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Frontal, amygdalar, and temporal convergence in the primate ventral striatum: implications for Huntington's disease

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Abstract

Huntington's disease (HD) is a debilitating neurodegenerative disease that is part of a class of diseases affecting the basal ganglia, a group of subcortical structures in the brain. Impaired negative emotion recognition is a common and early symptom of HD, and entails the patient being unable to properly identify negative emotions on human faces. Through analysis of cell label patterns in a macaque cortex with a retrograde tracer, a region in the ventromedial striatum has been identified with the potential to function as a critical hub in the emotion processing networks. This region of the striatum receives projections from cortical and subcortical regions involved with emotion processing, including the amygdala, ventromedial prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, and temporal lobe. Analysis of the region of the striatum receiving the projections above will be evaluated to provide more insight into the link between the progression of the pathophysiology of HD and the symptoms of HD over time. The identification of this hub has the potential to broaden our understanding of symptomatology and progression in HD, contribute to other projects in neuropsychiatric disorders involving the striatum, and provide further data for the study of brain connectivity as a whole.

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1. Introduction

Despite many years of dedicated study, the primate brain and its inner workings have proven difficult for researchers to understand. Connectome and projects like it focus on using modern advances in neuroimaging and other technologies to provide more information about the ways that primate brains take in and process information and put out behavior. These projects use different methodologies and approaches to gain more insight into the primate brain. Some study the behaviors directly, attempting to elicit specific behaviors in controlled environments. Others study the biochemistry of neurons and glia, attempting to understand more about the very basic functions that sustain and change the brain as an organ. Others focus on a middle ground between these extremely small-scale and large-scale approaches, the physical structures of the brain. Neuroanatomy, the study of these structures, is an on-going project with the eventual goal of mapping the location and nature of all functional and physical structures in the brain and their connections.

Studying NHP (see Figure 1 for explanations of abbreviations) anatomy has led to great insights regarding human neuroanatomy. In turn, these insights have led to further discoveries in other translational fields. Mapping the physical connectivity of brain regions can strengthen theories about particular networks and systems. However, these physical data are difficult to collect in humans. By studying the pathology and symptomology of certain diseases in humans allows researchers are able to access information about the connections between human anatomy and behavior. Through the coinciding study of HD and the basal ganglia, much has been revealed regarding the relationship between human anatomy and behavior. In the following project, I discuss the

implications that increasing knowledge regarding connectivity in the primate basal ganglia might have for the future study of HD.

1.1 The primate basal ganglia

The basal ganglia are a set of deep-seated subcortical nuclei comprising the striatum, nucleus accumbens, globus pallidus, subthalamic nucleus, and substantia nigra (Haber & Gdowski, 2004). Traditionally, the basal ganglia have been associated with motor control, due to the clear motor symptomatology of movement disorders such as Parkinson's Disease and Huntington's Disease, both of which include neuropathology of the striatum (Haber & Gdowski, 2004). However, it has become increasingly clear through further research that the basal ganglia perform an integrative function in the brain, connecting cognitive, motor, and limbic systems (Haber & Gdowski, 2004).

1.1.1 Function

In environments and with brains that provide near infinite possibilities for actions, the basal ganglia serve as an 'option-picker,' adding the filter of experience and habit to behavioral choices (Haber & Gdowski, 2004). These nuclei have long been identified as processes involved in coordinating movement and in the reward-guided learning circuit (Neubert et al, 2015; Haber et al, 2006; Jarbo & Verstynen, 2015).

The basal ganglia are most famous for their role in coordinated movement. A class of neurodegenerative disorders, including Parkinson's disease and Huntington's disease, primarily affect the nuclei of the basal ganglia. They are classified as movement disorders because of the obvious motor symptoms that accompany the progression of the disease. Research investigating the exact function of the basal ganglia in motor behavior

has shown that the functions are wide-ranging (Haber & Gdowski, 2004). Early studies showed that the basal ganglia primarily returned projections to the motor cortex (Nauta & Mehler, 1966). However, it was eventually determined that basal ganglia nuclei are also responsible for properly timing movements (Denny-Brown, 1962; Kornhuber, 1974; MacLean, 1978), proper motor planning, and the integration of sensory and motor networks (Marshall et al., 1971; Teuber, 1976; Marsden, 1984; Evarts *et al.*, 1985; Lidsky *et al.*, 1985; Marsden, 1985).

The basal ganglia were identified as key nuclei in the reward-guided learning—or reinforcement learning—circuit in the mid-20th century. However, it was later determined that the striatum is most functionally relevant for reinforcement learning (Graybiel, 1995). The ventral striatum was originally considered to be the more critical region for reinforcement learning. However, more recent studies provide evidence that regions outside of the ventral striatum, including the dorsomedial caudate, are also involved in reinforcement learning (Jarbo & Verstynen, 2015). Further, regions of the striatum have been identified as a possible substrate for direct overlap between executive controls and reward signals (Haber & Knutson, 2010; Haber *et al.*, 2006). This involvement in reinforcement has implicated the striatum in the pathology of addiction. Finally, striatal involvement in the development of addiction supports the theory that the striatum is critically involved in habit formation and reinforcement learning, as well as in integrating affective signals (Everitt & Robbins, 2005; Everitt, 2015).

1.1.2 Major nuclei

The striatum is the nucleus of the basal ganglia that receives cortical, thalamic, and brain stem input. The striatum consists of the caudate nucleus and putamen,

structures that are separated by bundles of the internal capsule. The nucleus accumbens and the ventral most portions of the caudate and putamen form the ventral striatum. The striatum projects to the globus pallidus and the nucleus accumbens projects to the substantia nigra. These connections and the striatum will be discussed in greater detail in sections 1.1.3 and 1.2, respectively.

Haber and Gdowski (2004) discuss the major basal ganglia nuclei in their chapter in *The Human Nervous System*. The first major nucleus, the globus pallidus—or pallidum—lies medial to and is capped by the putamen. The pallidum has an external and an internal segment. The external segment of the pallidum is a key process in the indirect cortico-basal ganglia-cortical loop, as seen in Figure 2 and described in section 1.1.3. Meanwhile, the internal segment of the pallidum is a process in the direct loop. Regardless of location in the internal or external segment, all pallidal neurons are GABAergic. The process as a whole serves a relay station between the striatum and the thalamus. The external segment projects to the subthalamic nucleus, and the external segment projects to the ventrolateral thalamic nucleus and the ventral anterior thalamic nucleus.

The subthalamic nucleus is located medial to the internal capsule and stretches posterior to rest above the rostral substantia nigra. This nucleus is a key process in the indirect loop, providing excitatory input to the internal segment of the globus pallidus. The STN receives GABAergic input from the pallidum, glutamatergic input from the cortex, and some minor afferent projections from other basal ganglia structures. Axons from neurons in the STN all bifurcate and project both to the pallidum and the substantia nigra.

The substantia nigra can be found dorsal to the cerebral peduncle, and is separated into the pars reticulata and pars compacta on the basis of differing chemical anatomy. Although the need for further subdivision in primates has been suggested, the pars reticulate typically refers to any neurons not belonging to the clearly identifiable pars compacta. The substantia nigra projects to and receives projections from the striatum, which form the striato-nigro-striatal loops involved in drug addiction, reinforcement learning, and habit formation (Haber *et al.*, 2000).

1.1.3 Connectivity

The basal ganglia are organized in two major cortico-basal ganglia-thalamo-cortical loops, as depicted in Figure 2. Haber and Gdowski (2004) describe these pathways as well. The direct pathway carries signals from the cortex through the striatum, the internal segment of the globus pallidus and the thalamus to excite the cortex. The indirect pathway passes through the striatum and into the external segment of the pallidum and then the subthalamic nucleus before passing through the thalamus to inhibit the cortex. Within this gross structure, there are also smaller loops, including the spiraling striato-nigro-striatal pathway (Haber *et al.*, 2000).

The direct loop is shown in Figure 2 and is comprised of the cortex, striatum, internal segment of the pallidum, and thalamus. The medium spiny neurons of the striatum receive glutamatergic inputs from the cortex. These neurons are classified as D1 due to the presence and type of dopamine receptors. The D1 neurons of the striatum project to the pallidum and provide inhibitory, GABAergic input. This inhibits the tonically firing, inhibitory pallidal neurons that project to the thalamus. This transient inhibition of tonic inhibition causes the thalamus to transiently excite the cortex, which

closes the loop.

The indirect loop has the exact opposite effect on the cortex. By involving the external segment of the globus pallidus and the subthalamic nucleus, the indirect loop produces net inhibition of the cortex. In this loop, D2 neurons of the striatum project onto and transiently inhibit the external segment of the pallidum. The neurons of the external segment tonically inhibit the subthalamic nucleus, which transiently excites the internal segment of the pallidum. Recall, the internal segment tonically inhibits the thalamus. By transiently exciting internal segment neurons, the subthalamic nucleus further inhibits the thalamus, which inhibits the cortex. It is important to note that in the study of both the direct and indirect pathways, researchers have primarily focused on motor systems. In more recent years, studies have been dedicated to applying this model to cognitive and affective functions with success. However, the body of evidence is still focused on motor function of the basal ganglia.

