**A review of preclinical and clinical findings of neurochemical influences on neurovascular activity: potential considerations for the dorsal striatum**

example: <https://link.springer.com/article/10.1007/s00429-021-02331-7>

Brittany M Katz1-4, Lindsay R. Walton2-4, Kaiulani M. Houston2,5, Domenic H. Cerri2-4, Yen-Yu Ian Shih1-4

1 Neuroscience Curriculum, University of North Carolina at Chapel Hill

2 Department of Neurology, University of North Carolina at Chapel Hill

3 Center for Animal MRI, University of North Carolina at Chapel Hill

4 Biomedical Research Imaging Center, University of North Carolina at Chapel Hill

5 Department of Neurology, New York University, NYC, NY

Corresponding author: Yen-Yu Ian Shih ([shihy@unc.edu](mailto:shihy@unc.edu))

**Manuscript contribution to the field (200 words limit):**

**Keywords:** Neurovascular Coupling, CPu, GABA, Glutamate, Dopamine, Acetylcholine, Peptides, Neurochemical,

**Acknowledgement:** This work was supported by National Institute of Neurological Disorders and Stroke (R01NS091236), National Institute of Mental Health (R01MH126518, RF1MH117053, R01MH111429, R41MH113252, F32MH115439), National Institute on Alcohol Abuse and Alcoholism (P60AA011605, U01AA020023, T32AA007573), and National Institute of Child Health and Human Development (P50HD103573).

**Abstract**

**Introduction**

Since its introduction in the late 1980s, functional magnetic resonance imaging (fMRI) has become a widely used tool to study human brain function and networks. fMRI gives an indirect measure of neuronal activity through measuring cerebral hemodynamics and its data are therefore interpreted according to the presumed relationship between neuronal and vascular responses, often termed as neurovascular coupling (NVC). In essence, NVC suggests that regional neuronal and hemodynamic activities are scalable under physiological conditions – a phenomenon attributed to the vascular signaling cascades following neuronal activation (Kannurpatti, 2017). However, even in healthy subjects NVC may not be regulated uniformly throughout the brain (Devonshire et al., 2012; Ekstrom, 2021), which may complicate fMRI data interpretation. This has clinical implications as dysregulated NVC can be brain region-specific and lead to energy deficits and eventually brain pathologies (Stackhouse and Mishra, 2021; Zlokovic, 2011). Exclusively considering neuronal activity in fMRI data interpretation excludes neuromodulatory and vasomodulatory influences from neurochemicals. It is therefore important to consider how neuronal activity and neurotransmission collectively produce a hemodynamic response in order to understand NVC in the healthy brain, with the hopes to differentiate between healthy and dysregulated NVC in clinical conditions where neurotransmission is also dysregulated.

One of the most commonly used fMRI modalities is blood-oxygenation-level-dependent (BOLD) imaging, where the BOLD signal reflects changes in the ratio of oxygenated and deoxygenated hemoglobin in the blood . Rather than reflecting a specific hemodynamic process, BOLD fMRI signal is the concerted result of cerebral blood volume (CBV), cerebral blood flow (CBF), and the cerebral metabolic rate of oxygen (CMRO2) (D’Esposito et al., 2003; Lindauer et al., 2010); however, modalities to look at each aspect individually also exist. Cerebrovascular and metabolic changes are tightly regulated in different ways via different cell types, which thus affect BOLD and its components (Hillman, 2014; Hosford and Gourine, 2019; Iadecola, 2017; Cauli and Hammel, 2010; Guerra et al., 2018; Atwell and Iadecola, 2002). Brain tissue does not store oxygen or glucose, so the energy consumed by active processes such as neuronal firing must be restored via targeted vascular delivery of fresh metabolic substrates (Iadecola, 2004). However, in addition to maintaining upkeep with metabolic demand, vascular changes can also be invoked by other cell types. Blood vessels are enveloped with perivascular cells that include endothelial cells, smooth muscle cells (SMCs), and pericytes that can modulate vascular tone, and these signals can be propagated along the blood vessel (Schaffer and Iadecola, 2021; Hillman, 2014; Hamilton et al., 2010; Hall et al., 2014; Lecrux and Hammel 2016). Excitatory neurochemical signaling can signal directly to endothelial cells or pericytes, or indirectly via astrocytes (Hillman, 2014; MAcVicar and Newman, 2015; Howarth, 2015; Krizbai et al., 2015; Negri et al., 2021; Lourenco et al., 2014). These neuronal or astrocytic signaling cascades lead to the production and release of vasodilatory neurochemicals such as nitric oxide (NO) and certain arachiodonic acid (AA) derivatives (e.g., prostaglandin) and vasoconstrictive neurochemicals such as the AA derivative 20-HETE (Wang et al., 2021, Mishra, 2017). Further, the vasomodulatory impact of other neurotransmitters such as dopamine must be considered if the area of interest expresses vascular receptors related to those transmitters and/or if the transmitters are also known affect the excitatory signaling cascades via neuromodulation. Examining how specific neurochemical signaling impacts the hemodynamic response at the level of individual brain regions will pave the way towards more accurate fMRI data interpretation.

The striatum, or the caudate putamen (CPu), in the rodent brain represents a brain region with distinct neuronal composition and neurochemical transmission as compared to the cerebral cortex. This review aims to discuss potential influences of neurochemicals on NVC in the CPu. The CPu serves as an integrative hub for cortical, limbic, and motor inputs, and is involved in motor learning, decision making, reward, and habit formation. Aberrant CPu function and connectivity also play a role in neurological disorders such as stroke, Parkinson’s disease, Huntington’s disease, Alzheimer's disease, and aging (Packard and Knowlton, 2002; Schultz et al., 1997; Yin and Knowlton, 2006), conditions that are also associated with dysregulated NVC ( Wang et al., 2018; Iturria-Medina et al., 2016; Hu et al., 2017;Junz and Iadecola, 2008; ) (Fig 1). While it is difficult to parse the contribution of specific circuit elements to NVC in the human brain, the use of animal models have enabled the careful dissection of CPu cell types and neurotransmitters. Unlike the cerebral cortex which is composed largely of glutamatergic neurons, the vast majority of CPu is composed of GABAergic cells including MSNs and GABAergic interneurons(refs) insert astrocyte density differences. In addition, the CPu GABAergic interneuron populations release neuropeptides known to have vasomodulatory or vasoactive properties in the cerebral cortex (Cauli et al., 2004; Krawchuk et al., 2020; Uhlirova et al., 2016), but their respective contributions in the CPu are incompletely documented. Further, the CPu contains the highest expression levels of cholinergic markers in the brain (Hebb and Silver, 1961; Macintosh, 1941) as well as the highest density of DA receptors (Dautan et al., 2020; Lecrux et al., 2017; Lee et al., 2018; Lim et al., 2014; Zuccolo et al., 2017). (Hurd et al., 2001; Mengod et al., 1992) . (Calabresi et al., 1989; Chen et al., 2005a; Choi et al., 2006; Gerfen et al., 1991; Surmeier et al., 2009; Suzuki et al., 2001). Given its unique composition outlined above, this review aims to describe the potential cellular and neurochemical influences on NVC within the CPu. Below we start by describing major neurons and interneurons that are identified in the CPu. We then summarize major neurochemicals in the CPu and discuss how they modulate neuronal and vascular responses. Finally, we summarize key findings in NVC changes in several neurological disorders, with the hope to shed light on neurochemical mechanisms influencing vascular dysfunction in CPu pathology.

1. GABA

2.1Origins of GABA in the CPu

Currently, most studies examining the contribution of gamma-aminobutyric acid (GABA) to NVC are performed in brain regions containing predominately glutamatergic principal neurons. However, because the principal neurons in CPu are GABAergic MSNs, accounting for over 95% of the neuronal population (Kreitzer, 2009), the GABAergic contribution to NVC in this region could very well be different from cortex. MSNs are divided into two subclasses based predominately, but not exclusively, on receptor expression and terminal projection site. Approximately half of MSNs express the DA type 1 receptor (D1R) and project axons directly to the basal ganglia output nuclei (i.e., substantia nigra pars reticulata and the internal capsule of the globus pallidus); for the remainder of the review, D1R-expressing neurons will be termed D1-MSNs. The remaining half of MSNs express the DA type 2 receptor (D2R) and project axons indirectly to the basal ganglia output nuclei via the external capsule of the globus pallidus and the subthalamic nucleus; for the remainder of the review, D2R-expressing neurons will be termed D2-MSNs. Further, GABAergic interneurons within the CPu are similar to interneurons within the cortex, and can be classified as either fast spiking or persistent low threshold neurons. Nonetheless GABAergic interneurons within the CPu might have different functionality compared to those found in the cortex due to the relative expression levels of neurotransmitters and receptors.

2.2 Influence of GABAergic transmission in CPu

The unique electrophysiological properties of GABAergic MSNs as compared to glutamatergic neurons may contribute to differences in NVC between CPu and cerebral cortex. MSNs are largely quiescent compared to other neurons in the cerebral cortex. The membrane potentials of MSNs transition between a resting down state and a depolarized up state (Wilson and Kawaguchi, 1996). Up and down state transitions can be accomplished via glutamatergic transmission or a shift in the balance between glutamatergic and GABAergic transmission (Di Filippo et al., 2009). An MSN downstate potential is more negative than the reversal potential of Cl- (Wilson, 2008), so GABAergic signaling depolarizes MSNs and elevates membrane potentials in sharp contrast to its hyperpolarizing influence on pyramidal neurons elsewhere in the brain. During the upstate, collaterals among MSNs aid in the formation of synchronous firing within the CPu via lateral inhibition of neighboring MSNs (Moyer et al., 2014). Therefore we think that the metabolic demand per neuronal activation might be different. Indeed a recent study by Vazquez et al., showed that neuronal activity, irrespective of excitatory or inhibitory nature, increases the cerebral metabolic rate of oxygen (CMRO2) – one of the metabolic parameters that significantly influences onset time… BOLD fMRI contrast (Vazquez et al., 2018). Optogenetic stimulation of GABAergic cortical parvalbumin (PV) interneurons using PV-cre mice increased CMRO2, but the responses were generally weaker than from optogenetic GABAergic interneuron activation paired with sensory stimulations or from sensory stimulations alone. This is in contrast to excitatory pyramidal neurons, where sensory stimulation evoked the weakest CMRO2 changes as compared to optogenetic or combined optogenetic and sensory stimulations (Dahlqvist et al., 2020). These results highlight that GABAergic transmission does not require copious amounts of O2 metabolism compared to glutamatergic signaling, and therefore the vascular effects of GABAergic transmission are unlikely to arise from metabolic signaling pathways.

