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Functional Interplay between Dopaminergic and Serotonergic Neuronal Systems during Development and Adulthood

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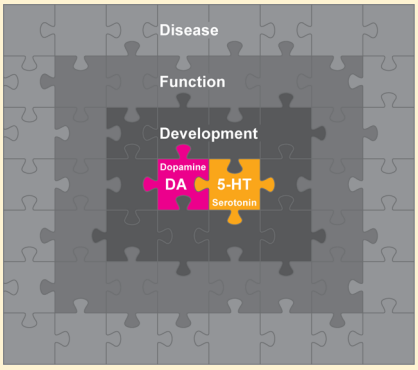
ABSTRACT: The complex integration of neurotransmitter signals in the nervous system contributes to the shaping of behavioral and emotional constitutions throughout development. Imbalance among these signals may result in pathological behaviors and psychiatric illnesses. Therefore, a better understanding of the interplay between neurotransmitter systems holds potential to facilitate therapeutic development. Of particular clinical interest are the dopaminergic and serotonergic systems, as both modulate a broad array of behaviors and emotions and have been implicated in a wide range of affective disorders. Here we review evidence speaking to an interaction between the dopaminergic and serotonergic neuronal systems across development. We highlight data stemming from developmental, functional, and clinical studies, reflecting the importance of this transmonoaminergic interplay.



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INTRODUCTION

Intermonoaminergic Function Is Crucial for the Development of Behavioral and Emotional Neuronal Processing Centers. Throughout history, neuroscientists and philosophers alike have sliced the mammalian brain, both literally and figuratively, along multiple axes. Neurons have been classified anatomically, chemically, electrophysiologically, and hodologically. Of particular interest have been the monoaminergic neurotransmitter systems, serotonin (5-HT), dopamine (DA), and norepinephrine (NE), which have long been viewed as the custodians of neuropsychiatric disease, playing key roles in modulating behavioral output. Until recently, most research has been aimed at understanding the function of these neurotransmitter systems independently. However, a growing body of evidence supports the notion that to fully comprehend the brain, we must understand the way that various neural systems *interact*.

Interaction among monoaminergic neuronal systems, both during perinatal development as well as in the adult organism, generates a spectrum of behavioral phenotypes which, at their polar extremes, extend into the realm of psychiatric illness. This Review focuses on the interaction between the DA and 5-HT systems, given the robust evidence for anatomical and functional overlap. The aim is to integrate existing data that speak to and demonstrate a functional interplay between the DA and 5-HT neuronal networks in the context of organismal behavior. We first review the architecture of each system in the adult rodent brain, noting the proximal nature of their localization and their overlapping projections. We then turn to the development of these systems, highlighting features shared by both during embryonic development, suggestive of cross-talk. This latter section lays the groundwork for sections that follow, with focus on the documented functional interplay between 5-HT and DA neurons during perinatal and adult phases as revealed through pharmacological, genetic, and clinical studies. We end with a discussion of the role to be played by modern genetic tools in the mouse, which have the potential to further delineate these trans-system interactions and the role that they might play in future treatment design.

ORGANIZATION OF THE MIDBRAIN DA (mDA) AND 5-HT NEURONAL SYSTEMS IN THE ADULT BRAIN DEMONSTRATES ANATOMY CONDUCIVE TO FUNCTIONAL INTERPLAY

Both the DA and 5-HT systems have documented involvement in a wide range of behaviors and physiology, extending from cognition to autonomic functions. This functional multiplicity can be understood in part when considered in the context of the associated anatomy: A sparing number of anatomically concentrated neurons constitute each system, while displaying extensive influence throughout the brain via vast axonal projections.

mDA and 5-HT Neuronal Nuclei Localize to the Brainstem. DA and 5-HT are separately produced by a relatively small number of neurons in the brain. The adult mouse brain contains about 30 000−35 000 DA neurons, of which about 75% are located in the ventral midbrain.[1,2] In this Review, we will focus on these midbrain DA (mDA) neurons since they are the best studied in terms of their importance for behavioral and emotional control. In the adult, mDA neurons