1.2 The primate striatum

The striatum is a particularly critical process of the basal ganglia, which can be inferred based on its position in the cortico-basal ganglia-cortical loops, seen in Figure 2. Studies that focus on subjects with injuries to the striatum find that widespread behavioral changes result from the injuries. In a seminal meta-analysis published in 1994, Kailash Bhatia and C. David Marsden found that injuries to the caudate and/or putamen in humans—typically due to stroke, hypoxia, or, occasionally, toxic damage—caused motor disorders in addition to a variety of cognitive or behavioral disorders, including confusion, abulia, disinhibition, obsessive-compulsive, speech disorders, depression, and

memory disturbances. However, these disorders were specific to the site of damage. Putaminal damage more often resulted in motor disorders, while damage to the caudate resulted in behavioral disorders. This functional specificity of the nuclei fits the current understanding of the connectivity of the caudate and putamen (Haber & Gdowski, 2004). Furthermore, the wide variety of these disorders support the conclusion that the striatum as a whole receives projections from almost all cerebral regions.

The caudate nucleus is an elongated, ovular nucleus that stretches along the lateral ventricle before looping back and terminating near the rostral end (Haber & Gdowski, 2004). The main region of gray matter located rostrally and dorsomedial to the putamen is referred to as the head of the caudate. Sections of the caudate further caudal to the head are called the body. The remaining small and ventral regions of the caudate are called the tail of the caudate. These sections of the caudate are labeled on a 3-D model in Figure 3. The caudate receives projections from much of the cerebral cortex, especially the frontal cortex (Haber, 2016). These anatomical results support the functional and clinical conclusions that the caudate is critical for integrating executive control and reinforcement with other systems. In humans with specific damage to the caudate, there is a high prevalence of cognitive and behavioral disorders including abulia, disinhibition, speech disorders, and depression (Bhatia & Marsden, 1994).

The putamen is the nucleus of the striatum that lies ventrolateral to the caudate nucleus and is a rounded, rather egg-shaped structure, as depicted in Figure 3. Almost all projections to the striatum from the motor and pre-motor regions terminate in the putamen (Haber & Gdowski, 2004). Additionally, the putamen receives input from the amygdala and several other limbic structures (Haber, 2016; Haber & Gdowski, 2004).

This connectivity matches functional data that show an association between the putamen and motor functioning. In a meta-analysis of lesions to the lentiform nucleus—the putamen and globus pallidus—humans showed extensive motor symptoms, including chorea, dystonia, and parkinsonism (Bhatia & Marsden, 1994).

The ventral striatum includes the nucleus accumbens and ventral portions of the caudate and putamen. This region of the striatum is strongly associated with habit formation and reinforcement learning (Haber, 2011). The ventral striatum receives input from the cerebral cortex, thalamus, and midbrain dopaminergic cells. These projections terminate in overlapping topographies. Efferent projections from the ventral striatum terminate in the substantia nigra and globus pallidus (Haber, 2011).

1.3 Convergence in the striatum

Despite this regional specificity, the striatum as a structure receives extremely widespread and diverse input from almost all cortical regions. Neuroanatomists have therefore concluded over the course of the past several decades that the striatum has the potential to serve as a site of convergence between cognitive, motor, and limbic systems by receiving projections from all of these regions. Seminal papers published in the 1970s and 1980s first demonstrated the potential for neural convergence in the caudate, and work identifying patterns of convergence has since continued, with great success. Regions of the striatum have been identified as critical hubs—defined in section 1.3.2—in a number of networks, including certain symptoms of OCD and visual attention in addiction.

1.3.1 History

From the discovery that the striatum received projections from nearly the entire cerebral cortex, researchers posited that this convergence of projections must serve some purpose. Yeterian & Van Hoesen (1978) identified early patterns of overlap in the striatum, followed by Selemon & Goldman-Rakic (1985) who started to map these topographies. These first discoveries identified a pattern of convergence in which cortical regions that have corticocortical connections also tend to project to similar regions of the striatum. This pattern has continued to be supported by the evidence as research has continued. These seminal studies have given way to a body of work dedicated to the identification of regions of especially high convergence in the striatum. These studies have identified potential anatomical substrates for symptoms of addiction and OCD, as well as reward-based learning and motivation (Calzavara *et al.,* 2014; Choi *et al.*, 2017; Haber & Knutson, 2010; Haber *et al.*, 2006). These regions of high convergence are referred to as critical hubs, a term that comes from the application of network theory to neuroscience.

1.3.2 Critical hubs

Network theory is a mathematical theory used to understand systems and their interactions. When applied to neuroscience, and specifically structural neuroscience, network theory maps brain systems as graphs with nodes and edges—where nodes are brain areas or regions and edges are the connections between these areas (van den Heuvel & Sporns, 2013). These nodes interact with one another via the edges, i.e. brain regions interact with other regions via the axonal projections between them. Nodes can be characterized in many different ways. Of particular interest to this project are connector

or critical hubs. Critical hubs have a high degree of diverse connection and must receive and put out connections to many regions of the brain that are in different systems (van den Heuvel & Sporns, 2013). These hubs serve as a connection point between different brain systems. These characterizations can be made based on functional or structural data. In this project, the data collected is structural, meaning the potential hub identified is structural as well. The application of network theory has been instrumental for furthering the understanding of complex brain systems and system interactions. Although brain regions and areas appear to have a certain degree of functional specificity, viewing the brain as a series of functionally specific units does not explain most of the phenomena the brain can produce (van den Heuvel & Sporns, 2013).

Some of these inexplicable phenomena include widespread deficits seen in neuropsychiatric disease (Albin *et al.,* 1989). Several disorders specifically affect the striatum and other nuclei of the basal ganglia. Drug addiction, Parkinson's disease, and HD all affect the cells of the striatum. HD is of particular interest because of its unique pathology—medium spiny neurons of the striatum are selectively killed. This makes HD a useful model for studying striatal connectivity in humans. By identifying particular symptoms of HD in humans and studying the neural correlates in the NHP model, regions that can potentially serve as critical hubs within the striatum are revealed.

1.4 Huntington's disease

Huntington's Disease (HD) is a devastating neurodegenerative illness that affects 3 people out of every 100,000 worldwide (Pringsheim *et al*, 2012). It is classified as a movement disorder because of its debilitating and degenerative motor symptoms.

However, HD has more recently been shown to have severe cognitive and affective symptoms as well (Ha & Fung, 2012). HD's pathology primarily affects the striatum, a region in the basal ganglia (Vonsattel, 1998). For this reason, HD has been used a model illness for studying the basal ganglia and striatum in humans.

Despite its practical function as a model illness, the actual mechanism by which the pathophysiology of HD causes the largescale symptomology is not well understood. The origins of misfolded proteins and HD's heritability have been linked to the huntingtin gene. Furthermore, much research has been devoted to identifying and cataloging changes in volume of certain brain regions over the course of the illness, as well as the symptomology of the disease. However, how exactly the expression of the huntingtin gene causes the widespread pathology remains a mystery.

1.4.1 Pathophysiology

The genetic cause of HD is well documented. In humans, HD results in individuals with 35 or more CAG repeats in the huntingtin gene, located on chromosome 4 (Zuccato *et al.,* 2010). These repeats are inherited and are considered too severe to occur at random. The huntingtin gene codes for glutamine, an amino acid, and the aberrant version of the gene codes for a polyglutamine tract causing misfolding of the final protein (Ha & Fung, 2012). It is known that the misfolded protein interferes with many different cellular functions, incuding axonal trafficking, NMDA receptor activation, protein-protein interactions, protein clearing, and gene transcription (Ha & Fung, 2012). The mechanisms by which these disruptions produce the neuropathology of HD are not understood. However, there are several mechanisms that have been identified and are being investigated for their potential use as a treatment site.

The primary mechanism of interest for this project that is currently under investigation is the excitotoxicity theory. This hypothesis suggests that selective cell death in the striatum is due to excitotoxicity from overactive glutamatergic projections from the cortex (Zuccato *et al.,* 2010). The medium spiny neurons of the striatum depend on these glutamatergic projections for survival, and in HD, it appears as though there is increased release of glutamate into the corticostriatal synapse, as well as decreased efficacy of the NMDA receptors on the postsynaptic surface (Zuccato *et al.,* 2010). This combination causes the death of the medium spiny neurons, the hallmark of HD. This hypothesis is promising, because it explains the breakdown of the corticostriatal synapse, as well as the cell death in the striatum. However, it does not explain the whole brain atrophy experienced by 80% of HD patients (Vonsattel & DiFiglia, 1998), which makes it unlikely that the excitotoxicity hypothesis paints a complete picture of the mechanisms in HD.