Though GABAergic signaling may not have intensive metabolic demand, it could still evoke robust hemodynamic changes through non-metabolic pathways. Histological staining identified GABAergic receptors in close proximity to the vascular bed and astrocytic end feet with (Vaucher et al. 2000), which have been implicated in changing and maintaining vascular tone (REFS), and therefore GABA could exert vasomodulatory actions by direct signaling on the vascular unit (He et al., 2018; http://betsholtzlab.org/VascularSingleCells/database.html). Indeed, stimulating GABAergic interneurons can evoke positive, negative, or biphasic vascular diameter changes within the cortex and hippocampus (Alborch et al., 1984; Fergus and Lee, 1997; Cauli et al., 2004; Jensen et al, 2015; Uhlirova et al. 2016, Bo et al., 2020, Moon et al. 2021; Kocharyan et al., 2008; Lecrux and Hamel, 2016). Anenberg et al., and Uhlirova et al., showed that specifically stimulating GABAergic interneurons within the cortex decreased neuronal activity but increased vasodilation (Anenberg et al., 2015; Uhlirova et al., 2016; Lee et al.,). Additionally, Uhlirova et al., show that stimulating cortical interneurons evokes a biphasic response in which vasodilation is followed by vasoconstriction (Uhlirova et al., 2016). Lee et al., show similar results using cell-type specific optogenetic somatostatin (SOM) or nNOS neuron stimulations. Both interneuron subtypes increased vasodilation when stimulated, but during long SOM neuron stimulations the increases had concurrent, surrounding decreases (Lee et al., 2020). There are various reasons why GABA can elicit such opposing vascular effects, including cell type specificity, inhibitory shunting, or differential activation of GABA receptor subtypes (GABAAR and GABABR); indeed, both receptor subtypes express ubiquitously throughout the CPu and are activated in a concentration-dependent manner (Jensen et al 2015). While these results lend credence to the possibility that GABA may contribute to the modulation of vascular tone by signaling to the vascular unit, they do not explicitly show the role of GABA in NVC.

2.2.1 GABAARs

GABAAR is an ionotropic receptor found on both neurons and astrocytes (Ishibashi et al., 2019; Terunuma, 2018) and also expressed on perivascular cells (fig 2 A). Immunohistochemical studies have shown that various GABAAR subunits express within the rodent CPu (Boccalaro et al., 2019, Fritchy and Mohler, 1995; Fritschy and Pazanelli, 2014; Hortnagl et al., 2013; Pirker et al., 2000; Schwarzer et al., 2001), yet a complete description of their cell type expression in the CPu remains to be determined. Thus far, 1-3 receptor subunits have been described in MSNs and CPu interneurons (Boccalaro et al., 2019). GABAAR activation induces rapid inhibitory postsynaptic membrane hyperpolarization in receptor-expressing cells (Ishibashi et al., 2019; Terunuma, 2018), which is responsible for feedforward inhibition of MSNs in CPu by GABAergic interneurons (Gettis et al., 2010, Koos and Tepper, 2010, Humperies et al., 2010) and inhibitory modulation of cholinergic, but not other interneurons by MSNs (Lim and Surmeier, 2021). Further, slice electrophysiology has shown that GABAARs mediate the IPSCs of NPY-expressing neurons in the CPu, and this signaling can be exacerbated in pathological conditions as exemplified in rodent Parkinsonian models (Rubi and Fritschy, 2020). GABAARs can be activated either tonically or phasically to affect gamma rhythms or decrease the likelihood of neuronal firing, respectively (Farrant and Nusser, 2005), and GABAAR-mediated lateral inhibition assists in tuning synchronous firing patterns within the CPu. ~~SP is co-released with GABA from D1-MSNs and aids in the excitability of cholinergic interneurons via inhibition of GABA~~~~A~~~~Rs (Govindaiah et al., 2010).~~

~~PV interneurons are self-regulating and do this via gap junction.~~

Direct application of muscimol, a GABAAR agonist, onto hippocampal slices dilates hippocampal microvessels and decreases LFP (Fergus and Lee, 1997). To examine whether such dilation is driven by nitric oxide (NOS), a known vasodilator, Fergus and Lee applied a NOS inhibitor (L-NNA) and found that the vasodilatory effect of GABAAR binding is independent of NO activity. Intriguingly, this conflicts with results from a more recent study by Vazquez et al., where the authors showed that L-NNA significantly diminished the vasodilatory response following optogenetic stimulation of GABAergic neurons in Vgat-Cre mice. We do not know the reason for these conflicting results, but NO may be specific to other classes of GABA receptors. ~~These conflicting results may be related to the involvement of glutamatergic signaling in the latter study, as NO has been linked to glutamate-induced vasodilation (Lourenço et al., 2014).~~ To determine how glutamate contributes to the vascular response during GABAergic cortical interneuron activation in Vgat mice, Anenberg et al. applied ionotropic glutamatergic receptor antagonists NBQX and MK-801, and reported no effect on the vascular response. Then, when GABAAR antagonist picrotoxin was applied in addition to a glutamatergic antagonist, they found minimal attenuation of the vasodilatory response driven by optogenetic stimulation of Vgat neurons (Anenberg et al., 2015). These results were nicely replicated by (Vazquez et al., 2018). Together, these results highlight that GABAaR-mediated vasodilation is independent from ionotropic glutamatergic signaling.

GABA influence over vascular tone should not only be thought of in a synapse specific manner, as GABA can also act as a volume neurotransmitter, and bind to receptors found on astrocytes and perivascular cells, but it is not clear how GABA volume transmission influences tone. To study the role of GABAergic volume neurotransmission or GABAergic currents at the GABAaR, Jessen et al. stimulated whisker pads at 0.5-5 Hz in the presence of GABAAR agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-o (THIP) or Zolpidem, in doses ranging from 1-100 µM and 0.5-10 µM, respectively. Though these activate extrasynaptic and synaptic GABAARs, respectively, both drugs augmented LFP changes at low (0.5-2 Hz) stimulation frequencies using low doses and attenuated LFP changes at higher frequencies using high doses within somatosensory cortex. Similarly, the CBF response was attenuated with high doses of either drug at high frequencies (Jessen et al., 2015). Though both drugs attenuated CBF at high doses at high frequencies, only the extrasynaptic GABAAR agonist THIP augmented CBF at low doses and low frequencies. Further, high doses of THIP bidirectionally augmented and attenuated CMRO2 at low and high frequencies, respectively, suggesting that NVC can be differently modulated via volume versus synaptic transmission GABAAR activation. Similar results have been seen in the cortex, where Mueggler et al. and Reese et al. both showed that vasodilation with elevating concentrations of systemic GABAAR antagonist bicuculline. Both studies also show that the response profile to bicuculline is brain-region specific (Reese et al., 1999, Mueggler et al., 2001), with cortex increasing in a time-dependent manner and CPu responding biphasically. Overall, these studies highlight that GABA can modulate neuronal activity, vascular tone, and/or metabolism via GABAAR signaling in concentration and brain-region dependent ways.

2.2.2 GABABRs

GABABRs are G‑protein-coupled receptors (GPCRs) and are found on axons and dendrites as well as neurons and astrocytes. They are composed of two different subunits which have differential cell-type-specific expression within the CPu (NG and Yung, 2001). GABABRs exert their actions by inhibiting neurotransmitter release and modulating neuronal excitability via inward rectifying K+ (GIRK) channels (Terunuma, 2018). GABA also diminishes glutamatergic signaling within the CPu, which is dominated by corticostriatal and thalamostriatal inputs, via the GABABRs found on cortical and thalamic terminals (C.J.Lacey et al., 2005, Logie et al., 2013). Additionally, MSNs elicit inhibitory feedback control onto each other via GABABR-mediated inhibition at MSN terminals (Ade et al., 2008) and their collaterals (Humperies et al., 2010, Tunstall et al., 2002). Perfusion of the GABABR agonist baclofen onto hippocampal slices constricts microvessels, in sharp contrast to the vasodilation seen by perfusing GABAAR agonist muscimol (Fergus and Lee 1997). However, such an effect could be region dependent, as electrical stimulation of Perkinje cells and climbing fibers in the cerebellum under applied baclofen, a GABABR agonist, alters synaptic activity but not CBF (Caesar et al., 2003). Baclofen application on preconstricted vessels in the *ex vivo* mouse retina has been shown to elicit vasodilation, vasoconstriction, or no response in distinct retinal arterioles (Hinds et al. 2013). Further, applying baclofen to ex vivo mouse retinas elicits either vasodilation, vasoconstriction, or no vascular responses in preconstricted retinal arterioles. These results collectively suggest that GABABRs influence NVC in ways that differ from GABAARs, but this needs to be interrogated more within the GABAergic CPu.

~~Due to the discovery of TH~~~~+~~ ~~GABAergic interneurons and NPY neuroglioform cells within the CPu further characterization is needed.~~

1. **Glutamate**

3.1 Origins of Glutamate in the CPu

Glutamate is a key regulator of MSN activity and a major excitatory neurotransmitter that increases neuronal activity by allowing Na+ and/or Ca2+ influx via ionotropic receptors, leading to depolarization. It also exerts excitatory effects via metabotropic receptors leading to transcriptional changes. Glutamatergic tone in the CPu comes from cortical and thalamic projections. Corticostriatal projections originate from sensory, motor and association regions across all mammalian species (Goldman-Rakic and Selman, 1986; Jones et al., 1977; Kemp and Powel, 1970; McGeorge and Faull, 1989; Oka, 1980; Royce, 1982; Tanaka, 1987; Veening et al, 1980). These regions are topographically arranged with sensorimotor cortices projecting to the lateral parts of the CPu and association cortices to the medial parts of the CPu (Reep et al., 2003). Like the corticostriatal projections, thalamostriatal projections are also organized topographically, with some differences across species. In primates, the centromedian and parafiscular (CM/PF) complex are distinct anatomical and functional parts of the thalamus. In rodents, this complex is composed exclusively of the PF, with the most lateral part of the PF being homologous to the primate CM (Galvan and Smith, 2011; Mandelbaum et al., 2019). Within the primate, the PF projects to the associative striatum also known as the caudate nucleus, while the CM projects to the sensorimotor striatum known as the putamen (Galvan and Smith, 2011; Mandelbaum et al., 2019). In rodents, the lateral part of the PF projects to the somatosensory striatum, while the central PF sends projections to the associative striatum and medial PF to the limbic striatum (Galvan and Smith, 2011; Mandelbaum et al., 2019).