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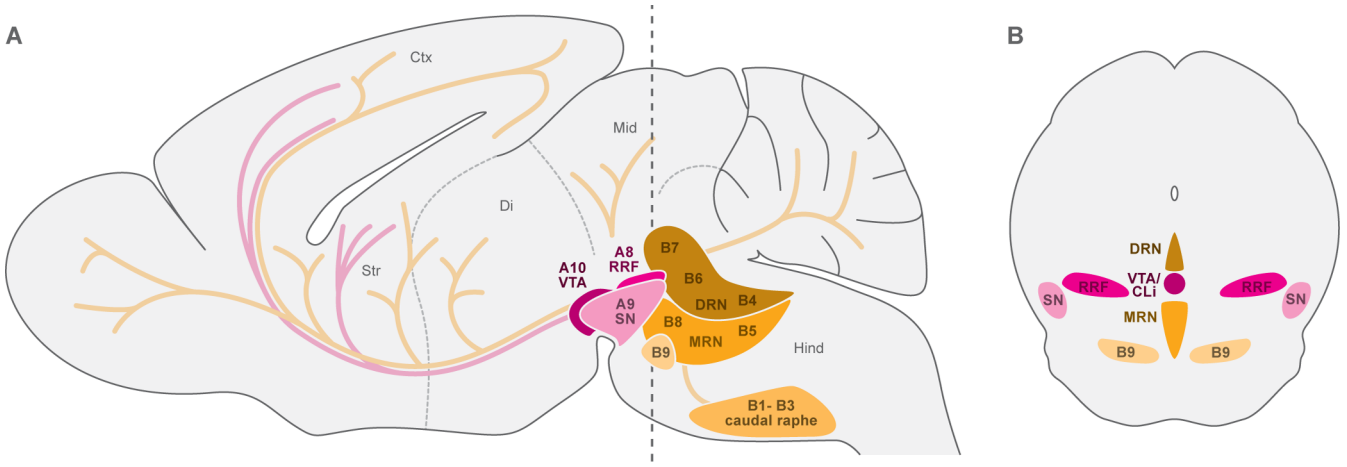

Figure 1. mDA and rostral 5-HT raphe nuclei are anatomically proximal. (A) Sagittal schematic shows the adult mouse brain, in a side view, with mDA nuclei and projections (magenta shades) and 5-HT nuclei and projections (orange shades) highlighted. It can be observed that the mDA and rostral 5-HT nuclei overlap near the midbrain–hinbrain boundary. VTA, ventral tegmental area (purple); RRF, retrorubal field (magenta); SN, substantia nigra (soft pink); DRN, dorsal raphe nucleus (brown); MRN, median raphe nucleus (orange); caudal raphe, raphe magnus (B3), raphe pallidus (B1), raphe obscurus (B2) (yellow). (B) Coronal schematic (dashed line in A) depicts the localization of the mDA and rostral 5-HT nuclei near the midbrain–hindbrain boundary. CLi, caudal linear nucleus (caudal VTA); Tel, telencephalon; Di, diencephalon; Mid, midbrain; Hind, hindbrain; Ctx, cortex; Str, striatum.

cluster in three anatomical nuclei, the ventral tegmental area (VTA; A10), the substantia nigra (SN, A9), and the oftentimes neglected retrorubral field (RRF; A8), all of which are located in close proximity to each other in the ventral midbrain (Figure 1). 5-HT neurons are similarly few in number, amounting to ~25 000 neurons in the adult mouse brain.[3] They are located in close proximity to mDA neurons in a region spanning the caudal midbrain through the hindbrain and forming a rostral cluster, including the dorsal raphe nucleus (DRN; B4, B6, B7) and the median raphe nucleus (MRN; B5, B8, B9), and a caudal cluster (B1–3) (Figure 1).[4] Indeed, immunohistochemical visualization of the two neurotransmitter populations reveals that the caudal extension of the VTA interweaves with the rostral region of the DRN.[5]

Though their numbers are small, their reach, both efferent and afferent, is astoundingly wide. Both the mDA and 5-HT neural systems receive inputs from many brain areas, which they integrate and relay to numerous brain areas involved in emotion, cognition, and motor behavior through elaborate axonal projections. This cytoarchitectural feat creates centers of information integration with the capacity to influence the activity of a myriad categories of neural function, rendering these populations as key neuromodulatory units and therapeutic targets.

