INVESTIGATING HPAB AS A MODULATOR OF GUT-BRAIN CONNECTIVITY THROUGH NEUROTRANSMITTER METABOLISM BY GUT MICROBIOTA

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TYLER MYERS

ADVISOR: HEIDI GOODRICH-BLAIR

UNIVERSITY OF TENNESSEE, KNOXVILLE

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Abstract

The gut-brain axis represents a bidirectional communication network linking the gastrointestinal tract and the central nervous system. A growing body of research implicates gut microbiota in modulating host neurochemistry, largely through microbial metabolism of neurotransmitters. This thesis investigates the flavin-dependent monooxygenase HpaB as a putative microbial enzyme involved in host dopamine metabolism, thereby influencing gut-brain signaling. Traditionally characterized for its role in the homoprotocatechuate (HPC) pathway, HpaB catalyzes the hydroxylation of 4-hydroxyphenylacetate (4-HPA) to 3,4-dihydroxyphenylacetate (3,4-DHPA)—a reaction structurally analogous to eukaryotic dopamine oxidation to DOPAC. This structural and chemical convergence suggests that HpaB may act on host-derived catecholamines. In vivo expression data, phylogenetic analysis, and gut colonization models for strains of human microbiota further the relevance of HpaB homologs in host-associated bacterial taxa. Notably, behavioral phenotypes in animal models resulting from host-associated strains overexpressing HpaB—such as impaired locomotion, altered sensory perception, and reproduction—mirror symptoms associated with dopaminergic imbalance. This work proposes a novel mechanism by which bacterial aromatic monooxygenases may deplete or modify host neurotransmitters in situ, with implications for host gut motility, immune function, and behavior. If validated through biochemical assays and animal models, HpaB could represent a novel enzyme-mediated mechanism at the interface of environmental metabolism and neurochemical regulation. The findings expand the conceptual framework of microbial endocrinology and open new avenues for targeting microbial enzymes to influence host neurological health.

Keywords: gut-brain axis, gut microbiome, dopamine, host-microbe interactions, HpaB

Introduction

Over the last two decades, the gut-brain axis has emerged as a dynamic bidirectional communication system, linking the gastrointestinal tract to the central nervous system through neural, hormonal, and immune signaling. Central to this axis is the gut microbiome, a diverse and metabolically active microbial community that influences numerous host processes beyond digestion, including neurodevelopment, stress response, and behavior (Wang et al., 2023; Tan, 2023). Recent studies have shown that gut-resident microbes can produce, degrade, or otherwise modulate neuroactive molecules—such as serotonin, gamma-aminobutyric acid (GABA), and dopamine (Barandouzi et al., 2022; Strandwitz et al., 2019; Otaru et al., 2021; Valles-Colomer et al., 2019)—thereby altering the host's neurochemical environment. These interactions are not merely passive but can shape emotional states and cognitive functions, emphasizing the microbiome's role as a neuroactive, or, at least, a neuromodulatory organ.

Microbial contributions to neurochemical signaling are often mediated by enzymes that transform small molecules exchanged between host and microbe. Enzymatic activities such as decarboxylation, hydroxylation, and oxidation are known to influence neurotransmitter pools within the gut, both through the synthesis of new neurotransmitter products and the degradation of already existing molecules produced (Gao et al., 2019). For example, a significant proportion of gut microbes, particularly those within the genera *Bifidobacterium*, *Escherichia,* and *Bacteroides*, are known to harness the synthesis of GABA as a mechanism for tolerating acidic conditions along the lower portions of the digestive tract (Strandwitz et al., 2019; Pokusaeva et al., 2017). Moreover, various intestinal trains belonging to genera such as *Streptococcus, Enterococcus, Escherichia, Lactobacillus, Klebsiella,* and *Morganella* have demonstrated the ability to produce serotonin (Özoğul et al., 2004, Shishov et al., 2009, Özoğul et al., 2012, Dinan

et al., 2015). While several microbial biosynthetic pathways for GABA and serotonin have been well characterized from amino acid substrates, far less is known about the bacterial enzymes that catabolize host-derived neurotransmitters, particularly the catecholamines. An interaction between catecholamine metabolism and microbial activity demands attention based on prior research demonstrating that the growth of gut microbes like *E. coli* responds favorably to increased levels of catecholamines in the intestinal tract (Freestone et al., 2002). Expanding the catalog of microbial enzymes that possess neurochemical activity is critical to elevating community understanding on how intestinal bacteria modulate animal host physiology and behavior.