* + 1. **Corticostriatal Circuit**

Corticostriatal projection neurons express the glutamate transporter VGlut1 (Doig et al., 2010) and are divided into two classes. Both classes send bilateral projections, and the class that sends collaterals into the CPu terminates in the brain stem (Shepard, 2013). The magnitude and duration at which corticostriatal neurons synapse onto MSNs plays a major role in MSN state transition. Integration between DA and corticostriatal transmission aids in increasing MSN membrane voltage and N-methyl-D-aspartate (NMDA) receptor expression (Surmeier et al., 2010; Cahill et al., 2014). Cortical inputs within the striatum synapse onto MSNs and predominately GABAergic interneurons (N.Abudukeyoumu et al., 2019; A. Kalfovi et al., 2005; C.J.Lacey et al., 2005 ). Specifically, corticostriatal efferents differentially target D1 or D2 MSNs (Lu et al., 2020) and fast spiking interneurons (FSI) and persistent low threshold spiking interneurons (PLTS) expressing SOM and/or NPY receptors. FSIs receive the largest input from corticostriatal synapses and exert the strongest feedforward inhibition onto MSNs as shown using whole cell voltage clamp (A.H. Gittis et al., 2010). PLTSs, however, weakly inhibit MSNs and CHINs. CHINs are shown to exert bidirectional influence on glutamate release via cholinergic metabotropic and nicotinic receptors (N. Abudukeyoumu et al., 2019). The influence of neurotransmitters released from these interneurons on striatal hemodynamics will be discussed later in this article.

**3.1.2. Thalamostriatal Circuit**

Thalamus is another major source of glutamatergic inputs to the CPu and is often thought of as a relay to convey information from the basal ganglia to the cortex and among cortical nuclei (Grant et al. 2012). In both primates and rodents, the dorsal striatum receives two predominant inputs from the thalamus, non-CM/PF projections and CM/PF projections. Some of the non-CM/PF nuclei that project to the CPu are rostral intralaminar, ventrolateral (in primates), and central lateral (in rodents) nuclei. Unlike cortical synapses within the CPu, thalamic inputs express VGluT2 vesicular glutamate transporters (E. Mestikawy et al., 2011; Fremeau, 2001). As a population, thalamic inputs make nearly equal synaptic contacts with both D1- and D2-MSNs (Doig et al., 2010), but contact fewer MSNs as compared to cortical inputs (Huerta-Ocampo et al., 2014). In agreement with many primate studies, recent works in mice have confirmed that PF thalamus discretely targets MSNs and striatal interneurons, with CHINs being predominantly innervated by central and lateral PF, and FSIs being innervated by lateral PF.

3.2. Glutamate receptor subtypes

The effect of glutamatergic signaling on vascular activity has been examined by microdialysis paired with fMRI, which revealed that cortical CBF increases with glutamate concentrations (Forman et al., 1998). Glutamatergic synapses can influence vasodilation in a number of ways: 1) release of nitric oxide (NO), 2) binding astrocytes to promote signaling cascade that promotes the release of arachidonic acid metabolites (MacVicar and Newman, 2014), or 3) binding endothelia cells directly to produce eNO (Lu et al., 2019). The CBF increase in sensory cortex induced during sciatic nerve stimulation can be abolished by applying the nitric oxide synthase (NOS) inhibitor L-NAME (Northington et al., 1992). These findings are corroborated by Dirnagl et al. using NOS inhibitor L-NNA during vibrissa stimulation (Dirnagl et al., 1993), suggesting that increases in glutamatergic transmission within the cortex result in vasodilation related to NO signaling. There are two predominate glutamatergic receptors within the brain: ionotropic (iGluR) and metabotropic (mGluR). While signaling via iGluRs and mGluRs both influence vasodilation, the underlying mechanisms differ.

3.2.1. iGluR Influence in NVC

The two iGluRs are NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (NMDARs and AMPARs, respectively), which are cation permeable, predominately postsynaptic, and responsible for transmitting quick excitatory responses. Both express on cerebral microvascular endothelial cells (Parfenova et al., 2003), but iGluR activation can also initiate signaling cascades to involve vasoactive second messengers (Attwell 2011/other reviews). Sensory stimulations paired with pharmacological manipulations highlight that both NMDA and AMPA receptors differentially contribute to hemodynamic responses resulting from somatosensory stimulation (Gsell et al., 2006). The following paragraphs will highlight the individual contributions of each receptor type to vasodilation.

A major mechanism by which glutamatergic NMDAR activation induces vasodilation is via the synthesis and release of NO by NMDAR-expressing neurons (Faraci and Breese, 1993). Vasodilation in the rabbit parietal cortex was abolished in the presence of either NMDAR antagonist MK-801 or tetrodotoxin (Faraci and Breese, 1993). Similarly, Yang and Chang blocked vasodilation in rat parietal cortex by applying either MK-801, tetrodotoxin, or the selective neuronal NOS (nNOS) inhibitor 7-nitroinda-zole (7-NI). To discern whether the NO-induced vasodilation was from nNOS and not endothelial NOS (eNOS), Yang and Chang applied nitroglycerin (NTG) and found that NTG-induced dilation was not attenuated by NOS inhibitor L-NNA. However, NMDARs also express on endothelial cells and isolated medial cerebral arteries dilate via eNOS in response to the co-binding of gliotransmitter D-serine and glutamate to NMDARs (laMaistre et al., 2012). To elucidate the involvement of eNOS in this cascade, eNOS inhibition by L-NIO significantly decreased vasodilation in response to astrocytic Ca2+ uncaging. Application of glutamate and D-serine mixture in the presence of L-NIO significantly reduces the vasodilatory response (Stobart et al., 2013). Together, these results suggest that while NMDAR activation leads to vasodilation, pharmacology must be used to determine whether dilation is from downstream nNOS or eNOS signaling.

Glutamate can also mediate vasodilation via AMPAR activation. Gsell et al., showed the involvement of AMPARs in glutamate mediated vasodilation via the reversable yet immediate attenuation of increased BOLD and CBV to forepaw stimulation by AMPAR antagonist GYKI-53655 (Gsell et al., 2006). Ohata et al., corroborated these results by showing that vasodilation increased in rat cortex following AMPA superfusion. NMDA superfusion also evoked vasodilation in the same experimental preparation, but while NOS inhibitor L-NNA attenuated the NMDAR-mediated vasodilation, the AMPAR-mediated vasodilation remained unchanged. Surprisingly, superfusion of adensosine A2A and A2B were also able to attenuate, but not abolish the AMPAR-mediated response (Ohata et al., 2006). These studies indicate that AMPARs can be involved in vasodilation in ways that differ mechanistically from NMDAR-mediated vasodilation; however, more work is needed to inform whether this is a result of direct signaling or indirect signaling via astrocytes.

3.2.2. mGluR Influence in NVC

Glutamate can also influence NVC via mGluRs, either through signaling cascades or modulating neuronal activity. Activation of mGluRs increases both Ca2+ and D-serine release (Mothet et al., 2005), and spur the production and release of vasoactive arachiodonic acid derivatives. Though adult astrocytes do not express mGluR1 or 5 (Calcinaghi et al., 2011, Sun et al., 2013), both murine bEND5 (Zuccolo et al., 2018) and human hCMEC/D3 (Negri et al., 2019) brain endothelial cells directly respond to glutamate application by increasing intracellular Ca2+ and releasing NO. These effects of glutamate on endothelial cells can be mimicked by applying a selective group 1 mGluR agonist (Negri et al., 2019) or blocked by BAPTA or mGluR1 antagonist MCPG (Zuccolo et al.; Negri et al.). As expected, NOS inhibitor L-NAME abolished the NO response. Together, glutamate receptors irrespective of subtype induce vasodilatory effects via the actions of NO, regardless of whether the NO is of neuronal or endothelial origin. Further studies are needed to examine the extent to which glutamate plays a role in vasodilation in various subcortical nuclei such as the CPu.

~~NO can be produced by endothelial cells, to assess whether this effect is carried out by neuronally released glutamate, as it has been shown that adult astrocytes do not express mGluR1 or 5 (Calcinaghi et al., 2011, Sun et al., 2013), Zuccolo et al., use in vitro models of endothelial cells, bEND5 cells, paired with pharmacology to show the direct involvement of mGluR1 in eNOS vasodilation. Specifically, the authors showed that the addition of 100 uM glutamate increases intracellular Ca~~~~2+~~~~, which is blocked by BAPTA. Increases in Ca~~~~2+~~ ~~can also be blocked by the addition of mGluR1 specific antagonist, MCPG. Through an elegantly aligned set of pharmacological manipulations, Zuccolo et al. show DAF-FM, a NO sensitive fluorophore increases in the presence of glutamate and could be blocked via BAPTA or NO inhibitor Ned-19.~~

1. **Acetylcholine**

**Origins of ACh in CPu**

Acetylcholine (ACh) neurotransmission is involved in sensory and attention processing (DaSilva et al., 2019; Jaminez-Martin et al., 2021; Hasselmo and McGauphy, 2004; Guillem et al., 2011; Herrero et al., 2008; Metherate, 2004; Gil et al., 2019;Veith et al., 2021), learning and memory (Hasselmo, 2006; Maurer and Williams, 2017; Crouse et al., 2020), entwined with dopaminergic neurotransmission (Vizi et al., 2017; Rizzi and Tan, 2017; Durand-de Cuttoli etal., 2018; Surmeier et al., 2012) and is dysregulated in several pathologies that affect the CPu (Lester et al., 2010; Lombardo et al., 2015; Freedman et al., 2000; Dziewczapolski et al., 2009; Davis et al., 2010; Hellstrom-Lindaul and Court, 2000). Compared to cortex, the CPu has high levels of ACh (ref), ACh receptors,(Hersch et al., 1994; Weiner et al., 1990), and other enzymes related to the synthesis and breakdown of Ach (Graybiel et al., 1986; Butcher et al., 1992; Zoli,2000; Wolf et al., 1991); thus, it follows that CPu function is heavily influenced by ACh signaling. ACh-releasing neurons are generally divided into cholinergic interneurons (CHINs) and cholinergic projection neurons. While CHINs are the major source of ACh in the CPu, the pedunculopontine and the laterodorsal tegmental nucleus send spatially organized cholinergic projections to CPu (Dautan et al., 2014). Brainstem cholinergic neurons innervating the striatum target various cell types with greater preference for CHINs, and are involved in habitual behavior (Dautan et al., 2020). While ACh is often considered an excitatory neurotransmitter, in many cases it can also downregulate or inhibit cellular activity due to the complex distribution of ACh receptor subtypes within cells, and the configuration of those cells within local circuits (Mallet et al., 2019; Belousov et al., 2001).