Further mechanisms that are more broadly of interest include the loss of BDNF, proteolysis, aggregation of the Htt protein, dysfunction of the mitochondria, and interruption of proper gene transcription (Zuccato *et al.,* 2010). In patients with HD, there is a widespread loss of brain-derived neurotrophic factor (BDNF), a neurotrophic factor that supports the survival of neurons around the brain (Zuccato *et al.*, 2010). At this point, it is unclear exactly how the aberrant huntingtin protein causes loss of BDNF or how the loss of BDNF contributes to the selective cell death seen in HD. However, it has been found that the administration of BDNF in models of HD has a neuroprotective effect (Zuccato *et al.*, 2010). The toxic fragment hypothesis suggests that the misfolded huntingtin protein becomes more neurotoxic when it is lysed in the cells. This view,

pioneered and supported by the Hayden group, argues that the lysis of the aberrant huntingtin protein allows the polyglutamine tracts to build up within the cell. These tracts encourage cellular mechanisms meant to be protective that lead to the eventual death of the cell. The aggregation hypothesis suggests neurotoxicity is associated with the aggregation of the whole aberrant huntingtin proteins, although the exact mechanism by which these aggregates cause cell death is unknown. The huntingtin protein is also known to affect mitochondria function by binding directly to the mitochondrion and interrupting normal metabolic activity as well as mobility within the cell. Finally, the aberrant huntingtin protein can also affect normal gene transcription, preventing normal gene expression.

These mechanisms all describe some piece of the larger mechanistic puzzle. With the current understanding of these theories, they provide disparate explanations for the neuropathology of HD. However, there is promise of eventual reconciliation of the different mechanisms into a singular view of the progression of HD.

1.4.2 Neuropathology

In contrast with the physiological mechanisms, the gross neuropathology of the HD is fairly well understood. The hallmarks of HD include whole brain atrophy, particularly in the frontal lobes and the striatum (Vonsattel & DiFiglia, 1998). Atrophy is also seen in the thalamus, white matter, and the globus pallidus. Atrophy of the striatum is particularly severe, occurring in 95% of cases (Vonsattel & DiFiglia, 1998). Given that the striatum is the most prominent site of active degeneration, the pattern of atrophy in the striatum has been well studied. Medium spiny neurons are selectively killed (Han *et al.*, 2010), starting in dorsolateral caudal portion of the striatum and progressing

diagonally to the ventromedial rostral portion (Hedreen & Folstein, 1995). This selective cell death preferentially involves the indirect cortico-basal ganglia-cortical loop, causing increased cortical excitation.

1.4.3 Symptomology

The neuropathology of HD leads to progressive and debilitating symptoms. The severity and progression of symptoms is subject to a number of variables, discussed in the next section. However, regardless of when the symptoms occur, they can all be sorted into three categories: motor, affective, and cognitive.

Motor symptoms of HD can be simply characterized as involuntary and extra movements, balanced with increasing dystonia and inability to move voluntarily (Roos, 2010). In the degeneration of the striatum, the cells that participate in the indirect loop as defined above are preferentially killed, causing increased cortical excitation. In terms of symptomology, this progression typically begins as chorea—extra, involuntary, repetitive movements—in the extremities and the small facial muscles (Roos, 2010). This progresses to include difficulty walking and eventually bradykinesia, dysarthria, and dysphagia (Roos, 2010). Further, patients typically experience increasing difficulty with dynamic balance, mobility, motor performance, and postural control (Ha & Fung, 2012). Each patient experiences a unique combination of chorea and hypokinesia, so generalizing across patients is difficult (Roos, 2010). However, almost all patients suffer the same symptoms in varying degrees. These motor symptoms are well-documented in the literature and were the first symptoms of HD studied. In fact, HD was known as Huntington's Chorea until more recently.

In contrast, affective or behavioral symptoms of HD are not well characterized or

understood. Despite the high incidence of behavioral symptoms, they continue to be considered a secondary priority in diagnosis and treatment. Up to 20% of clinical HD patients experience severe psychiatric disturbances, with many more experiencing more mild psychiatric symptoms (Ha & Fung, 2012). These can include suicidal ideation and attempts, irritability and aggression, psychosis, and depressive, anxiety, and affective disorders (Gray *et al.,* 2012). Clusters of these symptoms show up in meta-analyses of patients in the clinical phase and suggest that the symptoms are primary symptoms of HD (Rickards *et al.*, 2011). Furthermore, physicians are beginning to call for more rigorous inclusion of behavioral measures in the diagnosis process (Nagel *et al.*, 2014). The development of accurate behavioral measures and better mapping of symptom progression in coming years will likely prove interesting.

Similarly, cognitive symptoms in HD are still being identified. It is therefore much more difficult to concisely describe the host of symptoms a patient with HD will experience during the course of their disease. However, most patients will experience some combination of the following symptoms: deficits in visuomotor integration, psychomotor speed, executive function, and emotion recognition, as well as other predominantly frontal lobe deficits (Papoutsi *et al.*, 2014**)**. These cognitive impairments typically emerge in the pre-clinical phase (Papoutsi *et al.*, 2014) and mirror the preferential involvement of the indirect cortico-basal ganglia-cortical loop described in section 1.1. However, these different cognitive functions are difficult to measure even within individuals, as changes to cognitive processes exist in the normal aging process. Furthermore, certain cognitive functions that are distinctly cortical—and not subcortical—in nature are preserved throughout early stages of HD, including semantic

memory, language comprehension, and spatial awareness and orientation. All of these factors paint a complicated picture of cognitive deterioration in HD. The underlying neuroanatomy is missing from this picture, however, motivating the project presented here.

Some cognitive symptoms lend themselves better than others to longitudinal study. Deficits in emotion recognition are easily operationalized. Several studies have tested individuals with HD over the course of their illnesses to determine cognitive decline, and, in particular, decline in the ability to properly identify human emotions (Bora *et al.*, 2015; Labuschagne *et al.,* 2013**)**. These studies report changes in emotion recognition in pre-manifest patients with HD—meaning they are still in the pre-clinical phase. This symptom is interesting for two main reasons. First, the ability to perform socially is integral to the human experience, and deficits in emotion recognition can make it extremely difficult for patients to interact with other people in professional, personal, and social capacities. Second, this emotion recognition symptom tends to emerge earlier than motor symptoms, suggesting that the progression of this symptomology is linked to the progression of the disease pathology as well. Moreover, early deficits in emotion recognition selectively affect negative emotion recognition (Bora *et al*., 2015). The recognition of negative emotions involves networks such as face recognition and emotion processing that have been closely examined for many decades, making emotion recognition a particularly good model symptom for investigation here.

1.5 Neurobiology of emotion recognition

Emotion-face perception or emotion recognition is a complex process distributed

across many different regions of the brain. Proper emotion recognition appears to also require certain temporal associations, although these temporal associations fall outside of the scope of the following project (Vuilleumier & Pourtois, 2007). Across networks, regions associated with face recognition, sensory integration and processing, fear, and emotion integration and regulation are implicated in emotion recognition (Vuilleumier & Pourtois, 2007). All of these regions show enhanced activation when subjects are presented with emotional faces, specifically including the amygdala, ACC, vmPFC, OFC (including the OMPFC) and FFA (Vuilleumier & Pourtois, 2007). As a result, the amygdala, ACC, vmPFC, OFC, and temporal lobe are the cortical targets of this project.

The amygdala is famously associated with the fear response. However, more recent work has suggested involvement in reward and motivation circuits as well (LeDoux, 2007). The amygdala is a nucleated structure in the medial temporal lobe, and each nucleus has a unique set of inputs and outputs (LeDoux, 2007). Of particular interest for this project are the basal and lateral nuclei of the amygdala, because of their cortical connectivity. These nuclei receive input from sensory thalamic and cortical regions, as well as prefrontal cortex (LeDoux, 2007). Further, the lateral nucleus projects to the basal nucleus, which projects to the ventral striatum (LeDoux, 2007). These connections suggest the involvement of the lateral and basal nuclei in a potential critical hub in the ventral striatum.

The ACC is also involved in emotion recognition and is more generally associated with top-down emotion regulation (Etkin *et al.*, 2011). Top-down emotion regulation is typically understood as the application of cognitive processes—like attention switching—to emotion processes. The ACC has been functionally implicated in emotion reappraisal

and other emotion distancing tasks (Etkin *et al.*, 2011). Further, the ACC has functional connectivity to the amygdala, indicating involvement in negative emotion (Etkin *et al.*, 2011).

Similarly, the vmPFC is also involved in emotion regulation; however, functional characterization of the vmPFC is typically very broad (Roy *et al.*, 2012). These characterizations include producing and maintaining 'affect', regulation, and default-mode (Roy *et al.,* 2012). Some argue that the vmPFC is itself a site of integration for many different processes, including episodic memory, representation of affective valences of sensory events, social cognition, and affective behavioral and physiological responses (Roy *et al.*, 2012). These descriptions all vary, but they share the premise that the vmPFC is somehow involved in emotion processing.