Among the microbial enzymes that carries potential to be involved in this biochemical dialogue is HpaB, a flavin-dependent monooxygenase that catalyzes the hydroxylation of 4-hydroxyphenylacetate (4-HPA) to 3,4-dihydroxyphenylacetate (3,4-DHPA) as part of the homoprotocatechuate (HPC) pathway (Sparnins & Chapman, 1976). This pathway is used by many environmental and gut-associated bacteria to degrade aromatic compounds for energy and carbon. The crystal structure of HpaB has been extensively characterized in a range of bacterial species, spanning *Escherichia coli*, *Acinetobacter baumannii*, and *Thermus thermophilus* (Deng et al., 2020, Alfieri et al., 2007; Kim et al., 2007). Characterizations of this protein have left it notable for its catalytic versatility based on substrate promiscuity within its active site. Moreover, the protein has a conserved presence across diverse bacterial taxa—including many strains that are well-known for host association. Interestingly, 3,4-DHPA, the product of HpaB activity, bears striking structural resemblance to dopamine, and it bears identical structure to dopamine's oxidative metabolite, DOPAC, produced by the action of catechol *O*-methyl transferases (COMTs) and monoamine oxidases (MAOs) in mammalian neurons and astrocytes (Zhang et al.,

2019). A canonical suite of enzymes coordinates the generation of dopamine in neurons from the amino acid tyrosine, promotes its export into the synaptic cleft, and allows for degradation in both the presynatpic neuron and surrounding astrocytes (Fig 1.) In eukaryotic systems, this oxidation of dopamine to DOPAC by MAOs and COMTs converges with bacterial degradation of aromatic compounds at the catechol ring. This biochemical overlap suggests the possibility that HpaB, although classically associated with xenobiotic degradation, may also act on host-derived catecholamines. This thesis seeks to explore the hypothesis that HpaB contributes to gut-brain axis signaling through promiscuous enzymatic activity on host-derived catecholamines such as dopamine. By bridging environmental metabolism with host neurochemical pathways, HpaB may represent a previously unrecognized mechanism by which bacteria influence host behavior and neurological homeostasis.

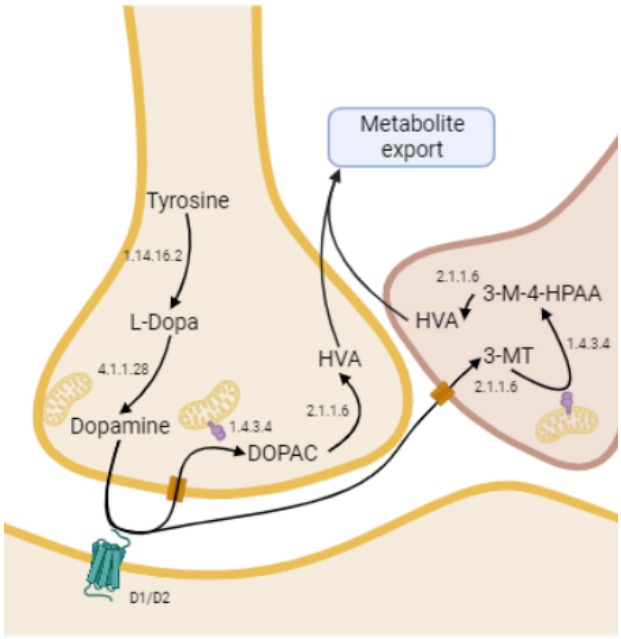

Figure 1: Schematic representation of the proposed mechanism of dopamine synthesis, transport, and metabolism in animal neurons (yellow) and astrocytes (red). Numbers represented enzyme classification (EC) numbers of conserved enzymes involved in these pathways, with the catechol O-methyltransferase and monoamine oxidase referenced in this article depicted by 1.4.3.4 and 2.1.1.6, respectively. The schematic was generated using Biorender (https://www.biorender.com).

