosis Monocytes. *JAMA Neurol, 74*(6), 677-685. doi:10.1001/jamaneurol.2017.0357

Zondler, L., Feiler, M. S., Freischmidt, A., Ruf, W. P., Ludolph, A. C., Danzer, K. M., & Weishaupt, J. H. (2017). Impaired activation of ALS monocytes by exosomes. *Immunology and Cell Biology, 95*(2), 207-214. doi:10.1038/icb.2016.89

Biography

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