Meanwhile, the OFC appears to play an integrative role as well. The OFC has anatomical connections to subcortical structures that are involved in emotion, behavior, and motivation (An *et al.,* 1998; Öngür *et al.*, 1998; Rempel-Clower & Barbas, 1998). Evidence suggests that the OFC integrates decision-making and emotion (Bechara *et al.*, 2000). The OFC is related specifically to fear circuits and motivation circuits through connections to the amygdala, striatum, and thalamus (Öngür & Price, 2000). Furthermore, the OFC projects to regions of the diencephalon and brainstem that mediate the physiological responses associated with affective states. This pattern of connectivity shows that the OFC is clearly involved in integrating the experience of emotions and cognition.

The final region of particular interest in emotion recognition is the temporal lobe. In humans, the FFA is responsible for face recognition and is a region of the fusiform

gyrus in the temporal lobe (Kanwisher *et al.*, 1997). This region has perhaps one of the strongest functional associations of any region of cortex and is selectively activated by visual exposure to faces (Kanwisher *et al.*, 1997). Recent work suggests that the temporal lobe is more generally associated with modality-specific and modality-general sensory processing, and that these associations are organized in a gradient by connectivity along the length of the lobe (Jackson *et al.,* 2017). In human studies, emotion recognition involves activation of the FFA. However, NHP temporal lobe anatomy is not identical. The macaque species examined in this project do not have a FFA, but rather a series of face patches (Tsao *et al.*, 2008).

1.6 The macaque model

The advantage of using NHP models for studying neuroanatomy is the highly conserved nature of subcortical structures across primate species. However, not all structures are conserved, and the frontal lobes in humans are particularly unique. Human brains have greater volumes and much larger surface areas than NHP brains, and as a result, have greater gyrification (Rilling, 2006). This greater surface area allows further parcellation of regions into specific anatomical areas. Furthermore, human brains are not simply scaled-up versions of NHP brains (Rilling, 2006). However, especially in the regions identified above, there are strong similarities between NHP and human patterns of connectivity. The primate basal ganglia are highly conserved (Haber & Gdowski, 2004), which suggests that results found in the NHP striatum are largely translatable to the human striatum. Furthermore, the connectivity of OFC circuits is fairly conserved across primate species as well (Öngür & Price, 2000).

The greatest difference between species in the anatomy discussed in this project is found in the face recognition system. While fMRI results show activation in the FFA when humans view faces, similar studies show activation in a series of face patches in the temporal lobe when macaques view faces (Yovel & Freiwald, 2013; Tsao, 2014). These face patches vary from animal to animal, although they are typically found in similar regions of the temporal lobe (Tsao *et al.,* 2008; Tsao, 2014). As a result of this within species variability, it is difficult to identify the face patches without performing live cell recordings or fMRI scans on each individual animal. However, three face patches are typically found in the anterior portion of the temporal area (Tsao *et al.,* 2008), so for the purposes of this project, the focus will be on the anterior temporal regions.

It would be false to claim either that the regions of the prefrontal cortex examined here are identical across species, or that connectivity in the NHP brain exactly maps onto connectivity in the human brain. However, by focusing on functional regions that share functional associations across species, conclusions drawn from projects working with the NHP model can still inform theories regarding human neuroanatomy and connectivity.

1.7 Hypotheses

Based on the synthesis of known information regarding the basal ganglia, HD, and emotion recognition, there appears to be a gap in the literature where the neuroanatomy of emotion recognition should be. The striatum serves as a site of cortical integration for many different networks, and it receives projections from many different regions involved in emotion recognition. Furthermore, deficits in emotion recognition occur in HD, a disease that causes the selective cell death of striatal medium spiny

neurons. All of this suggests a potential critical hub in the striatum.

The project laid out in this paper will primarily address two hypotheses regarding critical hubs in the macaque striatum. The first hypothesis is that there exists an anatomical substrate underlying the integration of emotion processing and face recognition in the ventral striatum. This substrate could serve as a critical hub connecting these networks and explaining the breakdown in the related capabilities in patients with HD and degeneration of the striatum. Further, the second hypothesis is that this substrate stretches further laterally in the striatum than the currently existing literature suggests, which explains the early occurrence of these symptoms in the progression of HD.

2. Methods

The first step for identifying a potential critical hub in any region of brain involves a retrograde analysis. This analysis requires the injection of a retrograde tracer into the region of interest, which will be taken up by any axon terminals in the region and transported back to the cell body. The cell bodies containing the tracer are labelled during a staining procedure, and they are identified and counted by a researcher viewing serial sections of the brain under a light microscope. The number of cells per standard anatomical area will quantify the strength of the connection, while the density of cell label can be used to qualify the connection as strong or weak. These qualifications can be used to compare relative strengths of connection between different areas and regions.

2.1 Project structure

In this project, six cases with retrograde tracer injections into different regions of the striatum were compared. All six cases showed cell label in the cortex, however, the

specific areas of cortex and distribution of the cells differed based on the placement of the injections within the striatum. The striatal regions with the strongest cell label in the regions of interest are the most likely to serve as a critical hub in the emotion processing network. In the case of these specific hypotheses, it is possible that more than one case or small region of the striatum could be involved in the hub. Figure 4 shows the location of the injections from each of the six cases within the macaque striatum.

2.1 Tissue preparation

All procedures listed in this section and section 2.2 are reported by Calzavara et al (2014). The six cases analyzed here were six adult, male macaques, of the following species: 1 *Macaca mulatta,* 2 *Macaca fascicularis,* and 3 *Macaca nemestrina*. All procedures, including those not outlined explicitly here, were approved by the University of Rochester Committee on Animal Resources and adhere to the ILAR *Guide for the Care and Use of Laboratory Animals*. All macaques received surgical procedures for the placement of tracers and sacrifice. The injection placement procedures were performed under sterile operating conditions while the animals were anesthetized with Ketamine, delivered intramuscularly at 10 mg/kg. Proper levels of anesthetization were maintained via isoflurane throughout the procedure.

Predetermined injection sites were mapped in two different ways. In older cases**,** physiological recording was used to locate the striatal injection sites. For these cases, macaques received intravenous pentobarbital at 20 mg/kg. In any newer cases, the injection sites were mapped using MRI imaging performed on the macaques in the days leading up to the procedure.

Throughout all procedures, temperature, heart rate, and respiration were monitored constantly. Macaques were placed in a stereotaxic apparatus from David Kopf Instruments and the surgeon performed a midline incision, with muscles and fascia displaced laterally. A craniotomy was then made over the region of interest and small dural incisions were made at the injection sites. All cases analyzed here were of either retrograde or bidirectional tracers-- wheat germ agglutinin (WGA), fluorescein (FS), or Lucifer Yellow (LY)-- which were placed via pressure injection over 10 minutes, with the syringe then left *in situ* for an additional 10-20 minutes.

After the injection placement, the macaques survived approximately 14 days in the vivarium with close monitoring of behavior, health, and surgical wounds, until the sacrifice. The macaques were anesthetized with Ketamine again, followed by pentobarbital. They were then transcardially perfused with saline followed by a 4% paraformaldehyde and 1.5% sucrose solution in 0.1M, pH 7.4. The brains were removed from the skull cavity and postfixed overnight. The brains were then cryoprotected in increasing gradients of sucrose solution.

The brains were cut into serial sections at 50 microns depth on a freezing microtome. Sections were sorted out so that staining procedures would be run on first one in 24 sections, then one in 8 sections per tracer. Immunhistochemistry procedures were performed on free-floating tissue sections. They were first treated with 10% methanol and 3% hydrogen peroxide (0.1M PB to inhibit endogenous peroxidase activity). They were then rinsed for 1-2 hours in 0.1M PB with 0.3% Triton X-100 solution (TX). The tissue sections were finally preincubated in 10% normal goat serum (NGS) and 0.3% TX.

After preincubation, tissue sections were incubated in primary anti-WGA, anti-FS, or anti-LY in 10% NGS. The immunoreactivity was visualized using a standard DAB (3,3'-diaminobenzidine tetrahydrochloride) procedure. The staining was then intensified by incubating the tissue in 0.05% DAB, 0.025% cobalt chloride, 0.02% nickel ammonium sulphate and 0.01% hydrogen peroxide for 1-15 minutes. After the IHC staining protocols, tissue sections were mounted onto gel-coated slides, dehydrated, defatted in xylenes, and coverslipped with Permount.

Once the slides were prepared and ready for analysis, the boundaries of injections were mapped using counterstained or adjacent Nissl-stained slices and standard anatomical classifications (Walker, 1940; Matelli *et al.*, 1985, 1991; Paxinos *et al.*, 2000). Cases with white matter or cortical contamination at the injection site were excluded from analysis, as well as cases missing pieces of cortical tissue.

2.2 Data collection

Labelled cortical cells were charted using StereoInvestigator under 4X and 10X objectives of a light microscope. They were marked using an optical fractionator guide to focus the eye and minimize distractions in order to achieve the most accurate count of cells. The cortex was segmented into the anatomical areas using standard anatomical classifications, and the boundaries of each area constrained the optical fractionator. Cells were marked by researchers trained to identify cell bodies with the particular staining protocols used here. These determinations were based on size, shape, color, and granulation and were made in bright field. The amygdalar nuclei were segmented using counterstained or adjacent Nissl-stained sections and the standard anatomical

classifications. The boundaries of the nuclei were then used to constrain the optical fractionator that was used for counting cells.