HpaB and the Homoprotocatechuate Pathway

The HpaBC complex plays a central role in bacterial degradation of aromatic compounds via the HPC pathway—a metabolic route critical for transforming phenolic substrates into intermediates of the tricarboxylic acid (TCA) cycle. This pathway is employed by many free-living and host-associated bacteria to catabolize 4-HPA, a compound derived from environmental sources or host metabolism. The complex consists of two components: HpaB, a flavin-dependent monooxygenase, and HpaC, a flavin reductase that regenerates the reduced

flavin cofactors ($FMNH_2$ or $FADH_2$) required for HpaB catalysis. HpaB initiates the catabolic sequence by hydroxylating 4-HPA to form 3,4-DHPA, effectively converting a monohydroxylated phenyl ring into a catechol. This transformation is essential for subsequent ring-cleavage reactions, which produce succinate and acetate—metabolites that feed directly into central metabolism (Sparnins & Chapman, 1976). The efficiency and specificity of this conversion would be vital for bacterial survival in phenol-rich environments, including the mammalian gut, where dietary and host-derived aromatic compounds are readily available for catabolism.

HpaB is a class A flavin-dependent monooxygenase, structurally characterized by a canonical Rossmann fold that binds flavin cofactors. As mentioned previously high-resolution crystallography in the model species *E. coli*, *A. baumannii*, and *T. thermophilus* has shown that HpaB forms a compact, globular structure with a solvent-accessible catalytic pocket capable of accommodating a range of aromatic substrates. All three HpaBC complexes are homotetrameric, in which HpaB forms an active homodimer that can stabilize reduced FAD in the absence of a substrate to prevent an autooxidation of the co-substrate. In its catalytic cycle, HpaB can receive either $FMNH_2$ or $FADH_2$ from its partner enzyme HpaC, depending on the strain source and enzyme subtype (Kim et al., 2007). Previous work has sought to gauge the necessity of HpaC in the catalytic function of HpaB, finding that 4-HPA hydroxylating activity can only occur when supplementation with HpaC takes place (Galán et al., 2000). Upon binding of reduced flavin and molecular oxygen, HpaB forms a C4a-hydroperoxyflavin intermediate that transfers an oxygen atom to the bound substrate, typically hydroxylating the *para* position of 4-HPA (Thotsaporn et al., 2014; Prieto & Garcia, 1994). The oxidized flavin is then recycled by HpaC, completing the redox cycle. Importantly, the active site contains several conserved residues that stabilize the

aromatic substrate and coordinate oxygen activation, allowing the enzyme to function efficiently under aerobic conditions (Kim et al., 2007). Flavin-dependent monooxygenases, including HpaB, are known for their relaxed substrate specificity. Broadly, HpaB homologs are characterized into two classes: those that are strictly FAD-dependent and those that are FAD/FMN-dependent, which are referred to here as type I and type II, respectively (Kim et al., 2007). While type I HpaB enzymes possess a buried binding site for FAD, type II enzymes possess a slightly more open site, burying only the isotetrameric ring portion and increasing its donor flexibility (Alfieri et al., 2006). Numerous studies have documented that these enzymes can hydroxylate a wide variety of phenolic and aromatic compounds, depending on the organism and environmental context (Lin & Yan, 2014; Shen et al., 2019; Chen et al., 2019). Moreover, the degree by which HpaB demonstrates substrate promiscuity varies across species, with that derived from *E. coli* (a type II enzyme) possessing far less specificity compared to that expressed by *T. thermophilus* (a type I enzyme) (Huang et al., 2013). This promiscuity is facilitated by a relatively spacious and chemically adaptive active site that tolerates substitutions at different positions of the aromatic ring.

The chemical logic underlying the hypothesis that the promiscuity of HpaB would allow for binding of catecholamine neurotransmitters, namely dopamine, and their metabolic derivatives is compelling. 3,4-DHPA, the product of HpaB and recognized as DOPAC in mammalian systems, contains a catechol functional group—an essential structural motif recognized by a wide range of bacterial dioxygenases. In both prokaryotic and eukaryotic systems, catechols are funneled into downstream degradation pathways that yield energy-rich intermediates. Given this convergence, it is plausible that HpaB participates in microbial catecholamine metabolism either directly, by hydroxylating dopamine to a trihydroxy derivative,

or indirectly, by transforming dopamine precursors or analogs. This participation has been confirmed in several studies involving the manipulation of *E. coli* genomes to possess full machinery required for dopamine synthesis. These studies routinely demonstrate the role of HpaB in performing the final conversion in generating an L-DOPA or dopamine product (Das et al., 2017; Fordjour et al., 2019; Nakagawa et al., 2021). Based on these experiments revealing the full capability of microbes harnessing HpaB to generate dopamine, HpaB would possess the qualities to act as a biochemical interface between host neurochemistry and microbial central metabolism, particularly in the gut environment where dopamine plays roles in motility, immune modulation, and local microbial dynamics, as will be explored the next sections