mDA and 5-HT Efferent Projections Target Common Forebrain Regions. The signals carried into mDA and 5-HT neurons are relayed to downstream target cell populations via widely projecting axonal processes. mDA neurons most prominently innervate the striatum and cortex. In rodents, dense cortical DA innervation is observed in the prefrontal cortex (PFC), perirhinal cortex, and cingulate cortex. Widespread but sparser innervation can be found in other cortical regions.[6] In addition to the striatum and cortex, other target regions include the hippocampus, lateral habenula, amygdala, and septum.[6] These projections are largely categorized into three pathways, the mesostriatal, also called nigrostriatal, mesolimbic, and mesocortical pathways, each modifying an associated set of behaviors. The prevailing view is that the mesostriatal pathway is formed by SN compacta (SNc) DA neurons projecting to the dorsal striatum and is critical for voluntary motor control. SNc DA neurons have been the subject of intense study since their selective degeneration is the cause for the motor defects observed in patients suffering from Parkinson's disease.[7,8] By contrast, DA neurons of the VTA form the mesolimbic and mesocortical pathways, by projecting to the ventral striatum (nucleus accumbens), and cortical regions, respectively. Their function is to modulate cognition-, emotion-, and motivation-related behaviors.

In contrast to this circumscribed number of identifiable mDA pathways, 5-HT neurons project more broadly and diffusely, and consequently seem to follow less defined pathways. Accordingly, serotonin modulates a wide range of physiological functions and behaviors, including respiration, thermoregulation, aggression, and anxiety.[9] While the common notion persists that the majority of 5-HT neurons in the rostral neuron cluster give rise to ascending axonal projections to the midbrain and forebrain, whereas caudal raphe nuclei send mostly descending axons within the brainstem and toward the spinal cord, there is substantial evidence that both clusters project broadly throughout the rostral and caudal brain.[10−12] This widespread innervation pattern is reinforced by intense collateralization of 5-HT neurons, allowing an individual 5-HT neuron to innervate multiple target regions, including other 5-HT raphe nuclei.[11,13−15] As a result, all brain regions innervated by DA neurons are also occupied by 5-HT projections; although the within-region target cell specificity remains largely uncharted.

Shared Afferent Projections Innervate mDA and 5-HT Nuclei. Both mDA and 5-HT neurons receive input from neural regions distributed throughout the brain, conveying information about a variety of different functional processes. Notably, tracing and immunohistochemical studies demonstrate that many of the afferent inputs into select mDA and 5-HT nuclei originate from the same neural regions: Recent monosynaptic viral tracing studies revealed that VTA DA neurons receive inputs from a remarkably similar set of brain regions as DRN 5-HT neurons,[16,17] highlighting the potential coregulation of both neurotransmitter systems and accounting