The contours and cell markers created in StereoInvestigator were transferred to Adobe Illustrator and modified in size, color, and thickness for ease of comparison. These contours were laid out next to one another in Figures 6, 7, and 8 as presented below. Patterns and density of cell label are compared across the different cases. Patterns of cell label refer to the areas and visual appearance of the cell label at the level visible with a macroscope. The density of cell label is used to determine the strength of identified connections. The assumption in these cases is that the greater the number of projecting neurons, the stronger the functional connection between locations in the brain. Density of cell label, then, is determined to be either dense or light. This determination is made by eye after training to do so. Figure 5 provides examples of both dense and light cell label.

3. Results

All injection sites received input from the prefrontal cortex, although with varying degrees of strength and different patterns of cell label. Only the ventromedial and ventrolateral striatal cases received projections from the amygdala. The control cases—MF170FS, MF170LY, and MN39WGA—all received inputs from different regions of the prefrontal cortex, while none received input from the amygdala. These preliminary results show that the ventromedial and ventrolateral striatum are uniquely connected to the cortical regions of interest investigated in this project.

3.1 Projections to the ventromedial striatum

Two of the six cases received injections to the ventromedial striatum, MR13WGA and MN33WGA. These injections included the nucleus accumbens, as well as the caudate nucleus. As seen in Figure 6, these cases had cell label in the vmPFC, OFC, and ACC. In MR13WGA, there was clearly dense cell label. MN33WGA had lighter cell label in the regions of interest. However, this case also had lighter cell label throughout the cortex, which indicates the difference in label density could be due to poor uptake of the tracer in the striatum. One particular difference between the two cases can be found in the ACC. MR13WGA has particularly strong label in the ACC in relation to MN33WGA. Nevertheless, clear and similar patterns of cell label are found in the PFC of these two cases.

As seen in Figure 7, these two cases also had cell label in the basolateral nuclei of the amygdala. The cell label in the amygdala of MR13WGA is more dense. In both cases, there are clear projections from the amygdala to the ventromedial striatum. Other nuclei of the amygdala also show cell label.

Figure 8 shows patterns of cell label from the temporal lobe of MR13WGA. Due to tissue damage, data from the temporal lobe in MN33WGA were excluded. The cell label in the temporal lobe in MR13WGA is light, despite the strength of cell label in the PFC, indicating weaker projections from the temporal lobe to the striatum. Although the macaque face patches of the temporal lobe cannot be properly identified without further examination, the cell label in this case appears to be in the region of the temporal lobe where the face patches are typically found.

3.2 Projections to the ventrolateral striatum

One case, MN40LY, received an injection to the ventrolateral striatum. This injection also appears to include parts of the nucleus accumbens and caudate nucleus, in addition to the rostral putamen. As seen in Figure 6, there are projections from many regions of the PFC to this region of the striatum. Unlike the first two cases, there is also cell label in the dorsal PFC of this case, due to the putaminal involvement. Additionally, the patterns of cell label in the vmPFC and OFC of MN40LY are different from both ventromedial striatal cases. There is diffuse or sparse label throughout the OFC, vmPFC, and ACC of this case.

Figure 7 shows very dense label throughout the entire amygdala, indicating very strong projections to the ventrolateral striatum. The strength of this connection is especially clear in comparison with the ventromedial striatal cases.

This region of the striatum also receives clear input from the temporal lobe, as seen in Figure 8. The label in this case is very densely distributed throughout the entire rostral portion of the temporal lobe, again indicating potential involvement of the face patches.

3.3 Projections to other regions of striatum

The other three cases included in this paper received injections to the dorsal caudate, putamen, and ventral putamen. These cases all saw different patterns of cell label. The dorsal caudate and dorsal putamen cases had dense cell label in the dorsal PFC, in stark contrast with the other four cases. Two of these cases, the dorsal caudate and putamen injections, had much more dense label in the ACC than all other cases, as well as the most dense label in the dorsal PFC. The third case, the ventral putamen injection, showed

the most sparse PFC label of all cases, likely due to its caudal location in the putamen. All of these difference in cell label pattern is clearly visible in Figure 6.

No temporal lobe data were collected for these cases due to incomplete tissue and time constraints. Additionally, there were no cell bodies found in the amygdala of these three cases either. As a result, they are not included in Figure 7 or Figure 8.

4. Discussion

This project has yielded several important insights. First, there is the potential for convergence of projections from regions involved in face and emotion processing in the ventral striatum. Second, this convergence could serve as a substrate for the breakdown of emotion recognition in HD. Third, further analysis of these results and further investigation is necessary for drawing strong conclusions.

4.1 Potential convergence in the ventral striatum

The results of this analysis indicate the potential for convergence in the ventral striatum. As discussed in the section 3, the ventral striatum in the six cases examined here received projections from the vmPFC, OFC, ACC, temporal lobe, and amygdala. Moreover, the potential region of convergence seems larger than what would be expected based on previous studies of corticostriatal circuitry (Haber, 2016). Based on the cell label patterns here, the potential critical hub could stretch most of the rostral ventral striatum. Further investigation is necessary to confirm this pattern, as discussed in section 4.5.

4.2 Strength of results

The prefrontal cell label was collected by another member of the Haber lab several years ago as part of a larger retrograde striatal connectivity study (Tanimura & Haber, unpublished). The amygdala cell label was collected as part of this project. In both cases, cells were rigorously counted. Further, the anatomical areas of interest in both these regions are well separated in the literature and easily mapped onto the cases at hand. For this reason, the patterns of cell label presented here are strong evidence of projections from the regions of interest.

The label in the temporal cortex is the weakest set of data in this project. While cell label in the temporal lobe is certainly promising, the macaque face patches are notoriously difficult to locate in individual macaques, which typically must be done through electrophysiology. As a result, these patterns of cell label in the temporal lobe shown in the macaques included in this study do not conclusively show involvement of the face patches in the critical hub. However, involvement of the temporal lobe at all is important. First, this shows involvement of more caudal regions of cortex, which is discussed further in section 4.3. Second, the temporal lobe is typically functionally associated with complex and abstract sensory processing. When integrated with emotion processing networks regardless of the specific sensory modality, this kind of sensory and emotion processing hub has the potential to explain symptoms in HD including deficits in emotion recognition as well as other cognitive symptoms (Bora *et al.,* 2015).

4.3 Implications for Huntington's disease

The results presented here provide a compelling explanation for the progression

of cognitive symptoms in HD that is currently lacking. The field has not reached a consensus regarding the specific physiological nature of these symptoms. Competing theories attribute changes in emotion recognition either to changes in parietal and occipital regions responsible for visual processing, or damage to the temporal regions responsible for face recognition (Papoutsi *et al.*, 2014). Here, we suggest a third possibility—these changes in emotion recognition are due to the same subcortical physiological changes causing the motor symptoms. Until recently, the striatum has typically been characterized as receiving strong projections from the frontal lobes. While this characterization is certainly fair given the overwhelming strength of those projections, it paints an incomplete picture of striatal connectivity. In more recent years, researchers have started investigating the projections from caudal regions of cortex to the striatum (Choi *et al.*, 2017). These investigations have yielded important insights regarding the striatum's role as a site of integration. Without this complete picture of corticostriatal circuitry, it is reasonable to assume that the etiology of symptoms like deficits in emotion recognition must lie in the cortex. However, given the evidence presented here and in other recent studies, subcortical etiology of symptoms like emotion recognition should be considered.

Furthermore, a hub in the ventral striatum serving as the substrate for the deficit in emotion recognition answers questions regarding the time course of HD that cortical etiology does not. Striatal etiology could explain the early emergence of this cognitive symptom. Cortical degeneration typically occurs well into the clinical phase of HD (Kassubeck *et al.,* 2004). However, striatal degeneration begins decades before the onset of the clinical phase (Vonsattel & DiFiglia, 1998).

Ultimately, the product of this project is to inform further investigation. As discussed in Section 1.4, cognitive and affective symptoms of HD are not well studied or understood. A more complex and holistic understanding of the anatomy affected by HD can help researchers create a more complete picture of the disease and its progression. Moreover, a better understanding of the anatomy can lead to new avenues for treatment by providing new targets for translational researchers to investigate.

4.4 Limitations of the project

This project was limited in its scope and power by time and methodology. Some of these limitations can be improved through further study, as discussed in section 4.5, however, other limitations are inherent to the neuroanatomical methodology employed here.

4.4.1 Normal neuroanatomy

In this study, only healthy adult male macaques were examined. For this reason, the implications for disease models are obviously limited. No conclusions can be drawn from this study regarding the progression of HD or its symptoms. Furthermore, it is not possible to test potential treatment methods. However, by understanding the location and strength of normal, healthy connections in the functioning primate brain, we can reveal information that can guide the study of HD animal models, producing more disease-specific results.