HpaB in the Context of Host-Microbe Interactions

In recent years, the notion that gut microbiota influence neurological function has transitioned from a provocative hypothesis to a well-supported framework. A central component of this framework involves microbial metabolism of neurotransmitters—either through biosynthesis, degradation, or transformation of neuroactive compounds that circulate within the host. Microbes have been shown to interact with a broad range of host neurochemicals, including serotonin, dopamine, norepinephrine, and GABA. These interactions are mediated by enzymes encoded by commensal bacteria that modulate neurotransmitter pools via pathways that often mirror or complement host metabolism. For instance, certain species of *Clostridium* use glutamate decarboxylase to produce GABA, thereby impacting host stress response (Braga et al., 2024). *Bacteroides fragilis* and related taxa carry monoamine oxidase-like enzymes that degrade serotonin and dopamine analogs (Yano et al., 2015). Such microbial activities influence not only local gut physiology—such as motility and immune signaling—but also systemic functions,

including mood and cognition, through neuroimmune and neuroendocrine pathways. However, while biosynthesis and canonical degradation routes are increasingly well characterized, little is known about the role of bacterial aromatic-degrading enzymes like HpaB in this context.

Unlike typical monoamine oxidases that oxidatively deaminate amine neurotransmitters, HpaB functions as a hydroxylase acting on the phenyl ring of aromatic compounds. Given the chemical similarities between 4-HPA, 3,4-DHPA, dopamine, and DOPAC, HpaB may catalyze transformations that mimic those of both host and microbial catecholamine pathways. For instance, HpaB could feasibly convert dopamine into trihydroxylated derivatives such as 3,4,5-trihydroxyphenylethanol or initiate degradation via ortho-hydroxylation—a known first step in bacterial aromatic catabolism (Dhammaraj et al., 2015). These reactions could deplete the local pool of bioactive catecholamines or generate novel signaling intermediates with as-yet-unknown physiological roles.

If HpaB does in fact metabolize host catecholamines like dopamine, its activity could exert profound effects on gut-brain signaling. Dopamine plays critical roles in regulating intestinal activity motility through inhibition of duodenal function, contraction of the circular layer in the ileum, and an overall reduction of peristalsis in the colon (Zizzo et al., 2016, Zizzo et al., 2010; Lim et al., 2008). With regards to a local immune response, dopamine modulates the function of various effector immune cells in the intestine and the production of cytokines through the activation of T-lymphocytes (Sarkar et al, 2010). Furthermore, dopamine levels in the intestinal region have a strong correlation with the composition of the gut microbiome, with strains capable of metabolizing dopamine growing at a higher rate compared to non-metabolizing strains (Strandwitz, 2018). Bacterial degradation of dopamine could therefore influence the availability of this neurotransmitter at the host-microbe interface, with downstream

consequences for both host physiology and microbial ecology. This perspective aligns with the broader concept of microbial endocrinology, wherein host hormones and neurotransmitters are treated not merely as internal signals but also as interkingdom cues that affect microbial gene expression and behavior (Boukerb et al., 2021; Neuman et al., 2015). In this light, HpaB represents a candidate enzyme mediating microbial response to—and manipulation of—host neurochemical states. Moreover, by degrading dopamine, HpaB may indirectly affect host neural signaling. Local depletion of dopamine in the gut, especially in the enteric nervous system, could alter peristalsis, gut permeability, or immune tone. These physiological disruptions could, in turn, feed back into host behavioral phenotypes—a connection supported by emerging research on the microbiome's influence on anxiety, depression, and neurodevelopmental disorders.