**Influences of ACh in CPu**

***CPu activity***

CHINs modulate and are modulated by components of the CPu microcircuit and exert substantial influence over local neuronal activity and metabolism as it relates to NVC. In the CPu, CHINs express DA receptors, iGluRs, GABAaRs, and ACh receptors, and are capable of co-transmitting glutamate or GABA with ACh depending on the origin of these neurons (Takacs et al., Klijaik et al., 2017; Granger et al., 2020) . These interneurons switch between rhythmic, autonomous single-spike firing and bursting activity (Bennett and Wilson, 1999; Sharott et al., 2012). The autonomous firing maintains a relatively high tonic level of ACh in CPu, which regulates glutamatergic tone within the striatum via pre and post synaptic mechanisms (Piciotto et al., 2012). Glutamatergic inputs onto CHINs, which are predominantly from thalamus (Johansson and Silberberg, 2020), contribute to both the bursts and pauses in CHIN firing. These pauses are well documented and modulate the synaptic activity of DAergic terminals in the CPu (Ding et al., 2010; Deng et al., 2007; Maurice et al., 2004). Optogenetic CHIN stimulation modulates DA release in a frequency dependent manner (Cachope et al., 2012; Threlfell et al., 2012), and the two neuromodulators have extensively been shown to exert both antagonistic and faciliatory effects on each other (Sumier and Graybeil, 2012; Gerfen and Surmeier, 2010; Threlfell et al., 2012; Aosaki et al., 1994). It is worth noting that though afferents from brainstem also provide cholinergic innervation in CPu, they do not modulate DA (Brimblecombe et al., 2018), but do inhibit MSNs and excite CHINs (Dautan et al., 2020). A CHIN subpopulation exists that responds strongly to cortical glutamatergic inputs and modulates MSNs via muscarinic receptors (Mamaligas et al., 2019); however, MSNs can also form synaptic connections onto CHINs (Gonzales et al., 2013; Chuhma et al., 2011) and exert inhibitory tone over them via GABAARs (DeBoer and Westerink, 1994). CHINs can also be inhibited via GABA co-released from SNc DA neuron terminals (Tritsch et al., 2012; Deng et al., 2007; Maurice et al., 2004), and via reciprocal connections with GABAergic interneurons (Sullivan et al., 2008; Vuillet et al., 1992). Inhibiting CHINs reduces tonic ACh levels in CPu and may facilitate changes in local microcircuitry that lead to aberrant behaviors or pathology (Austin et al., 2014). Though a small percentage of CPu neurons are CHINs, their extensive arborization, varied firing patterns, and co-release of GABA and glutamate with ACh enable these interneurons projection neurons to exert a wide range of neuromodulatory effects within the CPu circuit. The influence of ACh on CPu neuronal activity likely extends to local metabolic changes and subsequent vascular signaling, but could also affect metabolism and blood flow due to downstream signaling or the direct effects of acetylcholine on NVC .

***CPu vessels***

As mentioned above, ACh is vital to regulating basal ganglia neurotransmission, studies of how it affects NVC largely have taken place in glutamatergic brain regions such as hippocampus and cortex. While modern tracing, imaging, and optogenetics studies have shown that ACh can signal via fast synaptic transmission (Sarter et al., 2009; Colangelo et al., 2019; Obermayer et al., 2017), ACh has been classically considered a volume neurotransmitter. Thus, unlike the ubiquitous neurotransmitters glutamate and GABA, ACh could affect vascular tone directly by extending past synaptic spaces to reach blood vessels and perivascular cells in ACh-rich brain regions like the CPu. Axon terminals expressing choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, have been found in close proximity to endothelial cells (Parnevales et al., 1985), and endothelial cells within the rat cortex were also found to be immunoreactive for ChAT. As ACh is easily broken down in blood, it is posited that endothelial cells likely take up choline and then synthesize ACh. In addition, astrocytes and astrocyte endfeet in close proximity to vasculature express ACh receptors, and could regulate neuronal as well as vascular-related signaling through related pathways (REF). ~~Wilson et al., showed that applying ACh onto carotid artery endothelial cells increased Ca~~~~2+~~ ~~mediated vasodilation, and could be replicated by applying neostigmine, an ACh esterase inhibitor. Further, ACh mediated vasodilation in has been linked to Ca~~~~2+~~ ~~increases that result in NO release (Wilson et al., 2018).~~

Despite the evidence for ACh signaling to vasculature, only a few recent studies have examined acute cerebrovascular responses to ACh. In one study, exogenous ACh applied through a cranial window in anesthetized rats dilated cortical blood vessels (Sakata et al., 2021). However, Zaldivar et al. showed that injecting exogenous ACh into the macaque visual cortex elicited a more complex, spatially-specific response. Near the injection site, baseline CBF and BOLD increased, but vascular visual stimulation response amplitudes decreased, as did LFP and MUA power. Farther from the injection site, vascular baselines remained the same and both vascular and LFP evoked responses increased (Zaldivar et al., 2018). In another brain-wide fMRI study, Hoff et al., examined how the muscarinic ACh receptor (mAChR) agonist pilocarpine affects CBV in a region dependent manner. Interestingly, cortex and hippocampus both exhibited increases in CBV, yet CPu displayed robust vasoconstriction (Hoff et al., 2010). These observed vasomodulatory differences between brain regions and proximity to ACh release sites could be related to corresponding differences in the ACh receptor subtypes that are recruited; a difference which is especially pronounced in the distribution of nicotinic and muscarinic ACh receptors and their subtypes in the CPu as compared to the cortex(ref).

**Nicotinic Receptors**

Nicotinic ACh receptors (nAChRs) are ionotropic receptors responsible for fast, phasic cholinergic signaling (Obermayer et al., 2017, Arroro et al 2014). In cortex, nAChRs are found on pyramidal neurons and GABAergic interneurons that target NPY/SOM and VIP interneurons, but not PV interneurons (Askew et al., 2019)(ref), and …. nAChRs are in CPu are similarly expressed on … with these notable exceptions …. Activation of nAChRs on glutamatergic terminals leads to enhanced excitation (Campos et al., 2010). Nicotinic receptor activation on DA terminals has been shown to increase phasic changes in DA tone (Zhouh et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004). Add in new MRI nicotinic specific stuff. While nAChRs have been identified on vascular beds within the central nervous system (Kalaria et al., 1994), their function yet to be established, and therefore nAChR-mediated changes in vascular signaling are most likely downstream of circuit-level changes in neuronal activity.

**Muscarinic Receptors**

Muscarinic ACh receptors (mAChRs) are G-protein coupled receptors that play a role in tuning excitatory and inhibitory synapses (Jamenez-Martin et al., 2021). The five sub-receptor types are categorized as either group 1 (M1, M3, and M5) or group 2 (M2 and M4) mAChRs, which couple to stimulatory Gq or inhibitory Gi/o proteins, respectively, and express nonuniformly across brain regions and cell types (Volpicelli and Levey 2004). Unlike cortex, where mAChR expression predominantly includes M1s on post-synaptic and M2s on pre-synaptic neurons(Volpicelli and Levey 2004), the CPu broadly expresses both group 1 and group 2 mAChRs (Zhanget al., 2002; Zhou et al., 2003; Pisani et al., 2007; Langmead et al., 2008; Bonsi et al., 2011). D1- and D2-MSNs both express M1 receptors that lead to [facilitate?] excitation in both cell types, but because D1-MSNs also express M4 receptors, CHINs can exert bi-directional control over MSN subtypes. [talk about M2,M3 and M5 too!]. [talk about mACHr on GABA tone and CPu activity via interneurons signaling here]. [talk about mAChR effects on glutamatergic inputs here]. DA tone can also be enhanced through M5 muscarinic receptor signaling (Weiner et al., 1990). While not expressed on the vasculature directly, it is possible that M4 signaling via MSNs could indirectly influence vascular tone, especially when muscarinic activation of either M2 or M4 receptors leads to decreased glutamate release probability (Ding et al 2010). Likewise, Mx-mediated changes to DAergic signaling could alter vasculature signaling indirectly by tuning neuronal activity in CPu, or via direct DAergic influences on vascular tone, discussed below.

Several studies point to the potential for mAChR modulation of vascular tone. Human PCR studies have shown that human cerebral microvessels express M2 and M3 receptors, endothelial cells express M2 and M5 receptors, and smooth muscle cells express all mAChRs except M4 (Elhusseiny et al., 1991). Lecrux et al., reported that during whiskerpad stimulation, increasing ACh within the cortex results in higher CBF responses, and this effect can be attenuated by the nonselective mAChR antagonist scopolamine, but not the nAChR antagonist MEC/MLA (Lecrux et al., 2017). [add additional, mechanistic studies from ctx and cpu studies here!][Elaborate on m5-NOS interaction here!] A mechanistic study using selective control/manipulation tools are needed to reveal the causal mechanisms that lead to this intriguing difference in cholinergic influence over vascular activity in CPu versus other brain regions.