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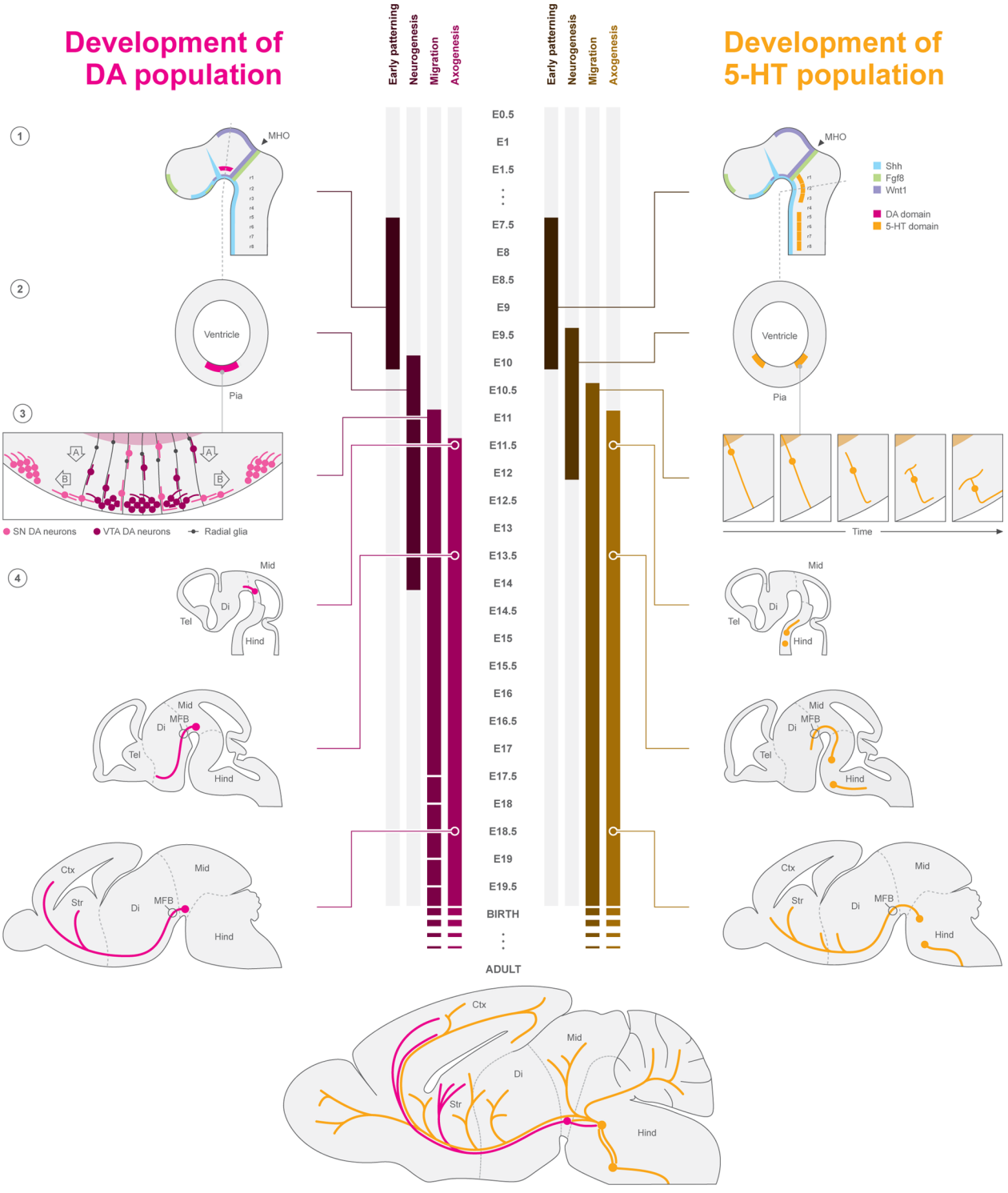

Figure 2. Parallel development of the mDA and 5-HT neurons results in two proximally located populations with shared projection targets. Figure plots development of the mDA (magenta, left) and 5-HT (orange, right) populations throughout embryonic development in the mouse. (1) Shown in a sagittal view of the embryonic brain are the regions in which mDA neurons and 5-HT neurons are to be generated: the mDA progenitor domain resides rostral and the 5-HT domain caudal to the midbrain-hindbrain organizer (MHO). (2) As seen in cross section, mDA progenitors are generated from FP cells in the developing midbrain, whereas 5-HT progenitors are located in two domains on either side of the FP. (3) Once postmitotic, both mDA and 5-HT precursors migrate ventrally, either along radial glia cells or via somatranslocation. Following their initial ventral migration (A), mDA neurons destined to form the SNc undergo a subsequent lateral migration (B). Similarly, following ventral migration, those 5-HT neurons constituting raphe nuclei situated medially move inward toward the midline, resulting in fusion of what were once separate lateral populations (not shown). (4) Although mDA and 5-HT axons begin to appear at approximately the same time (E11.5), mDA projections reach forebrain targets earlier. Both populations continue to extend axons beyond embryonic development and well into the postnatal period. The result is an intricate array of neural circuits with a considerable degree of overlap in their reach. Events with an undefined end point or that persist postnatally are indicated by disrupted bars. Tel, telencephalon; Di, diencephalon; Mid, midbrain; Hind, hindbrain; Ctx, cortex; Str, striatum; MFB, medial forebrain bundle.

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for the considerable overlap in affective and cognitive functions influenced by each system. Furthermore, there exist extensive interconnections between mDA nuclei (VTA, SN) and rostral raphe nuclei − DRN and MRN.[16−26] Some of these connections are monosynaptic/direct, some indirect via GABAergic interneurons.