While HpaB has not traditionally been considered in the context of host neurotransmitter metabolism, its structural and functional properties make it a plausible candidate for such a role. A hypothetical mechanism for dopamine degradation by HpaB might involve hydroxylation of the catechol ring to form a trihydroxybenzene derivative, which could then enter standard bacterial catechol degradation pathways involving dioxygenases and hydrolases and, subsequently, substrate pooling for the citric acid cycle. These transformations would allow the bacterium to extract carbon and energy from host neurochemicals, while simultaneously altering the neurochemical environment of the intestine. Alternatively, HpaB may not fully catabolize dopamine but rather convert it into modified intermediates that exert their own distinct physiological effects on the host animal. Moreover, HpaB may act on other, similar aspects of dopamine metabolism with supplemental function, such as converting tyrosine directly in L-DOPA in particular environmental or concentration-based conditions (Fig. 2). These phenomena would reflect the high degree of chemical similarity between each of the elements of

this pathway and promiscuity of the enzyme. Such molecules could act as either partial agonists or competitive inhibitors at host dopamine receptors, alter oxidative stress levels in the gut epithelium, or modulate the degree of host immune signaling. In either case, HpaB-mediated biotransformation of catecholamines would represent a novel intersection between bacterial metabolism in the intestinal tract and host neurochemistry.

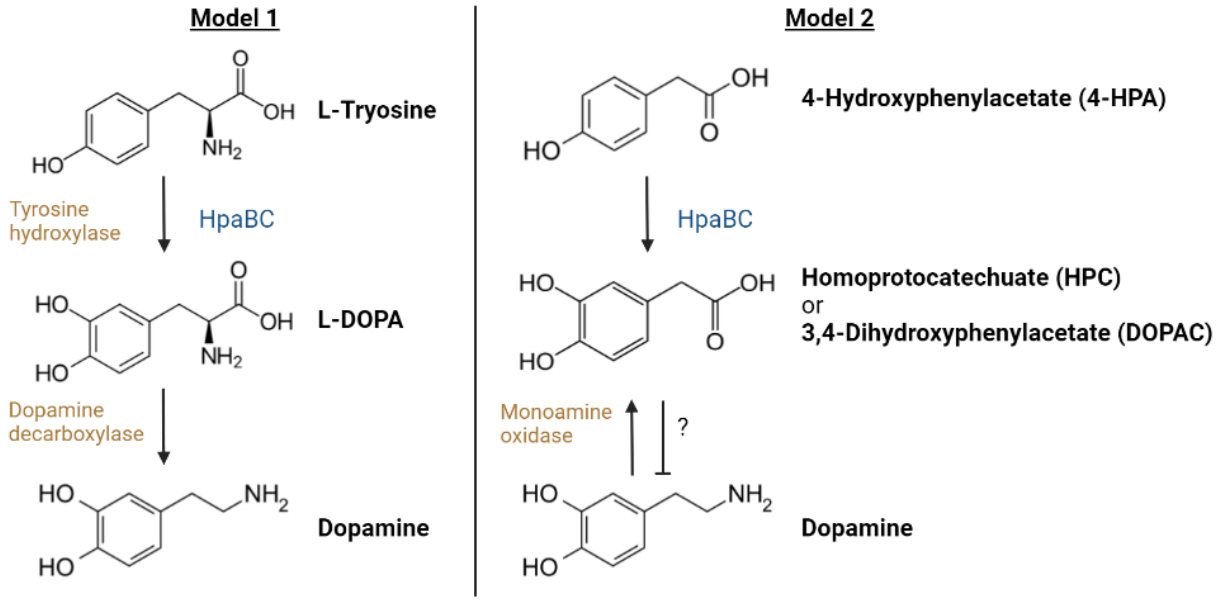

Figure 2. Depiction of two hypothetical mechanisms by which HpaB (within the HpaBC complex written in blue) may interplay with dopamine synthesis and metabolism. This schematic was generated using BioRender (https://www.biorender.com).

Implications for HpaB in Driving Human Health

The human gut microbiome is increasingly viewed not just as a digestive and immune regulator, but as a neuroendocrine organ that produces, transforms, and degrades signaling molecules that influence brain function. Microbial metabolites—ranging from short-chain fatty

acids (SCFAs) to neurotransmitter analogs—can interact with host cells locally or systemically through endocrine, immune, and neural routes (Dalil et al., 2019). Enzymes responsible for these metabolic transformations thus act as pivotal interfaces between microbial activity and host physiology. Among these interactions, the microbial metabolism of catecholamines such as dopamine and norepinephrine is particularly compelling. These neurotransmitters, while classically associated with the central nervous system, also play key roles in gastrointestinal function, including motility, barrier integrity, and mucosal immunity. Microbial enzymes that alter catecholamine concentrations may therefore exert both local and systemic effects, contributing to the broader network of gut-brain communication.