**Dopamine**

**Origins of DA in CPu**

The striatum receives some of the highest DA tone in the brain (Silberberg and Bolam et al., 2015). DAergic neurons project to the dorsal CPu primarily from the SNc (Dahlstrom and Fauxe, 1964) and sparsely from the SNr (Deutch et al. 1986), and to the ventral CPu from the ventral tegmental area (VTA; cite). Earlier retrograde studies suggested that SNc DA neurons could be divided along a dorsal/ventral axis (Fallon and Moore, 1978), with only dorsal neurons expressing calbindin (Gerfen et al., 1987a,b; Neuhoff et al., 2002). SNc DA neurons characteristically have aspiny dendrites, with a few passing into the SNr (Juraska et al., 1977; Kitta et al., 1986; Tepper et al., 1994, 1987). Once they reach the CPu, the axons of these neurons show symmetric synapses on the spines or the necks of spines of MSNs, and some make synapses by terminal boutons (Freund et al., 1984; Groves et al., 1994; Pickel et al., 1981). Similar to CHINs, SNc DA neurons fire spontaneously (Bunney et al., 1973a,b). DA neurons display 3 modes of firing, listed here in order from most to least common: irregular firing marked by long periods of afterhyperpolarizations, pacemaker activity, and bursting activity (Tepper et al.,1998) and remain in to active states tonic or phasic. In the following section, regulation of DA release at the axonal level will be discussed further.

Dopamine release within the CPu is also highly regulated by local presynaptic signaling. Glutamate can inhibit DA release via iGluR and mGluR activation, as shown via microdialysis and FSCV studies (Avshalumov et al.,2003; Kulagina et al., 2001; Wu et al., 2000). This conflicts with the fact that DA axons do not express ionotropic receptors (Bernard and Bolam, 1998; Chen et al.,1998), and suggests that this effect is indirect. For example, AMPARs that express on MSNs enhance membrane-permeable H2O2 release (Bao et al., 2009) when activated, which then inhibits DA release via ATP K+ channels (Patl et al., 2011). Activating type 1 mGluRs (mGLUR1s) that express on DA axons (Paquet and Smith, 2003) inhibits DA release in CPu (Zhang and Sulzer, 2003); conversely, activating mGLUR1s that express on DA neurons in SNc facilitates somatodendritic DA release (Patel et al., 2009). GABA also inhibits DA release postsynaptically by inhibitory interneurons via GABAARs (Ronken et al., 1993). CHINs, as discussed earlier, can bi-directionally influence DA excitability within the CPu. DA firing is enhanced when nAChRs on DA terminals are activated (Grenhoff et al., 1986; Threlfell et al., 2012), but single-pulse or low frequency stimulus evoked DA release is suppressed if nAChR antagonist is on board (Rice and Cragg, 2004; Zhang and Sulzer, 2004; Zhou et al., 2001). During high frequency stimulations, the block is released and DA neurons fire at a higher frequency than during nAChR activation (Patel et al., 2012). In addition to modulating DA neurons via nAChRs, CHINs also modulate DA neurons via M5 mAChRs that express on DA neurons (Vilaro et al., 1990; Weiner et al., 1990) and DA terminals (Shin et al. 2015). FSCV studies revealed that M5 receptors modulate ACh release to further modulate DA terminals via nAChRs (Sulzer et al., 2017). Though DA axons express both KORs and DORs, but not MORs (Svingos et al., 2001; Trovero et al., 1990), all three receptor types inhibit DA release (Schlosser et al., 1995). SP is shown to enhance DA release within striosomes but has no effect on DA terminals in the matrix (Crittenden and Graybiel, 2011). [something about how these complex infuences on DA release in striatum have important functional implications for DA-mediated changes in activity to tie into next part]

**Influence of DA in CPu**

***CPu activity***

Dopamine is a neuromodulator that influences activity via 5 GPCR receptors (D1-D5). D1/D5 are excitatory Gq-coupled and characterized as D1-type receptors while D2-D4 are inhibitory Gi-coupled and characterized as D2-type receptors. [This section is in desperate need of more DA effects on CPu neuronal activity in general (not receptor-spectic); see Lindsay’s comment above to get started; don’t forget a summary sentence tying DA regulation of CPu activity to vascular responses like we have for ACH and GABA above]

***CPu vessels***

DA neurons have long been shown to modulate vasomotor responses within the cerebral cortex. Light and electron microscopy showed that DAergic varicosities are in close proximity to capillaries (Krimer et al., 1998). In an *in vitro* pharmacological study, both DA and D2R agonist epinine were used to examine the role of DA receptors in the vasomotor response. It was shown that vasodilation and vasoconstriction are specific to which receptors were activated (Edvinsson et al., 1978). Krimer et al., employed iontophoresis and showed that applying DA elicits vasoconstriction in cortical vessels that respond to exogenous DA (Krimer et al., 1998). Conversely, Chen et al., showed in a pharmacological fMRI study that amphetamine evoked robust CBV increases across multiple brain areas including the cortex and CPu (Chen et al., 2005). However, D1Rs and D2Rs have different affinities for DA (cite), such that lower concentrations of evoked DA release are thought to preferentially occupy D2Rs before D1Rs. Indeed, low-dose amphetamine challenges increase DA concentrations and evoke vasoconstriction in CPu, but higher doses that increase DA concentrations by several orders of magnitude instead evoke vasodilation in CPu (Ren et al., 2009). These conflicting results may be the result of differential receptor activation.

**D1-like Receptors**

Pyramidal cell excitability within the primate PFC increases in the presence of DA in a concentration specific manner and has been shown to rely on D1 receptor activation as D1R antagonist SCH23390 but not D2 receptor sulpiride (Henze et al., 2000). In prepubescent rodent PFC, D1/D5R activation increases neuronal excitability and decreases the post-hyperpolarization of pyramidal neurons (Yi et al., 2013). This contrasts with the effect of applying the D2 agonist quinpirole, which increases excitability in murine motor cortex pyramidal neurons (Vitrac et al., 2014). These results were replicated by Swanson et al, who also showed that D1R activation increased membrane hyperpolarization and input resistance (Swanson et al., 2021). DA also plays a role in inhibitory transmission. D1R activation increases the excitability of PV interneurons and GABAA amplitudes in postsynaptic neurons (Seamans et al., 2001).

Administering D1/D5R agonist results in vasodilation (Choi et al., 2006), highlighting the specificity of DA receptors in the modulation of vascular tone. D1Rs have the highest expression throughout the brain (Bhatia et al., 2021)

**D2-like Receptors**

D4Rs have the lowest expression levels for DA receptors in the brain (Bhatia et al., 2021). and D2R decreases GABA release and GABAA mediated responses (Seamans et al., 2001). One mechanism regulating DA release is through DA autoreceptors. D2 and D3 autoreceptors are found on the axons of DA neurons (Gainetdinov et al., 1994; Sokoloff et al., 1990; Tepper et al., 1997; Zapata et al., 2001). Pharmacological manipulation using 7-OH-DPAT paired with FSCV in slices showed that both D3 and D2 receptors play a functional role in decreasing DA release. D3 receptors, like D2 receptors, are coupled to inward rectifGIRKs and cause hyperpolarization and current shunting (Kuzhikandathil and Oxford , 1999, 2000; Kuzhikandathil et al., 1998). This is in direct contrast with a study showing that D3 immuno-reactivity is not detectable on axons in the striatum (Diaz et al., 2000). This lends credence to the idea that autoreceptor-dependent decreases in DA concentrations are predominately carried out by D2Rs.

Choi et al., further observed CBV increases in parts of the cortex and CPu following use of the D3R antagonist PG-01037, which was reversed by applying the D3R agonist 7-OH-DPAT, resulting in negative CBV (Choi et al., 2010).

**Peptides**

~~CHINs are also modulated by the endogenous opioids released by D1- and D2-MSNs, dynorphin and enkephalin, respectively (Gagnon et al., 2019). mu opioid receptors (MORs) on CHINs (Le Moine et al., 1994; Jabourian et al.,2005) to decrease cholinergic vesicle release and increase CHIN firing, respectively. Though kappa opioid receptors (KORs) respond selectively to dynorphin secreted from D1-MSNs (Fallon and Leslie, 1986; Mansour et al., 1994), there is no evidence that KORs are expressed on CHINs (Lim et al., 2014).~~

**Persistent Low Threshold Spiking Interneurons**

PLTS interneurons can be classified by their expression of SOM, NPY (Johansson, 1983; Vincent et al., 1983), and/or NOS (Hope et al., 1991). Identifying GABA and GAD expression within PLTS interneurons was a challenging task (Kubota et al., 1993) until EM later revealed axonal immunoreactivity for GABA following pretreatment with an axonal transport blocker, colchicine (Kubota and Kawaguchi, 2000). With the use of biotin filling, these cells were discovered to possess two axons (Kawaguchi, 1993). Electrophysiological studies found these interneurons have a depolarized resting membrane potential (~ -56 mV), high input resistance , low threshold Ca2+, and long action potential durations (Kawaguchi, 1993). It has been shown that the CPu has varying numbers of SOM+ and NPY+ positive interneurons (Rymar et al.,1996) in addition to PLTS expressing varying amounts of SOM and NOS but were not immunoreactive for NPY (Figueredo-Cardenas et al., 1996).

Anatomically, PLTS neurons receive numerous afferent synaptic contacts on both proximal and distal dendrites from both DAergic neurons and CHINs (Kubota et al., 1988; Vuillett et al., 1989a,b, 1992). Pharmacological studies have demonstrated that DA facilitates excitation via D1Rs (Centonze et al., 2002) while CHINs presynaptically gate PLTS interneurons via M2 muscarinic receptors (Bernard et al., 1998). PLTS have also been shown to receive GABAergic input from MSNs and the (GPe) along with weaker inputs from the cortex (Partridge et al., 2009; Gittis et al., 2010). The principal targets of PLTS interneurons are MSNs. They form symmetric synapses on the spines and dendritic branches of MSNs, but not on somas (ref). It has been reported that PLTS interneurons make weak and sparse IPSCs in MSNs (Gettis et al., 2010), possibly because GABA is not the primary molecule released from these neurons. This suggests the possibility that neuromodulators released from these neurons may gate MSNs. .

**Parvalbumin Fast Spiking Interneurons**

FSIs are characterized by short duration action potentials and rapid after-hyperpolarization discharging (Freund abd Buzaski, 1996; Galarreta and Hestrin, 1999, 2001,2002). FSIs have notably low input resistance and a resting membrane potential of –80 mV, and thus, do not display spontaneous firing (Kawaguchi, 1993). These interneurons have been found to form gap junctions with other FSIs and because these connections show synchronized depolarization spiking, it is thought that they exert strong and synchronous control over MSN spike timing (Koos and Tepper, 1999; Berke, 2008). FSIs are known to exert strong IPSPs onto MSNs and paired recordings in culture show low failure rate (Plenz and Kitai, 1998; Koos and Tepper 2002). This is in contrast to, lateral inhibition that MSNs weakly exert onto each other (Tunstall et al., 2002).