The anatomical proximity and common projection targets of the mDA and 5-HT neurotransmitter systems in the adult brain positions them to impact similar physiological and behavioral processes. Indeed, the two systems are known to modulate many of the same, yet very diverse, behaviors and processes, such as aggression, reward processing, and locomotion. As we shall see, it is becoming increasingly apparent that the comodulation of these processes involves, to some extent, an interaction between mDA and 5-HT neurons. In order to understand the potential for such cross-talk, we must consider the developmental processes by which the adult monoaminergic circuits are constructed (Figure 2). The following section details the parallel development of these two neuronal systems, revealing that they are in the right place at the right time to interact during a developmentally dynamic period.

■ mDA AND 5-HT NEURONS DEVELOP IN PARALLEL AND IN CLOSE APPOSITION

In this section, we describe and contrast the developmental processes of the mDA and 5-HT neurons on a molecular, mechanistic and temporal level. We will largely focus on prenatal events including early ventral patterning, neurogenesis, migration, and axogenesis (Figure 2).

Early Ventral Patterning Events Influence the Generation and Localization of Both mDA and 5-HT Neurons. The development and localization of mDA and 5-HT neurons are controlled by a shared set of anatomical structures and the signaling molecules they secrete, though these neurons are generated in separate parts of the developing neural tube, namely the ventral mid- and hindbrain regions, respectively. The specification of these two regions relies on appropriate patterning along the anterior-posterior and dorsoventral axis, which is controlled by signals coming from three key structures in the early embryo: the floor plate, the notochord and the isthmus organizer. The floor plate (FP), present along the length of the developing neural tube, and the notochord, axial mesodermal cells located underneath the neural tube, secrete Sonic hedgehog (Shh) starting at embryonic day (E) 8.5 or 1 day earlier, respectively, in the mouse (Figure 2.1).[27−29] Shh has been shown to be crucial for the determination of ventral characteristics in the developing brain and spinal cord. This ventralizing activity of Shh affects the generation of mDA and 5-HT neurons. Overexpression of a constitutively active form of the Shh receptor, Smoothened, exhibited large numbers of ectopically located DA and 5-HT neurons in the dorsal midbrain and dorsal hindbrain, respectively, in addition to the normal ventral location of these two neuronal systems.[30] Conversely, absence of Shh results in a lack of mDA and 5HT neurons.[31]

The isthmus organizer, also called the midbrain−hindbrain organizer (MHO), lies between the midbrain and the hindbrain and is critical for the development of these two brain regions.[32,33] mDA neurons are specified rostral to the MHO, and 5-HT neurons, caudal (Figure 2.1). The two key molecules in establishing the MHO are the homeodomain transcription factors Otx2 and Gbx2. Otx2 is selectively expressed rostral to the MHO in the fore- and midbrain,[34,35] while Gbx2 is expressed caudal to the MHO, in the anterior hindbrain.[36] Gain-of-function studies demonstrated that the correct positioning of the MHO at the midbrain-hindbrain boundary is determined by the mutual repression of these two genes.[37−39]

Once the Otx2/Gbx2 boundary is created, a number of other factors, secreted or transcription-related, start to be expressed at the MHO, such as fibroblast growth factor 8 (*Fgf8*), Engrailed1 (*En1*), Engrailed2 (*En2*), *Wnt1*, *Lmx1b*, *Pax2*, and *Pax5*. Most have been identified to play multiple roles in patterning of the midbrain/hindbrain territory and/or the development of mDA and 5-HT neurons.[33,40,41]

The accurate positioning of the MHO critically impacts the development of mDA and 5-HT neurons. This was highlighted by work from the Wurst lab, which showed that moving the Otx2 expression boundary caudally by ectopic expression of Otx2 in the rostral hindbrain not only results in a caudal shift of the MHO and its associated genes, but also caused a caudal expansion of the mDA neuronal population at expense of the rostral hindbrain 5-HT cell group.[37,42] Notably, in these mutant mice, the mDA and 5-HT populations are still separated by the MHO, and these alterations were maintained through adulthood. Conversely, mutants in which Otx2 expression and the MHO were shifted rostrally, the mDA and 5-HT neuron domains shifted with it and resulted in an increase in rostral 5-HT neurons and a reduction of mDA neurons. These results highlight the critical importance of MHO positioning for normal mDA and 5-HT neuron generation. They furthermore show that, at this early developmental stage, the territory producing mDA and 5HT neurons is still plastic and that both populations are affected in a complementary manner by changes in the MHO position.