Multiple lines of evidence support the claim that microbial metabolites can affect host neurophysiology. For one, *Lactobacillus* and *Bifidobacterium* species have been shown to produce GABA, which modulates host anxiety and stress responses via the vagus nerve (Braga et al., 2024). Moreover, *Clostridium sporogenes* metabolizes tryptophan into indoles (Wikoff et al., 2009; Williams et al., 2014; Dodd et al., 2017), which cross the blood-brain barrier and influence serotonin signaling (Zhao et al., 2022; Wang et al., 2023). Additionally, an interspecies pathway for the metabolism of levodopa, a precursor to dopamine, in the gut microbiome has been observed between *Enterococcus faecalis* and *Eggerthella lenta*, which *E. faecalis* mediates the conversion of levodopa to dopamine through a pyridoxal phosphate-dependent tyrosine decarboxylase. This dopamine is then transformed into *m*-tyramine by a molybdenum-dependent dehydroxylase expressed by *E. lenta* (Maini Rekdal et al., 2019). These examples underscore a critical point: the neurochemical influence of microbes is often strain-specific and depends on the presence of specialized metabolic enzymes. Given its catalytic role and structural

promiscuity, HpaB may join this growing list of enzymes with the capacity to influence host neurochemistry through the metabolism of catecholamines.

Such microbial influences on behavior through monoamine metabolism have been recorded in other animal model systems. Notably, the nematode *Caenorhabditis elegans* provides an elegant model for studying microbe-induced behavioral change. A landmark example of this model in action involves colonization by *Providencia alcalifaciens*, a Gammaproteobacterium that natively colonizes the nematode intestinal tract and synthesizes tyramine, a monoamine structurally similar to dopamine. Tyramine mimics host signaling molecules, altering *C. elegans*' olfactory preferences in a way that benefits both host and microbe by strengthening food preferences for the nematode (Samuel et al., 2016). Such an example illustrates behavioral manipulation that confers increased fitness, providing an evolutionary argument that favors the promotion of microbial interactions by the host animal (O'Donnell et al., 2020). Intriguingly, search queries through NCBI BLAST indicate that the majority of documented *Providencia* strains encode homologs of *hpaB* with conserved active site residues (Camacho et al., 2009). This raises the possibility that, in addition to synthesizing neuroactive monoamines, *Providencia* and its related taxa may degrade them through aromatic hydroxylation. If so, these microbes might fine-tune host behavior by controlling the balance of neurotransmitter synthesis and degradation.

Although direct evidence for HpaB activity in the human gut microbiome has not been specifically probed, the presence of *hpaB* homologs in closely related, gut-associated genera such as the Gammaproteobacteria *Providencia*, *Morganella*, and *Escherichia* suggests functional conservation of this enzyme across host-associated environments. Many of these genera are found in the mammalian gut and have been implicated in health and disease. For example, *E.*

coli, a ubiquitous gut resident, harbors multiple aromatic degradation pathways, including the *hpa* operon, as mentioned previously. The related genus *Providencia* has been isolated from patients with urinary tract infections and gastrointestinal disorders (Klein et al., 2024). Furthermore, *Morganella* strains, particularly *M. morganii*, have been detected in the gut microbiota of individuals with inflammatory and neurodevelopmental conditions (Bang et al., 2025). These neurodevelopmental conditions often present comorbidly with inflammatory disease in the gut, suggesting a future avenue for investigation of a potential mediary role of *Morganella* between these ailments.

Of particular interest is the correlation between microbial taxa that are known to express *hpaB* and neurological conditions such as autism spectrum disorder (ASD), irritable bowel syndrome (IBS), and Parkinson's disease. Each of these conditions are often associated with both dopamine dysregulation and gut microbial imbalances, suggesting that microbial modulation of dopamine levels—potentially through HpaB or similar enzymes—could play a role in disease etiology or symptomatology (Taniya et al., 2022; Kwon et al., 2024; Cheng et al., 2024; Barandouzi et al., 2022). If validated experimentally, HpaB and its homologs may represent a novel class of microbial enzymes that influence host behavior and brain function via catecholamine metabolism. This opens several avenues for biomedical research, namely in the context of expanding diagnostic procedures on the functional makeup of the gut microbiome. The presence or activity of *hpaB* homologs in stool samples could serve as biomarkers for microbiome-associated neurological disorders, just as options for diagnostic measures are being explored with other bacterial products through single-protein detection for microbes associated with disorders such as inflammatory bowel disease and whole-microbiome sequencing (Lloyd et al., 2024; Schmartz et al., 2024). Conversely, from a therapeutic context, directly modulating