FSIs receive both symmetric and asymmetric synaptic connections, which are largely found on the dendrites with fewer inputs found on the soma. Approximately 2/3 of the asymmetric synapses formed on FSI spines are from cortical projections (Kita et al., 1990) that make multiple synaptic connections onto FSIs (Ramanathan et al., 2002) compared to the sparse glutamatergic input from the thalamus (Kitta et al., 1993). In addition to cortical innervation, FSIs also receive input from DA neurons, with DA modulating FSIs both at the pre- and post-synaptic level. DA exerts bidirectional modulation of FSIs by increasing FSI firing rates post synaptically via D5Rs and decreasing FSI firing via presynaptic activation of D2Rs (Centonze et al., 2003). Additionally, while FSIs express both muscarinic and nicotinic cholinergic receptors (Koos and Tepper, 2002; Lou et al., 2013; Ibanez-SanSandoval et al., 2015), and EM show cholinergic synapses and pharmacological studies show cholinergic terminals synapsing onto FSI (Chang and Kita, 1992), neither electrical (Koos and Tepper, 2002) nor optogenetic stimulation of CHINs (English et al., 2012) drive FSI firing. For this reason, it is thought that FSIs are innervated by cholinergic neurons arising from the pedunulopontine nucleus (Dauntan et al., 2014). Lastly, FSI are innervated by GABAergic neurons arising from the GPe(Bevan et al., 1998).

**Influence of peptides on CPu vascular activity**

All of these neurons are known to have vasoactive roles (Cauli and Hamel, 2010), but the precise effects of each may differ. Studies using immunocytochemistry and radioimmunoassay examining cerebral arteries reported that NPY across species induces vasoconstriction, albeit the density of NPY receptors on vessels may vary (Edvinsson et al., 1987). It has been shown that perfusion of NPY onto the microvessels caused vasoconstriction (Cauli et al., 2004). This robust constriction effect was later observed within the CPu, and the effect was proven to be independent of oxidative metabolism (Tuor et al., 1990). More recently, a study using 2 photon imaging showed that optogenetic stimulation within the sensory cortex induces a biphasic vascular effects, with the negative phase being attributable to NPY (Uhlirova et al., 2016). Similarly, SOM receptors are also found on the vasculature and elicits vasoconstriction mediated through the GABAA receptor (Cauli et al., 2004; Kocharyan et al., 2008).

[Transition paragraph to Disease States section --- some lame suggestion below]

Pharmacology and genetic modification techniques continue to be used to obtain progressively reductive models and clarify the role of specific cell types or receptors in NVC. Understanding the interconnectedness of neurotransmitters signaling and ultimately influencing vascular responses in healthy conditions can lead to better understanding the etiology or progression of neuropathologies. Some disease states are primarily categorized as pathologies with dysregulated neurotransmission, others are primarily categorized as pathologies with dysregulated vascular function; however, the truth is likely a cooperative effect from both. The striatum is affected in several disease states where neurotransmission and vascular regulation are impaired. Here, we describe a subset of these pathologies with both neurochemical and hemodynamic perspectives.

**Parkinson’s disease**

Summarize disease and why it is important ( so just facts people know ). Go to preclinical mechanisms that are already talked about above in the disease, specifically geared to vasculture and/or cell types. Then link them together. Talk about the progression and how it may start as one (vascular or neuronal) and transition to the other, as neuronal and vascular changes are usually related; can talk about bidirectional interactions too at different “stages” of disease, like onset/risk factors, clinical symptoms/detection, progression/recovery of symptoms/pathology .

An example is (this would be multiple paragraphs filled in):

Onset of PD is realated to a loss of DA innervation to striatum, and the data that says that this can influence neurons, but also the vasculature- some recent studies even link the onset of PD to loss of DAergic signaling to vasculature first, then neurons second. Indeed, vascular Parkinsonism, linked more specifically to vascular dysfunction, has been described as.... Of course DA loss also leads to motor deficits and has various effects on neurons which then effect metabolism, local neurotransmission, and hemodynamics (preclinical mechanisms discussed before). These aspects of parkinsons (see “stages” above) have been associated with the things we just mentioned (specifics), and therefor could also be related to neurotransmission effects of vasculature in striatum. Finally, these data suggest tht vascular markers could be good screening/diagnostic tools, and vascular targets could be treatment focus for these specific aspects of parkinsons.

<https://movementdisorders.onlinelibrary.wiley.com/doi/10.1002/mds.22937>

<https://www.frontiersin.org/articles/10.3389/fnana.2014.00084/full>

Imaging NVC associated pathogenesis represents a challenge in Parkinson’s disease (PD). Regional hypermetabolism with intact flow-metabolism coupling, determined by simultaneously increased CBF and glucose utilization, has been observed using PET in the pallidum, thalamus, and pons of untreated PD patients with concomitant hypometabolism in the lateral premotor cortex and parietal association areas. Expression of this PD-related spatial covariance pattern (PDRP) increases with age and can be consistently quantified using PET and/or CBF MRI with a high degree of correlation between the two modalities (Hirano et al. 2008 and Ma et al. 2010). Hirano et al. using PET with [15O]-H2O, a tracer for CBF, and [18F]- Fluorodeoxyglucose (FDG), a tracer for cerebral metabolic rate of glucose (CMRglc) observed flow-metabolism dissociation in the putamen and pallidum occurring during levodopa infusion (Hirano et al. 2008) but not globally. This dissociation was marked by increased in CBF proportionate to decreased in CMRglc, a phenomenon not observed 12 h prior to treatment. While dissociation was noted in several regions of the PDRP, only in the putamen and pallidum did CBF increase significantly more in patients with levodopa-induced dyskinesias than in treated patients without dyskinesias or in the healthy controls. As these regions overlapped with known local L-aromatic acid decarboxylase activity (AADC), it is possible that low doses of levodopa were converted to dopamine at axon terminals adjacent to local vasculature, causing vasodilation. Notably, flow-metabolism dissociation was not observed in PD patients treated using STN DBS.

Neuroinflammation from cytokine-mediated activation of astrocytes and microglia and neurotransmitter imbalances are major contributors to clinical decline. Decreased dopaminergic and GABAergic signaling accompanied by increased cholinergic and glutamatergic tone in the extrapyramidal tracts are also responsible for both motor and cognitive symptoms (Lotankar et al. 2017). Recognition of mitochondrial dysfunction and changes in NO activity gave rise to toxin-induced animal models for PD including the commonly used 6-hydroxy-dopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and has since gained traction in human studies for its contribution to alpha-synuclein accumulation (Kalia and Lang 2015 and Lourenço et al. 2017). Pathological dopaminergic denervation in the striatum, alterations in cholinergic and monoaminergic neuronal signaling, and neuronal NO accumulation in the striatum and cortex contribute to decreased functional connectivity and grey matter atrophy (Lourenço et al. 2017). One point of ongoing debate is whether NVC in PD is strictly secondary to neuronal loss. Evidence of VEGF-driven angiogenesis, leaky BBB, and fluctuations in CBF contributing to the motor symptomatology of PD suggest that there are concomitant vascular pathologies contributing to NVC dysfunction (Lourenço et al. 2017 and Lagana et al. 2020).

Studies using toxin-based animal models provide further evidence of local vascular changes associated with L-DOPA treatment. In the rhesus monkeys, striatal hypermetabolism and shifts in DA receptor density have been found after chronic dopamine depletion. Treatment of MPTP-lesioned rhesus monkeys (primate PD model) with L-DOPA lead to a change in BOLD response in the ipsilateral putamen and caudate (Chen et al. 1996) when compared to the contralateral unlesioned striatum. The individual contributions of the CMRO2, CBF, CBV, and glucose utilization were not measured, thus the hemodynamic origin of such BOLD changes has yet to be identified. 6-OHDA lesioned rat and immunohistochemistry in post-mortem PD patient brains demonstrated increased angiogenesis and BBB leakage in basal ganglia output nuclei after chronic levodopa treatment (Ohlin et al. 2012).

Owing to PD being primarily a disease of middle and older age with increasing risk in cardiovascular disease, it is unclear if having comorbid cerebrovascular disease (CVD) with PD has any effect on changes in CBF and flow-metabolism decoupling. CVD patients with recent strokes or transient ischemic attack (TIA) have demonstrated prolonged arterial arrival time (AAT) quantified by arterial spin labeling (ASL) MRI when compared to healthy controls. This increased AAT is thought to be related to chronic vasodilation, flow through collaterals, blood brain barrier (BBB) leakage sites, and possibly increased vascular tortuosity. Quantification of AAT in idiopathic PD patients without CVD revealed prolonged AAT compared to healthy controls and non-PD patients with chronic CVD (Al-Bacharach et al. 2017). In this same study, increased CBF in the putamen and GPe was observed in the postural instability and gait difficulty (PIGD) phenotype but not in the milder tremor predominant (TD) phenotype. This difference may have been caused by larger usage of levodopa in the PIGD cohort when compared to patients in the TD group. Additionally, occurrence of increased CBF and AAT extending beyond areas of grey matter atrophy raises the possibility that microvasculature dysfunction precedes neuronal loss, and may contribute to neuronal dysfunction rather than simply being a consequence of neuronal loss. Further development of imaging methodology is paramount to further understanding the etiology of PD and for the ongoing development of sensitive imaging-based biomarkers, and protocols for assessing disease progression and treatment effectiveness.

In the comparison of 5 L-DOPA treated PD patients with L-DOPA-induced dyskinesia (LID) and 5 without, Aljuaid et al. developed the putamen-to-thalamus hyper-perfusion/hypo-metabolism index (PHI) as a PET-fMRI-based biomarker for LID with 80% sensitivity (Alijuaid et al. 2019). As the flow-metabolism dissociation seen after treatment with L-DOPA is presumed to be mediated by D1R-driven vasodilation and angiogenesis in the striatum, they chose the thalamus as the baseline comparison site because of its relatively low density of D1 receptors. Additional longitudinal study with larger sample sizes are needed to determine if PHI is a useful tool for predicting which patients will develop LID and would benefit from anti-angiogenic and vasoconstrictive therapies (Alijuaid et al. 2019 and Ohlin et al. 2012). Investigation of L-DOPA functional connectivity modulation in patients with LID, produced a protocol able to predict the development of LID with 91% sensitivity and 100% specificity using resting-state fMRI (Herz et al. 2016). Decreased functional connectivity between the most affected putamen and primary sensory motor cortex after L-DOPA administration was observed in patients with LID while increased functional connectivity along the same corticostriatal pathway was observed in patients without LID. Notably, it has been observed that patients with LID have rapid increase in putamen dopamine levels shortly after administration followed by a dramatic decrease while non-LID patients maintain steady state concentrations (Herz et al. 2016 and Alijuaid et al. 2019). It remains unclear why some patients are able to maintain a reservoir of exogenous dopamine but understanding the mechanism could lead improved therapeutics.