4.4.2 Variation across species

Although much can be learned from studying non-human primate neuroanatomy, there are obvious differences across species. This is a problem for this project in several

ways. First, HD appears to be a largely human disease. Animal models of the disease do exist, however, the primary motivation for study is developing treatment for human patients. Second, the regions of cortex examined in the course of this project are regions that are largely variable across species. In section 1.6, the specific details of this variability are discussed. As a result of these differences, all comparisons between NHP and human anatomy must be read carefully. Fortunately, the basal ganglia and the general patterns of connection have been shown to be highly conserved across primate species, allowing for a fair amount of confidence in the cross species applicability of the results of this kind of project (Haber & Knutson, 2010).

4.4.3 Microcircuitry

Importantly, these types of neuroanatomical studies do not reveal any details regarding the specific microcircuitry of the striatum. Conclusions regarding convergence in the region require the assumption that the microcircuitry is complex and integrated; i.e. that cells receiving projections within the striatum are connected to one another, as well as to nearby cells receiving projections from other regions of cortex. This assumption has been generally shown to be true **[add citation here for paper about micro]**. However, nothing about the specific microcircuitry that is relevant in the ventral striatum is revealed during the course of this project. Therefore, it is impossible to strongly conclude that a critical hub does in fact exist in this region of striatum. Rather, this region of striatum may potentially serve as an anatomical substrate for convergence of the emotion processing and face recognition networks.

4.5 Future directions

The first step in continuing this project will be conducting the stereological count of cortical cells. Some of the cell counts have been completed, especially in the prefrontal cortex, however, there are not complete cell counts for each region of interest. By counting the cells using stereological techniques, the density of cell label in a particular anatomical area can be reconstructed and quantified in 3D. This would quantify the connection strength from regions with cell label to the ventral striatum, allowing for statistical analysis.

Furthermore, the results of this project have indicated that an anterograde study should be completed in order to fully comprehend the convergence of projections in the striatum. The retrograde tracer study on its own cannot produce sufficient evidence that a critical hub indeed exists in the ventral striatum, nor can it provide any details regarding the quality of the hub. However, the results can indicate a potential substrate for network integration. In order to determine the degree of overlap in the projection areas, in addition to the size, shape, and location of the specific topographies from each target cortical region, anterograde tracer studies must be conducted.

Such a study would involve the injection of anterograde tracers in regions of the vmPFC, OFC, amygdala, and temporal lobe. Tracer would be taken up by cell bodies in these regions and transported down the axon to the terminal bouttons. These labeled terminals would create terminal fields in the striatum, which could be marked, collected, and overlaid with terminal fields of other cases to create a heat map of projection from the cortical regions of interest to the ventral striatum.

5. Conclusion

Guided by the deficits in emotion recognition experienced by patients with HD, this project has provided evidence for a potential anatomical substrate for integration of the emotion processing and face recognition networks necessary for proper emotion recognition in the ventral striatum of the macaque. Further study should include stereological analysis and anterograde anatomical, electrophysiological, and disease model studies.

Figure Legends

Figure 1

This table contains the abbreviations used in this manuscript and their meanings, listed in alphabetical order.

Figure 2

This figure from *Neuroscience* (2008) depicts the major basal ganglia nuclei and their connectivity. The figure shows the indirect cortico-striato-cortical loop (in beige) and the direct cortico-striato-cortical loop.

Figure 3

This figure shows a 3-D representation of the right hemisphere of a macaque cortex, as well as a macaque striatum removed and to the left. The striatum is labeled with the caudate and putamen to illustrate the size, shape, and location of the nuclei in relation to the rest of the cortex.

Figure 4

This figure shows 3-D representations of the injection sites from all six cases merged onto a digital global model of the macaque striatum, showing different views. Each color corresponds to a particular case. Blue- MR13WGA; Teal- MN33WGA; Green- MN40LY; Yellow- MF170FS; Goldenrod- MF170LY; and Orange- MN39WGA. (A) The lateral view reveals the rostral-caudal and dorsal-ventral spread of the different injections. (B) The rostral view shows the medial-lateral spread of injections on a coronal section. (C) The dorsal view shows the medial-lateral and rostral-caudal spread.

Figure 5

These photomicrographs show the difference between regions with dense cell label and light cell label. What constitutes dense versus light cell label differs from region to region and between tracers, therefore the exact standard is determined and agreed upon by neuroanatomists on a case by case basis. (A-D) Are photomicrographs showing cell label in the following regions: (A) dense cell label in the vmPFC; (B) light or patchy cell label in the vlPFC; (C) dense cell label in the amygdala; (D) light cell label in the amygdala.

Figure 6

This figure shows a selection of coronal cross sections from the frontal lobes of all six cases. The columns have cross sections that are roughly equivalent in their rostral-caudal location. Rows have cross sections from the case noted in the leftmost column. Major regions of the frontal lobes are labeled in the top row. The injection sites of the first three cases comprise the target region in the striatum. The next three cases are controls.

Figure 7

Similar to Figure 6, this figure shows the patterns of cell label in the amygdala of the cases comprising the target region of the striatum. All three cases had significant cell label in the amygdala. The three control cases had no cell label in the amygdala and are therefore excluded.

Figure 8

This figure is the final results figure, and it shows the pattern of cell label in the temporal lobe of two of the target cases. The third case was excluded due to extensive tissue damage. Notably, the more lateral injection in MN40LY shows significantly greater cell label in the temporal lobe.

Figures

Figure 1

Abbreviation	Meaning
ACC	anterior cingulate cortex
BDNF	brain derived neurotrophic factor
CAG	cytosine, adenine, guanine-- the unit of base pairs repeated in aberrant huntingtin gene
D1	striatal medium spiny neurons, expressing D1-type dopamine receptors
D2	striatal medium spiny neurons, expressing D2-type dopamine receptors
DAB	3,3'-diaminobenzidine tetrahydrochloride
dPFC	dorsal prefrontal cortex
FFA	fusiform face area
FS	Fluorscein
GABA	gamma-aminobutyric acid, a neurotransmitter
GPe	globus pallidus, external segment
GPi	globus pallidus, internal segment
HD	Huntington's disease
Htt	Huntingtin
ILAR	Institute for Laboratory Animal Research
LY	Lucifer Yellow
MRI	magnetic resonance imaging
NGS	normal goat serum
NHP	non-human primate
NMDA receptor	glutamate receptor, named for its agonist N-methyl-D-aspartic acid
OCD	obsessive compulsive disorder
OFC	orbitofrontal cortex
OMPFC	orbitomedial prefrontal cortex
PB	phosphate buffer
PFC	prefrontal cortex
SN	substantia nigra
STN	subthalamic nucleus
vmPFC	ventromedial prefrontal cortex
WGA	wheat germ agglutinin

Figure 2

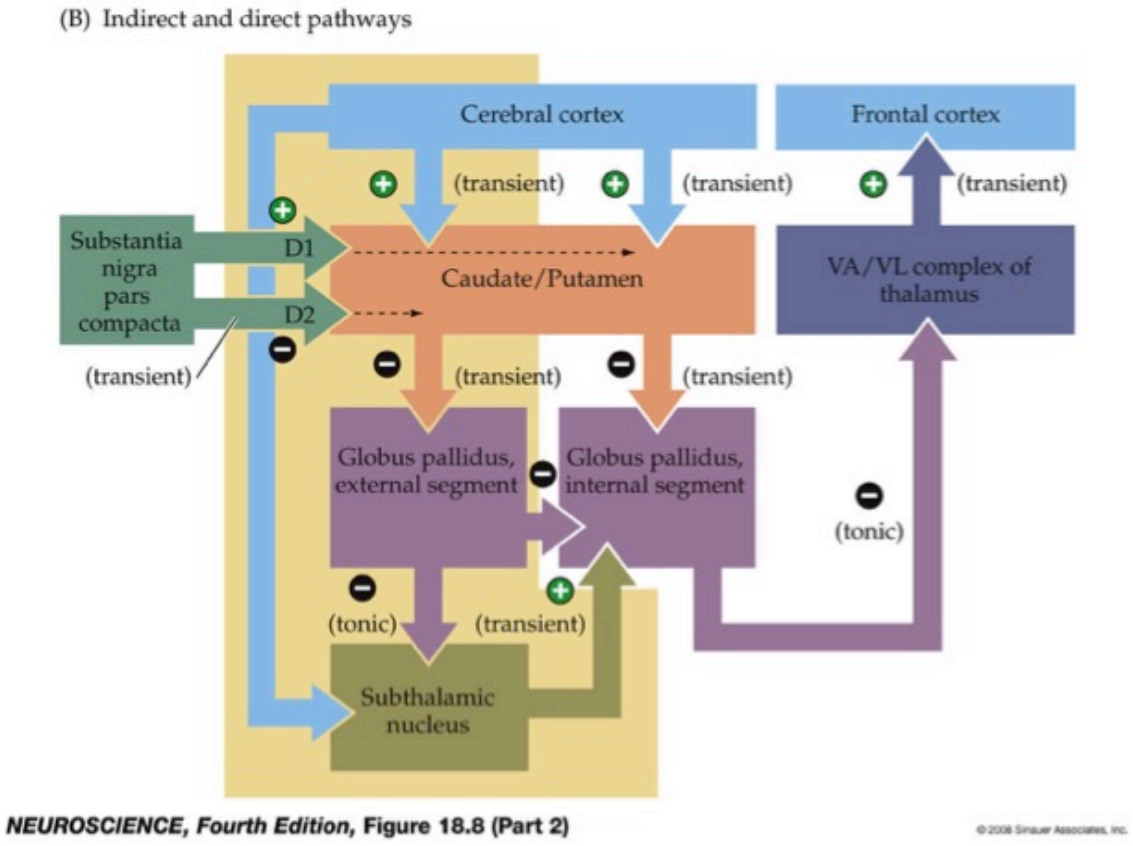

(B) Indirect and direct pathways
Cerebral cortex
Frontal cortex
(transient)
(transient)
(transient)
Substantia nigra pars compacta
D1
D2
Caudate/Putamen
VA/VL complex of thalamus
(transient)
(transient)
(transient)
(transient)
Globus pallidus, external segment
Globus pallidus, internal segment
(tonic)
(tonic)
(transient)
Subthalamic nucleus
NEUROSCIENCE, Fourth Edition, Figure 18.8 (Part 2)