HpaB activity through probiotics, small-molecule inhibitors, or engineered microbes could offer new strategies to influence host neurochemistry. Such intervention through intentional shifts of the gut microbiome would require intensive longitudinal trials with participants across numerous backgrounds to support the efficacy and safety of bacterial protein induction within individuals. Lastly, speaking in ecological terms, understanding how microbial catecholamine metabolism shapes microbial competition, quorum sensing, or host preference could illuminate broader principles of host-microbe coevolution and the degree by which microbiome composition and activity drives host fitness (Gould et al., 2018). In sum, the functional repertoire of enzymes like HpaB extends beyond classical, environmental metabolism, positioning these molecules as potential modulators of host neurobiology across animal-microbe systems.

Open Questions and Experimental Strategies

Although computational modeling, structural prediction, and docking simulations provide compelling support for HpaB's potential role in dopamine metabolism, *in silico* findings require empirical validation. Central to confirming the physiological relevance of this enzyme is the question of whether dopamine and its primary metabolite, DOPAC, are true catalytic substrates for HpaB or merely exhibit high-affinity, non-productive binding. Addressing this qualification would require *in vitro* biochemical assays, likely performed on recombinant HpaB. To this end, HpaB from animal host-associated strains can be heterologously expressed and purified within *E. coli*, and its activity assayed against dopamine, DOPAC, the canonical substrate 4-HPA, and any number of other candidate aromatic substrates. Enzyme kinetics may be quantified via colorimetric or LC-MS-based assays, and the formation of hydroxylated or otherwise modified products can be verified through high-resolution mass spectrometry or NMR spectroscopy, as

defined by protocols already established to molecularly probe the gut microbiome (Jiang et al., 2024; Yasuda et al., 2020). Importantly, confirming the necessity of flavin cofactors such as $FMNH_2$ or $FADH_2$—typically regenerated by the partner subunit HpaC—would help establish whether dopamine metabolism proceeds via the same redox cycle as 4-HPA hydroxylation. Beyond *in vitro* characterization, determining whether HpaB activity influences host physiology and behavior requires *in vivo* experimentation. Germ-free animal models such as *Caenorhabditis elegans*, *Drosophila melanogaster*, or mice can be selectively colonized with wild-type bacteria expressing HpaB, isogenic Δ*hpaB* mutants, or complemented strains with inducible HpaB expression. These models offer opportunities to assess behavioral phenotypes tied to dopaminergic signaling, such as chemotaxis, egg-laying, and basal slowing response in nematode models as previously developed, or anxiety-like behaviors and gut motility in mice (Ezcurra et al., 2011; Weinshenker et al., 1995; Pribadi et al., 2023; Sawin et al., 2000;). Measuring neurotransmitter levels in host tissues via LC-MS, in parallel with the deployment of existing transcriptional analyses of host dopamine pathway genes, would provide strong evidence for a causal link between microbial HpaB activity and host neurochemical balance (Fiorenzano et al, 2024). To uncover the mechanistic basis of HpaB's substrate scope, structural biology and protein engineering will be essential. Although AlphaFold and Phyre2 models currently provide confident predictions of the crystal structure of HpaB across many strains of Gammaproteobactera, empirical structures obtained through X-ray crystallography or cryo-EM—especially in complex with dopamine, DOPAC, or 4-HPA—would clarify substrate binding modes and catalytic geometry (Jumper et al., 2021; Kelley et al., 2010). Site-directed mutagenesis of key active site residues, guided by these structures and supported by molecular dynamics simulations, can test how specific amino acids contribute to substrate selectivity and

catalytic efficiency and how that efficiency subsequently drives host phenotype. This technique was previously performed *in vivo* on both the genes encoding β-lactamase and curli in *E. coli* colonizing a murine model, by which CRISPR-Cas9 base editing allowed for manipulation of gene expression within the gut microbiome (Brödel et al., 2024). Such studies would not only elucidate the enzyme's potential role in catecholamine metabolism but also define the structural determinants of flavin monooxygenase promiscuity more broadly.