Contrasting Mechanisms, But Coincident Timing, of mDA and 5-HT Neural Progenitor Specification, Migration, and Differentiation. Once the appropriate patterning of the midbrain−hindbrain region has occurred, developmental programs involving a coordinated sequence of gene expression establish the identity of mid- and hindbrain neural progenitors that ultimately generate mDA and 5-HT neurons, respectively. Though identity of the relevant signaling molecules only partially overlaps between the two monoaminergic populations, the timing of these developmental phenomena coincides, such that mDA and 5-HT neurons emerge in parallel.

Coordinated expression of Shh from the FP and Fgf8 from the MHO are able to induce fore- and midbrain DA neurons as well as 5-HT neurons of the rostral cluster (B4−9) in vitro.[43] Specifically, culturing of ventral midbrain explants of rat E9 embryos in the presence of Shh and Fgf8 led to the induction of DA neurons. Similarly, culturing the rostral portion of the ventral hindbrain in the presence of these two factors resulted in the generation of 5-HT neurons (rostral cluster B4−9). By contrast, the induction of the caudal cluster of 5-HT neurons (B1−3) in caudal hindbrain explants was less reliant on Fgf8, but instead depended on Fgf4, which is secreted earlier in development by the primitive streak juxtaposed to the posterior neural plate, and might in so doing prepattern the hindbrain neuronal tissue. Thus, induction of the caudal 5-HT neurons seems to be controlled by the combined activities of Fgf4 and Shh, with the former expressed prior to the latter.[43] These findings show that the same set of factors can induce a mDA or a rostral 5-HT phenotype in differentially patterned tissues, which originate just rostrally or caudally to the MHO.

mDA progenitors are generated from Shh expressing FP cells in developing midbrain starting around E10 in mouse (Figure

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2.2).[44−46] Unlike the hindbrain FP, the midbrain FP has been found to be highly neurogenic and to generate several distinct midbrain neuron populations.[46−48] This difference in neurogenic capacity of the FP can, in part, be ascribed to a dynamic interplay between differential canonical Wnt/ß-catenin and Shh signaling in these regions. In wildtype embryos, Wnt1 and other Wnts are expressed in the ventral midbrain at the beginning of mDA neurogenesis, whereas Shh levels begin to be downregulated in this region at this time. By contrast, the hindbrain FP is characterized by extremely low expression levels of canonical Wnt ligands and subsequent ß-catenin signaling, but strong Shh expression. Conditional loss of ß-catenin function diminished midbrain FP neurogenic capacity resulting in fewer mDA neurons.[47,49] Conversely, ß-catenin gain-of-function in the hindbrain FP resulted in molecular similarity to the normal midbrain FP: mDA progenitor genes are induced, and Shh is downregulated throughout the rostrocaudal axis of the hindbrain-generated progenitors that resemble the midbrain FP.[47,50] Interestingly, the differential potential of mDA progenitor cells to generate VTA or SN mDA neurons was shown by using genetic inducible fate mapping studies to be associated with the spatiotemporally dynamic expression of Shh.[51,52] Thus, the neurogenic potential of the midbrain FP and the induction of mDA neurons are largely attributable to appropriately balanced Wnt and Shh signaling from the FP, different regions of which preferentially produces VTA or SN neurons in a temporally controlled fashion.

In contrast to mDA neurons, 5-HT neurons are born caudal to the MHO in two longitudinal domains on either side of the FP (Figure 2.2). During embryogenesis, the hindbrain is transiently compartmentalized into rhombomeres (r). r1, r2, and r3 produce the rostral cluster of raphe neurons, while rhombomeres 5 and beyond give rise to the caudal raphe cluster. Here we reference the most caudal rhombomere as r8, noting that some continue subdividing, resulting in r9, r10, and r11 (Allen Developing Mouse Brain Atlas, http://developingmouse.brain-map.org/).[53] The first progenitors competent to produce 5-HT neurons appear in r1, from about E9.5 to E10.5.[54] In r2, r3, and r5−8, this competency begins 1 day later.[55] Intersectional genetic fate mapping studies have revealed the link between rhombomeric origin of 5-HT neurons and their final anatomical location in the adult.[56] The dorsal raphe nucleus (B4, B6, B7) is solely composed of r1-derived 5HT neurons. The median raphe nucleus (B5, B8, B9) constitutes a combination of r1-, r2-, and r3-derived 5-HT neurons, whereas more caudal rhombomeres (r5 and caudal) contribute to the medullary raphe nuclei (B1−3).