Figure 3

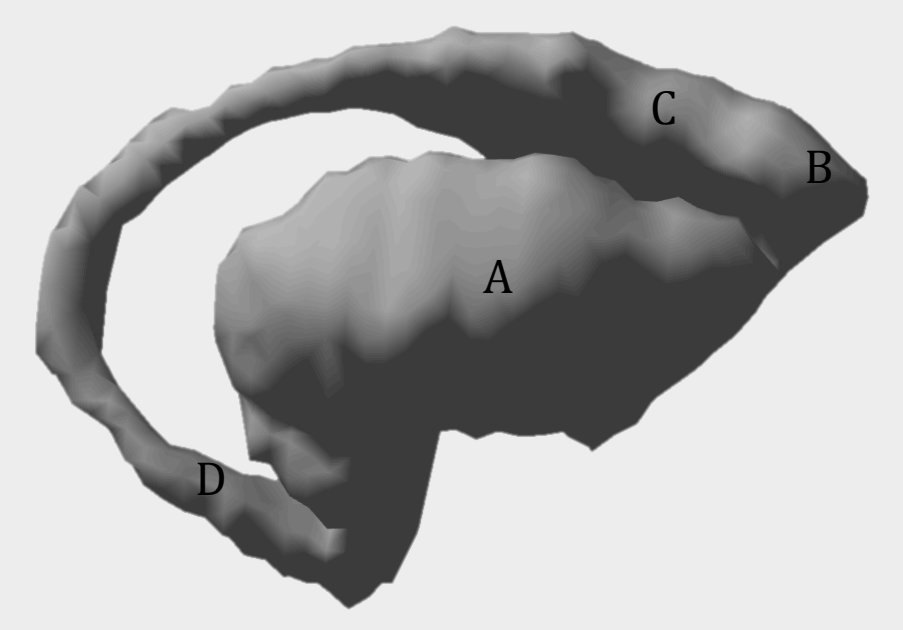


Figure 4

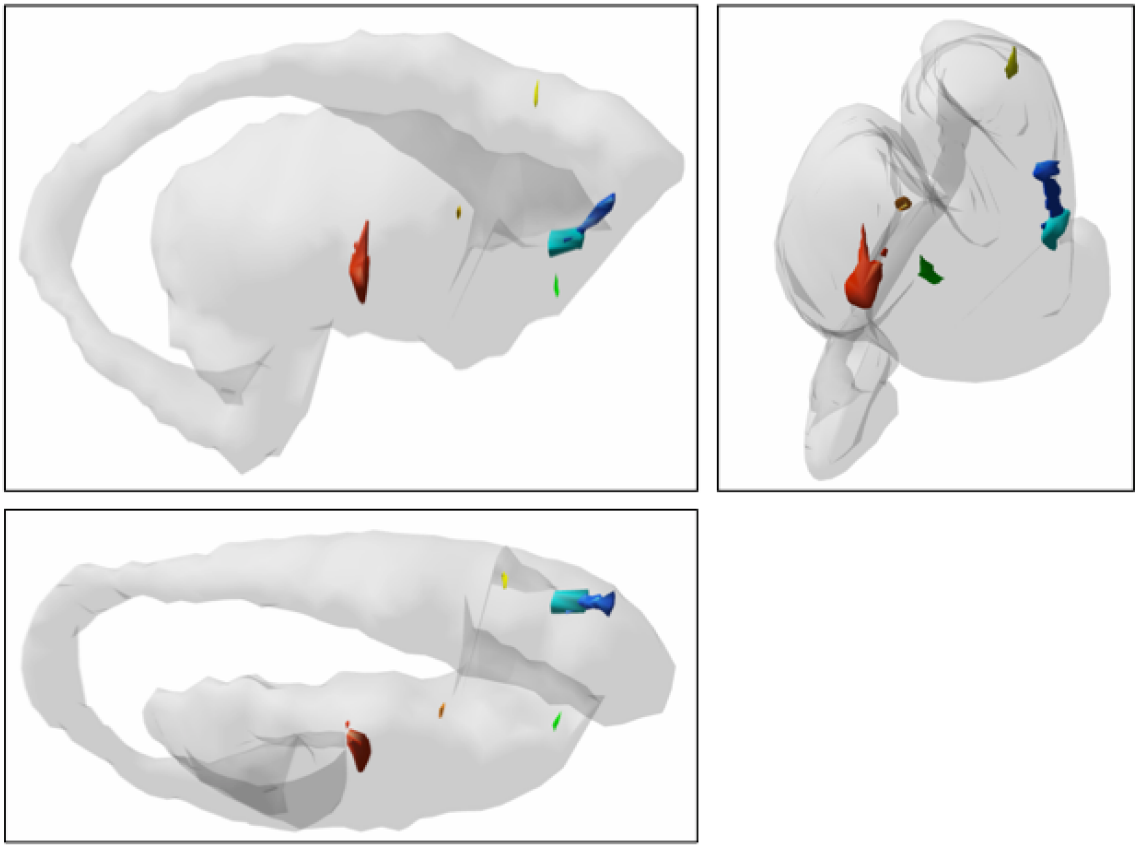


Figure 5

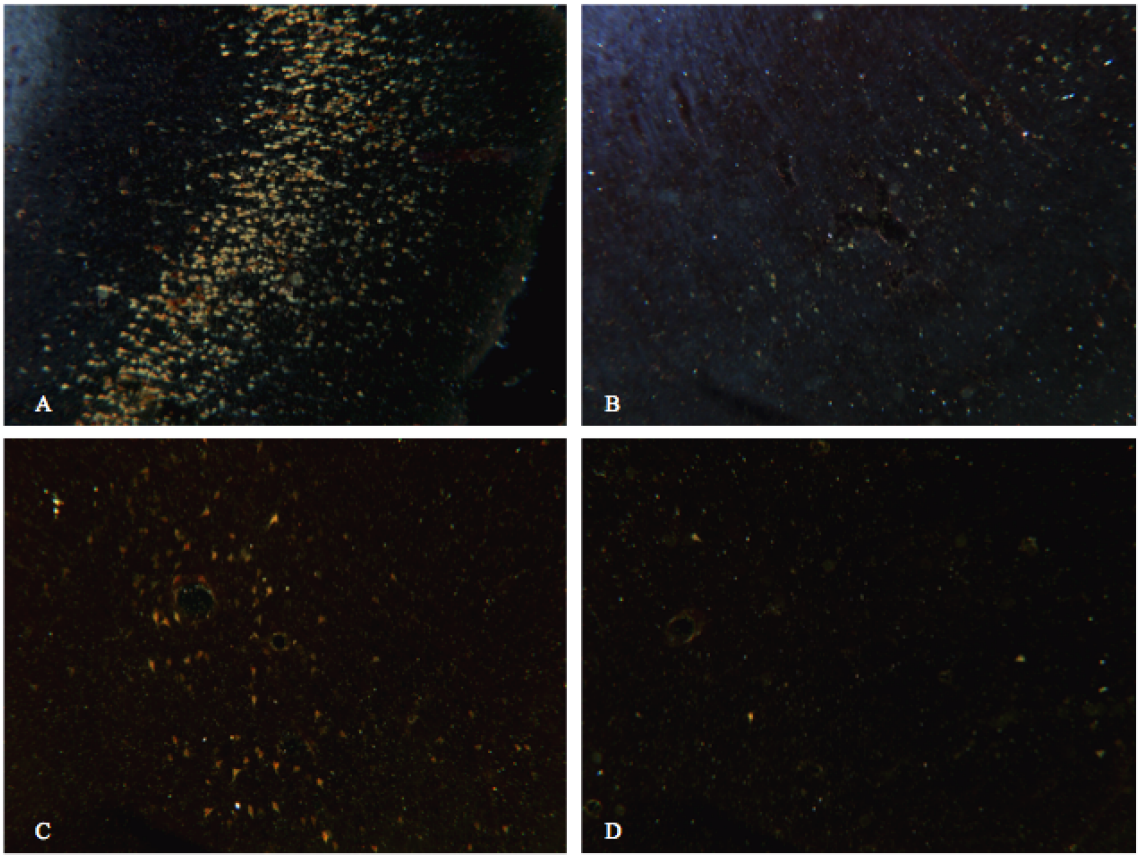


Figure 6

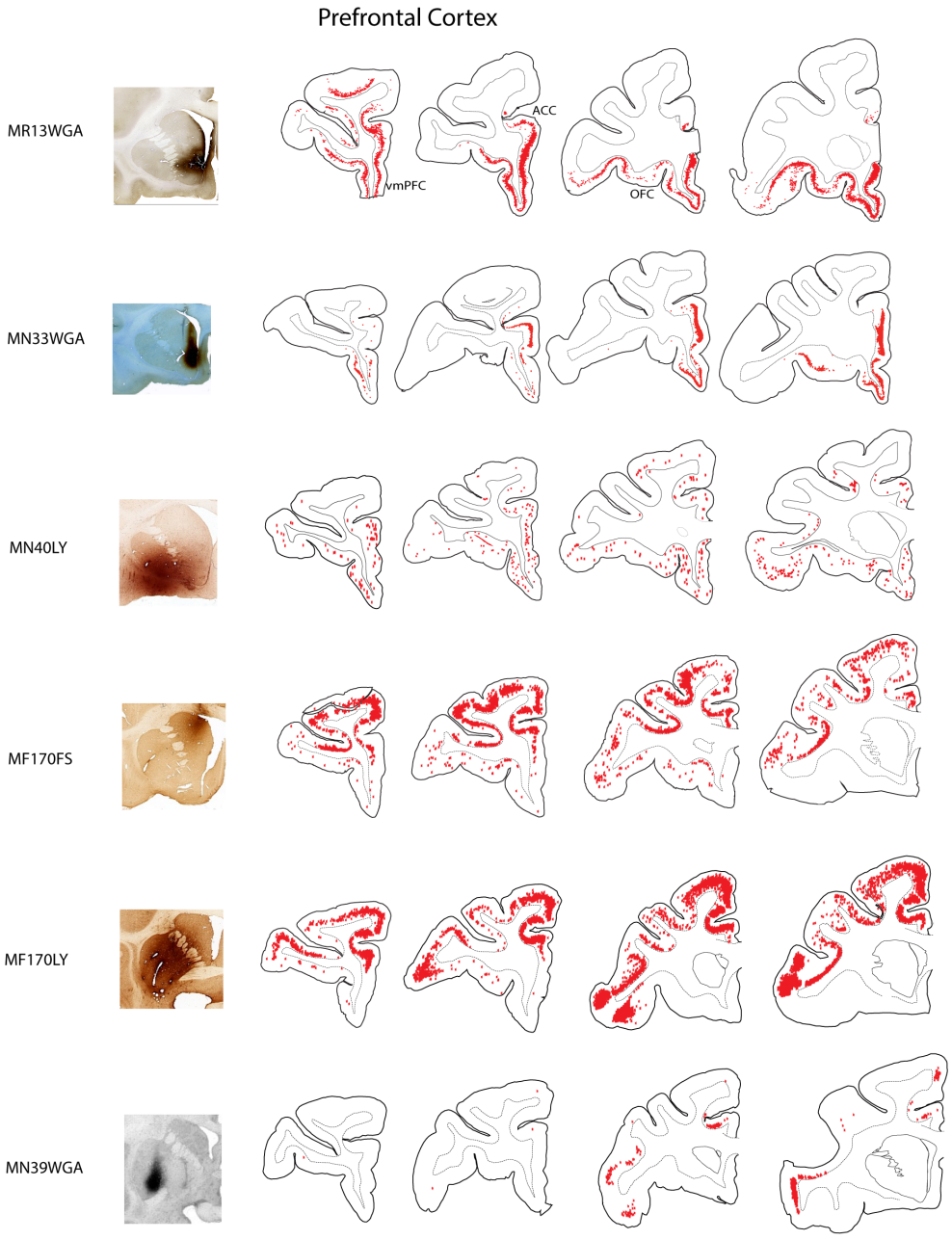

Prefrontal Cortex