Another key issue is determining when and where *hpaB* is expressed in microbial cells, particularly under conditions mimicking the host gut. Expression analysis using RT-qPCR or RNA-Seq, as applied on other gut microbiome studies, can assess *hpaB* transcript levels in response to gut-like environmental cues, including exposure to host neurochemicals, bile salts, or oxidative stress (Ostan et al., 2015; Nyangale et al., 2014). Additionally, the curation of enteric strains with target protein-GFP promoter fusions has been routinely employed as a technique for identifying the localization of proteins expressed by gut microbiota (Teh et al., 2016; Scott et al., 2000). Constructing *hpaB*-GFP promoter fusions would allow live imaging of gene expression in bacteria colonizing host tissues. Considering that GFP functions optimally only in aerobic conditions, alternative methods would be required for fluorescent scanning of *hpaB* expression in anaerobic environments, such as through derivatives of bilin-binding fluorescent proteins (Chia et al., 2020). Further dissection of regulatory elements, including the roles of upstream genes like *hpaA* and stress-responsive regulators such as *rpoS*, may reveal whether HpaB expression is induced during host colonization, thus supporting its ecological and functional relevance *in vivo*.

Finally, the relevance of HpaB to human health hinges on its presence and expression in the human gut microbiome. While the gene encoding HpaB has been documented in bacterial genomes canonically associated with human gut microbiota (e.g. *E. coli* and *A. baumanii*), its

expression must be experimentally validated. Functional metagenomic datasets from large human cohorts (e.g., HMP, MGnify) can be mined for *hpaB* homologs using BLAST or hidden Markov models, a method already performed for the detection of viral proteins and dietary fiber catabolic enzymes, among many other examples (Richardson et al., 2023; Barrientos-Somarribas et al., 2018; Tasse et al., 2010). Phylogenetic analysis of these sequences would identify taxonomic distribution, while metatranscriptomic data could reveal whether *hpaB* is actively expressed *in situ*. Critically, the abundance or expression of *hpaB* could then be correlated with host clinical metadata—such as diagnoses of autism spectrum disorder, Parkinson's disease, or irritable bowel syndrome—conditions frequently associated with both dopamine imbalance and microbial dysbiosis (Taniya et al., 2022; Kwon et al., 2024; Cheng et al., 2024; Barandouzi et al., 2022). This integrative approach would provide the strongest evidence to date that HpaB functions at the intersection of bacterial metabolism and host neurochemistry, with implications for diagnostics, microbiome-targeted therapeutics, and elevating a broader understanding of microbial contributions to the gut-brain axis.

Conclusion

Microbial influence on host neurobiology is no longer a speculative concept, but an increasingly well-supported paradigm. The gut-brain axis, once thought to be regulated solely by host-driven endocrine and immune pathways, is now recognized as a bidirectional communication network in which microbial enzymes and metabolites play crucial roles. Within this framework, the work presented in this thesis provides strong evidence for considering HpaB—a bacterial aromatic monooxygenase—as a novel modulator of neurotransmitter metabolism and, by extension, a potential regulator of host neural signaling. Traditionally, HpaB

has been studied for its role in the homoprotocatechuate pathway, catalyzing the hydroxylation of 4-hydroxyphenylacetate (4-HPA) into catecholic intermediates. However, findings presented within this review expand this perspective, suggesting that HpaB possesses the structural features, substrate affinity, and taxonomic conservation necessary to interact with host-derived catecholamines—most notably, dopamine and its metabolite DOPAC.

Importantly, this work proposes a new mechanistic framework for microbial contributions to the gut-brain axis: aromatic-degrading enzymes, originally evolved for environmental metabolism, may be repurposed to engage host neurochemistry in ways that affect development and behavior. This insight carries broad implications for human health, especially in the context of microbiome-associated conditions like Parkinson's disease, autism spectrum disorder, and mood disorders, where dopamine dysregulation and microbial imbalance routinely demonstrate comorbidity. Future studies proposed within this article—including biochemical validation, *in vivo* host assays, structural analysis, and ecological surveys—will be necessary to confirm the full extent of the role of HpaB in host-microbe communication. If substantiated experimentally, HpaB may emerge as a targetable enzyme for microbiome engineering, diagnostics, or therapeutic modulation of host catecholamine balance for treatment of neuropsychiatric and gastrointestinal disease. In sum, this body seeks to advance a novel perspective on microbial enzymatic participation in neurochemical signaling, positioning HpaB as a compelling candidate at the frontier of microbial endocrinology and gut-brain axis research.

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