**Huntington’s Disease**

Huntington’s Disease (HD) is a fatal autosomal dominant neurodegenerative disease caused by a mutant huntington protein (mHtt) ubiquitously expressed in the CNS including in all major cell types of the neurovascular unit (Chan et al 2021). HD is characterized genetically by an abnormal CAG trinucelotide repeat expansion on the exon of chromosome 4. Scoring systems such as the CAG-Age Product or CAP score (age of patient x (CAG repeat length – L)/S, where L and S are constants) 3 can be used to predict the age of symptomatic disease onset (Ross et al. 2014) . The imaging hallmark of HD is caudal atrophy followed by neuronal cell death in the cortex and other subcortical nuclei, along with demyelination and axonal degeneration in later stages. The symptomatology of HD is characterized by progressive loss of motor control, and executive function, resulting in uncoordinated movement, psychological disorders, and ultimately death. Loss of motor control in HD is hyperkinetic initially due to the death of iMSNs in the striatum manifesting as distinctively ballistic and choreiform movements (Gittis and Kreitzer 2012). Hypokinesia is seen in later stages with progression of widespread neuronal loss (Ross et al. 2014). Caudal atrophy, as measured by volumetric MRI, as well as changes in hemodynamics and neuronal activity can be observed years prior to symptom onset (Gregory and Scahill 2018). Below we will discuss evidence of striatal neurochemical and microvascular changes that probe our understanding of NVC in HD.

Simultaneous increases in CBV and LFP in cortical regions of transgenic HD mouse models indicates that NVC may be preserved despite pathological changes to the microvasculature and synaptic plasticity (Cepeda-Prado et al. 2012). The degree of NVC in striatum of HD patients however remains unclear. This growing body of knowledge describing changes in synaptic activity and the microvasculature of the dorsal striatum associated with HD provides further impetus to continue investigating NVC in the dorsal striatum to allow for accurate interpretation of BOLD fMRI data in disease processes specific to the basal ganglia.

Understanding vascular activity and integrity is necessary for the proper interpretation of functional imaging in HD. In the striatum, increased perivascular spaces (also known by the eponym Virchow-Robin spaces) in the putamen of symptomatic patients was noted to be proportional to the degree of caudal atrophy (Chan et al. 2020). Investigations of CBF using ASL fMRI and structural MRI in HD patients with striatal atrophy demonstrated parenchymal volume loss and proportional reduction in CBF (Chen et al. 2012). Impaired cortical cerebrovascular responsiveness to mild hypercapnia in HD patients was observed after the administration of exogenous CO2 using BOLD fMRI (Chan et al. 2021). Utilization of 3D-Triple-acquisition-after-Inversion-Preparation (3D-TRIP) MRI developed by Klinkmueller and colleagues allowed for the investigation of CBV, CBF, CMRO2, and BOLD separately in a pre-manifest and early-manifest HD patients during visual stimulation (Klinkmueller et al. 2021). While significant differences in CBF, CMRO2, and BOLD were observed in HD patients when compared to healthy controls, arteriolar CBV was impacted to a greater degree than the other parameters, showing a ~10% increase in HD patients than in healthy volunteers during stimulation. Their findings of increased arteriolar CBV in cortical regions of HD patients using 3D-TRIP was consistent with previous investigations using the inflow-based vascular space occupancy (iVASO) MRI technique to assess cortical CBV in prodromal patients without observable atrophy (Hua et al. 2013). Notably, their data demonstrated correlation between decreased CMRO2 and patient CAP scores and years-to-onset of symptomatic disease. Though this investigation did not include the striatum, the regions studied are known areas of striatal input, providing evidence that further investigation of pathological hemodynamic changes that may affect NVC in the striatum is warranted.

Impairment in synaptic plasticity of striatal MSNs is evident prior to neuronal death and caudal atrophy in HD (Dijak et al. 2019). Specifically, changes in dopaminergic, glutamatergic, cholinergic and GABAergic tone have been observed in transgenic mouse models of HD and HD patients. Some studies in transgenic HD mice demonstrated increased dopaminergic input into the striatum prior to neuronal loss (Dijak et al. 2019). Increased dopamine from the nigrostiatal pathway would result in LTP of D1-MSNs and LTD and inactivation of D2-MSNs at corticostriatal synapses. Cholinergic interneurons of the striatum also express D2R, resulting in decreased release of acetylcholine when bound. Increased striatal dopaminergic tone early in the disease prior to appreciable caudal atrophy may contribute to prodromal HD. Clinically, these changes could manifest as increased involuntary movements or difficulty with learning and habit formation (Kreitzer and Malenka 2008). In a 3-year study using [11C] -raclopride PET to measure D2R binding potential changes in the striatum and cortex over time, annual decreases in D2R binding correlated with increasing Unified Huntington’s Disease Rating Scale (UHDRS) scores, demonstrating a correlation between increasing D2R dysfunction in the striatum and patient functional decline (Pavese et al. 2003). This is consistent with reports that D2-MSNs of the indirect pathway are most vulnerable to mHtt toxicity. The loss of the D2-MSNs followed by the loss of D1-MSNs is thought to be responsible for the transition form hyperkinesia to bradykinesia with disease progression in adult-onset disease (Dijak et al 2019). Given the distinct vasomodulatory effects following activation of distinct DA subtype receptors (see xxx), xxxxx

Evidence of impaired glutamine reuptake at corticostriatal synapses was observed in HD patients and in transgenic mice. In addition, increased prevalence of extrasynaptic NMDA receptors on MSNs was observed in YAC128 mouse models resulting in increased MSN intracellular stress. The potential excitotoxic effects of decreased glutamine reuptake at corticostriatal synapses and activation of apoptotic pathways via glutaminergic activation of extrasynaptic NMDA receptors provide two possible pathways for the striatal atrophy observed in HD (Kreitzer and Malenka 2008 and Dijak et al 2019). There is evidence of increased GABAergic tone from striatal interneurons in HD mouse models, possibly due to the relative preservation of interneurons when compared with MSNs and increased GABA release (Kreitzer and Malenka 2008). This shift in GABA release could serve as a compensatory mechanism to offset the hyperkinesis caused by increased activity of dMSNs. However, GABA may also be dampened or only target iMSNs, leading to further suppression of inhibitory signals (Dijak et al 2019). Further investigation of GABA mediated signaling is needed for understanding it’s role in HD-associated synaptic activity, neuronal metabolism, and associated vascular responses.

**Stroke**

The necessity of targeting treatments to repair injury to all parts of the neurovascular unit (NVU) while maintaining the delicate balance of its intercellular communication is demonstrated perhaps most clearly in the management of acute stroke. Classically, stroke is categorized as ischemic, thromboembolic arterial occlusion resulting in the death of the surrounding tissue, or hemorrhagic caused by the rupturing of a weakened vessel resulting in leakage of intravascular contents into the surrounding tissue and decreased downstream blood flow. Both ischemic and the less common hemorrhagic strokes involve anatomical and physiological disruptions the NVU. It has been demonstrated that the caudate followed by the putamen are the more susceptible to ischemic injury from hypoperfusion after acute stroke (Payabvash et al. 2011).

In a review by Zoppo (2010), the importance of the dynamic structural relationship of the NVU in the setting of hemorrhagic stroke and focal ischemia. In experiments investigating effects of proximal middle cerebral artery (MCA) occlusion in nonhuman primates on areas supplied by the distal lenticulostriate arteries (LSAs), the distance between striatal neurons and their most proximal microvessel was a strong determinant of susceptibility to focal ischemia. Interestingly, though some neurons farther from their blood supply proved to be most vulnerable, the effects were heterogenous with some nearby NVUs with longer microvessel-to-neuron distances spared from neuronal injury. Neuronal metabolism and CBF in the striatum differ from that of cortical regions (Mabuchi et al. 2005, Zoppo 2010). While the arteriolar and capillary architecture of the striatum results in lower baseline CBF – likey because of the low supply needed for its quiescent baseline neuronal activity (see discussion in section xxx above), striatum possesses greater sensitivity to proximal occlusion. The heterogeneity of neuronal injury within the ischemic territory remains unclear. Importantly, loss of matrix-adhesion receptors, basal laminar matrix proteins, and increased activity of matrix metalloproteinases (MMPs) was observed in the ischemic territory and correlated with hemorrhagic conversion, one the most detrimental complications of ischemic stroke and its treatment with antifibrinolytics. Degradation of the basal lamina matrix as can be seen in stroke, causes spillage of blood contents in to neighboring tissue resulting in infarction. The demonstrated loss of adhesion molecules connecting the astrocyte end feet and endothelial cells has implications for BBB breakdown and neurovascular decoupling as the vessels and neurons are connected via intervening astrocytes. Therapeutic bolstering of the basal lamina and BBB integrity could prevent or reduce the risk of hemorrhagic conversion in ischemic stroke. Finally, endothelial oxidative stress and response to inflammatory signals may be partially responsible for why some microvessels in the striatum remain patent while adjacent ones are occluded after proximal vessel occlusion (Mori et al 1992 and Zoppo 2010). The intravascular activation and accumulation of local inflammatory cells in response to ischemia-induced damage to endothelial cells could be partially responsible for the propagation of distal vessel occlusion hours after the initial infarct (Zoppo 2010). Taken together, the heterogeneity of CBF, neuronal injury, matrix integrity, and endothelial response within a focal ischemic region provides overwhelming evidence that recovery of neurologic function after stroke is likely dependent upon the baseline health of the affected NVUs and the effectiveness of treatment to preserve the architecture and restore coupling.