MR13WGA
MN33WGA
MN40LY
MF170FS
MF170LY
MN39WGA
ACC
vmPFC
OFC

Figure 7

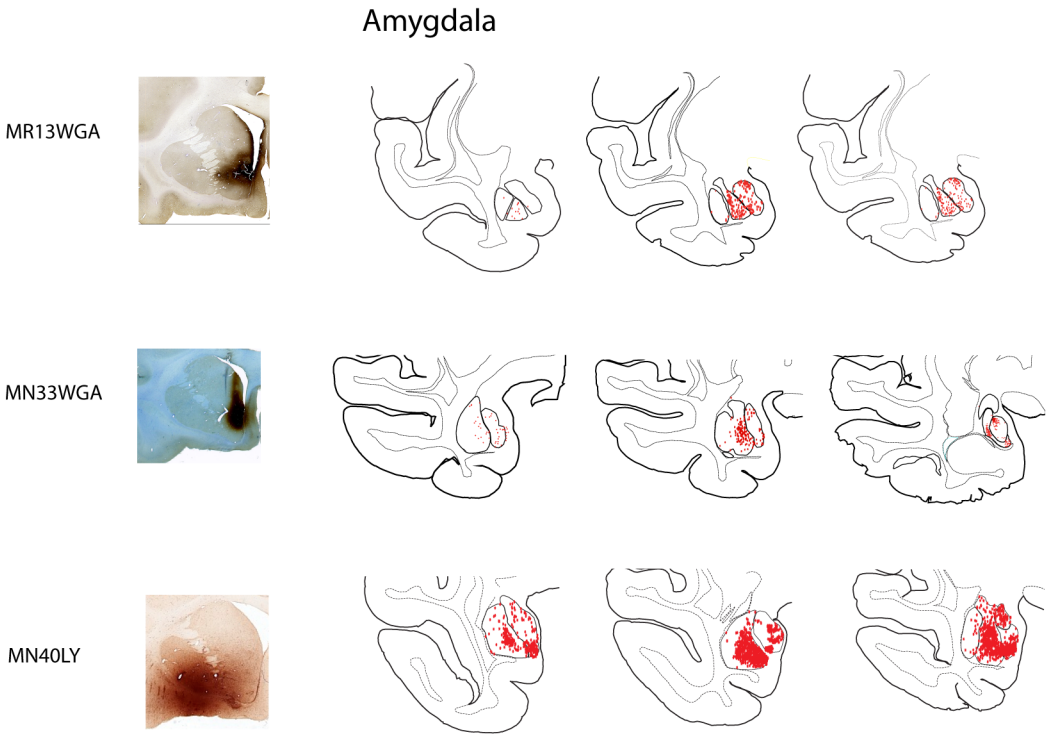
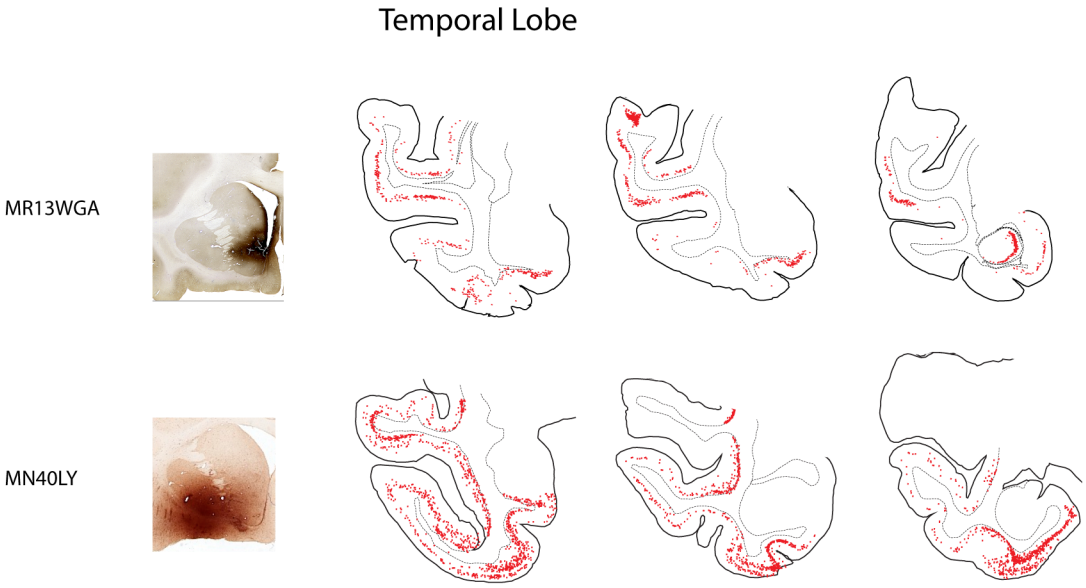


Figure 8



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