To date, the mainstay treatments for ischemic stroke mostly target CBF restoration. The primary goal in treatment of acute ischemic stroke is to recanalize and reperfusion the occluded vessel pharmacologically within 3-4.5 hours of symptom onset or treatment or via mechanical thrombectomy within 24 hours (NIND rt-PA Stroke Study Group, 1995, Hacke et al. 2008, Nogueira et al. 2018). While reperfusion with fibrinolytics and antiplatelet agents has been overwhelmingly successful, it has not entirely prevented the spread of infarction from the initial core to the penumbra or “peri-infarct tissue” (Ramos-Cabrer et al. 2011). The penumbra, visibly evident on MRI in acute stroke, is an area of presumably endangered but salvable tissue characterized by hypoperfusion, metabolic derangements, local depression, decreased protein synthesis, and increased expression of MMP. Similarly, to the outcomes of focusing solely on reperfusion, trials with use of neuroprotectant and neurorepairing agents without addressing the damage to other parts of the NVU, have been met with limited success (Zoppo 2010, Ramos-Cabrer et al. 2011). The quest to salvage the penumbra and contain infarction continues to be the primary driving force behind attempts to understand how brain ischemia and hemorrhage affect the involved neurons, astrocytes, endothelial cells, their shared basal laminal, the BBB, mural cells, neighboring microglia and oligodendrocytes as individual units and their relationship in totality. Further investigation of a multipronged approach with combination treatments targeting CBF, neuronal activity, BBB stabilization, and inflammation, in the setting of acute stroke may prove to beneficial in penumbra rescue and as secondary prevention.

Two of the most devasting sequelae of infarction and disruption of the NVU in the dorsal striatum are the subsequent development of Vascular parkinsonism (VP) and subcortical vascular dementia (sVAD) (Kalra et al. 2010, Caruso et al. 2019). First described by MacDonald Critchely in 1929 as “arteriosclerotic parkinsonism”, VP is a syndrome of alpha synucleinopathies but is a result of extensive CVD in the basal ganglia rather than nigrostriatal denervation (Critchley 1929 and Kalra et al. 2010). There have been numerous attempts to establish clear guidelines for diagnosis of VP which continue to come up short. Formerly called “lower body parkinsonism”, VP classical presents with more lower extremity involvement than PD, including symmetrical gait and postural instability, multiple falls, incontinence, pyramidal signs, in addition to pseudobulbar palsy with less rigidity than is seen in idiopathic PD. Cognitive decline, seen late in the disease course of PD, presents earlier in VP. The age of disease presentation is up to a decade later in VP when compared to PD. Patients are less responsive to L-DOPA which aligns with the SPECT imaging studies that showed symmetrical reduction in dopamine reuptake to be more prominent in PD than in VP (Kalra et al. 2010). However, the majority VP patients (up to 96%) demonstrated infarction and/or white matter lesions on MRI in comparison to <25% of PD and for many the symptom onset was within 1 year of an acute stroke (Winikates and Jankovic 1999, Zijlmans et al. 2004, and Kalra et al. 2010).

Multiple subcortical strokes in a brief period (<3months) can precipitate the acute onset of vascular dementia (Caruso et al. 2019). Over 20 million people are living with some type of vascular dementia associated with history of chronic CVD or stroke. Vascular dementia is thought to arise from neurovascular decoupling secondary to the loss of vasoreactivity and vasoregulation. Chronic hypertension, diabetes mellitus, and hyperlipidemia, in conjunction with advanced age result in the stiffening and partial occlusion of vessels, particularly the lenticulostriate arteries through hyalinosis and atherosclerosis. In addition, loss of cholinergic vascular afferents also reduce vasoreactivity to changes in the metabolic demands of the adjacent neuron. In sVAD affecting the dorsal striatum, patients present acutely (usually within 1 month of multiple stroke) with decreased executive function, planning, poor memory retrieval, decreased processing speed, and behavioral changes most notably disinhibition. Motor signs such as hypokinesis and gait instability may also be seen (Caruso et al. 2019). Treating chronic CVD with agents that effectively preserve NVC may lower the incidence of sVAD.

**Aging**

A lot of the research focused on the effects of aging in the dorsal striatum was done in the context of cerebrovascular disease, Parkinson’s disease, and Huntington’s Disease as these are amongst the most prominent chronic and neurodegenerative diseases associated with pathological aging within the caudate and putamen. However, there is overwhelming evidence to suggest that aging in otherwise healthy adults without clinically relevant neurodegeneration or history of stroke is also associated with neurovascular decoupling. As stated previously, appropriate NVC relies on a delicate concert between neurons, astrocytes, endothelial cells, pericytes/SMCs, and perivascular macrophages (Iadecola 2017 and Tarantini et al. 2017). Age-related changes and loss of any of these cell types in the NVU can result in decreased NVC and BBB breakdown (Zlokovic et al. 2008, Iadecola 2017, Tarantini et al. 2017 and Yabluchanskiy et al. 2021).

Appropriate astrocytic function is necessary for maintenance of NVC. Astrocytes are involved in maintenance of neuronal synapses, recycling of neurotransmitters, maintenance of the BBB, and participation in microglia-induced neuroinflammatory responses after trauma (Clarke et al. 2018). Amongst the many roles of astrocytes is maintenance of optimal synaptic iron concentration as iron is needed for synthesis of neurotransmitters but in excess catalyzes the oxidation of neurotransmitters and can thus impair neurotransmission (Wu et al. 2004 and Kalpouzos et al 2017). Striatal iron accumulation increases with age and may be responsible for the impairment in frontostriatal activity and pathways associated with overt-motor inhibition (Steiger et al. 2016, Kalpouzos et al 2017). Kalpouzas et al. using mental imagery task-based fMRI and MR relaxometry (to approximate of striatal iron concentration) showed increased iron accumulation in otherwise healthy older adults when compared with younger adult controls. Interestingly they also demonstrated a reduction in frontostriatal activation associated with decreased memory recall of imagery task events associated with motion and overt-motor inhibition (participants were asked to imagine themselves in a situation requiring them to be motionless). The difference in activation was particularly evident in the putamen, a known participant in overt motor inhibition in young adulthood, even when corrected for age-related volume loss (Kalpouzos et al 2017). Ghadery et al. (2015) also used MRI R2\* metric as a surrogate for iron content and found increased iron accumulation in the putamen and caudate of otherwise healthy adults when compared to younger adults some of whom were younger first-degree relatives. Higher dorsal striatal iron content was associated with decreased executive function and psychomotor speed but they did not find a correlation with memory impairment. Further analysis with much larger participant groups and clear age delineation between the population of interest and controls are needed to resolve this discrepancy. However, it is clear that astrocytic-neuronal dysfunction plays a role in age-related reduced function of the dorsal striatum Clarke et al. (2018) used RNA sequencing to compare the cortical, striatal, and hippocampal transcriptomes across the lifespans of mouse models. Their data demonstrated the greatest age-associated changes in genetic expression occur in the striatum and hippocampus with few changes in the cortex. Importantly, striatal astrocyte activation in mice in aged mice correlated with a shift toward the gene expression profile of A1 astrocytes, a neuroinflammatory phenotype associated with complement-driven synaptic degradation and neurotoxin-mediated neuronal death. Notably, A1 astrocytes have been implicated in PD, HD, and Alzheimer’s disease ( in only animal models?). Disproportionate astrocyte-driven synaptic degradation and decrease astrocyte-mediated neuronal synapse formation in the striatum when compared to the cortex, could play a prominent role in regional NVC dysfunction.

The contribution of anatomical changes to vasculature (decreased arterial capacitance and compliance) and atrophy of the caudate and putamen associated with healthy aging are unclear (Gunning-Dixon et al 1998, Caruso et al. 2019, Xu et. al 2021). Characterization of lenticulostriate arteries (LSAs) using 3D high resolution black blood MRI in adults aged 50-82 years old with normal cognition, demonstrated the fewer supplying the dorsal striatum was associated with smaller diameters in the proximal ACA and MCA (Xu et al. 2021). Additionally, their results were consistent with decreased vascular compliance and lower vascular tortuosity in those with hypertension. This provides evidence that differences in LSA morphology as it direct influences CBF may partially explain variations in the resiliency of NVC during aging. Substantial asymmetric shrinkage of the dorsal striatum is associated with age (Gunning-Dixon et al 1998). The clinical relevance of less severe shrinkage than what is seen in neurodegenerative disorders remains unclear but presumably is affected by the integrity of the remaining NVUs.

There is evidence to support NVC may be a compensatory mechanism in aging the MCA territories (Sorond et al. 2011). As part of the MOBLIZE Boston study the relationship between hemodynamics, MRI white matter hyperintensities (WMH), and gait speed in older adults was investigated (Sorond et al. 2010 and Sorond et al. 2011). Based on prior results associating slow gait speeds and falls with decreased MCA vasoreactivity (as determined by TCD) and higher probability of having WMH (Sorond et al. 2010), the investigators attempted elucidate the relationship between WMH and NVC as measure by MRI (Sorond et al. 2011). Unexpectedly WMH was not the strongest correlate with slow gait speeds, though slower walkers were much more likely to have increased WMH than fast walking older adults (age >70 years). Importantly, they found an inverse relationship between L MCA blood flow and age. Notably, older adults with high density of WMH and adequate BF velocity associated with activity had maintained high fast gait speeds, thus NVC may serve a compensatory mechanism allowing them to be high functionally cognitively and maintain mobility. There was no correlation between BF velocity and chronic conditions such as hypertension and diabetes mellitus typically seen in older populations regardless of gait speed. The data from this study may have implications for NVC in the dorsal striatum as the lenticulostriate arteries supplying the caudate and putamen are branches of the MCA and ACA.

**Conclusion**

The promise of BOLD fMRI as a beneficial translational medicine and clinical tool for the investigation, tracking of disease progression, and assessment of treatment effectiveness in neurological diseases, is bound to our understanding of NVC in such states. NVC relies on the interdependence of regional metabolism and hemodynamics both of which are disrupted or dysregulated in neurogenerative disease, stroke, and with increasing age. The relationship between neuronal atrophy and local changes in neurometabolism and hemodynamics remains to be elucidated. Further investigation of neurochemical, synaptic, and microvascular changes, and their relationship to volume loss in dorsal striatal pathologies and aging is necessary for the accurate interpretation of functional imaging in affected patients.

Further investigation of neurochemical, synaptic, and microvascular changes, and their relationship to volume loss in dorsal striatal pathologies and aging, is necessary to accurately interpret functional imaging in affected patients