Once neural progenitors of the midbrain FP are specified to produce DA phenotypes, these mDA neural progenitors give rise to postmitotic mDA progeny between E10-E14 in mice (E12−E16 in rats).[57−59] The induction of tyrosine hydroxylase (TH; the rate limiting enzyme in DA production) expression is the first sign of DA neuronal phenotype acquisition, and occurs shortly after the final mitosis of mDA neural progenitors, beginning at E11.5 in the mouse, while they are actively migrating to their final positions.[60,61] The correct specification and hence onset of DA-type genes, such as TH, requires expression of several transcription factors, which include Lmx1b, Nurr1 and Pitx3.[41,59,62] The process of migration of mDA neurons from the FP ventricular zone to the future VTA and SNc involves two steps (Figure 2.3): All DA neurons initially migrate ventrally along radial glial processes toward the pial surface (Figure 2.3A). Once they have reached the basal part of the ventral midbrain, neurons that will ultimately constitute the SN migrate tangentially away from the midline resulting in the separation of SN and VTA (Figure 2.3B).[51,60,63−65]

The generation of postmitotic 5-HT precursors, their ventral migration and further differentiation is remarkably synchronous with mDA neuron development. Similar to mDA neurons, shortly after 5-HT progenitors have been specified, they undergo final mitosis and start migrating away from the ventricular zone, where they were born. It is during the initial migration event away from the ventricular zone toward the ventral surface that 5-HT postmitotic neuronal precursors start to express 5-HT type genes, such as tryptophan hydroxylase (Tph2), the rate-limiting enzyme in 5-HT production. 5-HT is first detected at E10.5 in the mouse.[66] The onset of 5-HT genes depends on several transcription factors, such as Pet1, which is the only known transcription factor present exclusively in 5-HT neurons, and Lmx1b, which is expressed more broadly in the developing brain and is also crucial for the development of mDA neurons.[67−73]

In contrast to mDA neurons, 5-HT neurons travel by soma translocation mediated partially by intracellular dynamin activity, rather than by radial glia-guided migration from E10.5 until at least E13.5 in mice (Figure 2.3).[66] This movement of 5-HT neurons occurs sequentially according to their date of birth: The oldest, and hence earliest, migrating neurons will be the first to reach and remain closest to the pial surface. Later born neurons migrate after the older neurons, and thus ultimately reside in a position more distant from the pial surface. The effect is a piling up of newer born neurons on top of older neurons.[66] During a subsequent secondary migration occurring in a rostral-born to caudal-born gradient from E17.5 through P6 in rat, 5-HT neurons destined to populate 6 of the 9 B-nuclei migrate toward the midline resulting in a medial fusion of what were previously separate lateral nuclei.[74]

Thus, although distinct factors affect the development of each population, mDA and 5-HT postmitotic precursors are generated and subsequent migration occurs in a temporally parallel fashion. As we describe in the next section, this side-by-side development of mDA and 5-HT neurons continues to be observed during their extension of axonal projections and innervation of target regions.

mDA and 5-HT Axogenesis Initiates during Embryonic Development, and Continues Well into Postnatal Development. To become a functional component of neuronal circuitry, mDA and 5HT neurons must innervate downstream target regions. While most detailed studies of developing mDA and 5-HT axonal pathways have been performed in the rat, several reports confirmed a similar timeline for the development of mouse mDA and 5-HT projections. Here we summarize the ontogeny of rat mDA and 5-HT axonal projections with an attempt to refer to the corresponding mouse time points whenever possible.

There are three main steps in mDA axonal pathfinding during rat and mouse embryogenesis (Figure 2.4).[75] First, at E13 in rat and E11.5 in mouse, mDA neurons start to extend axons dorsally, which then turn rostrally to take a ventrorostral course through the diencephalon toward the telencephalon.[62,76−80] Second, these axonal processes grow longitudinally through the midbrain and the diencephalon to form the medial forebrain bundles (MFB), which run along the ventrolateral

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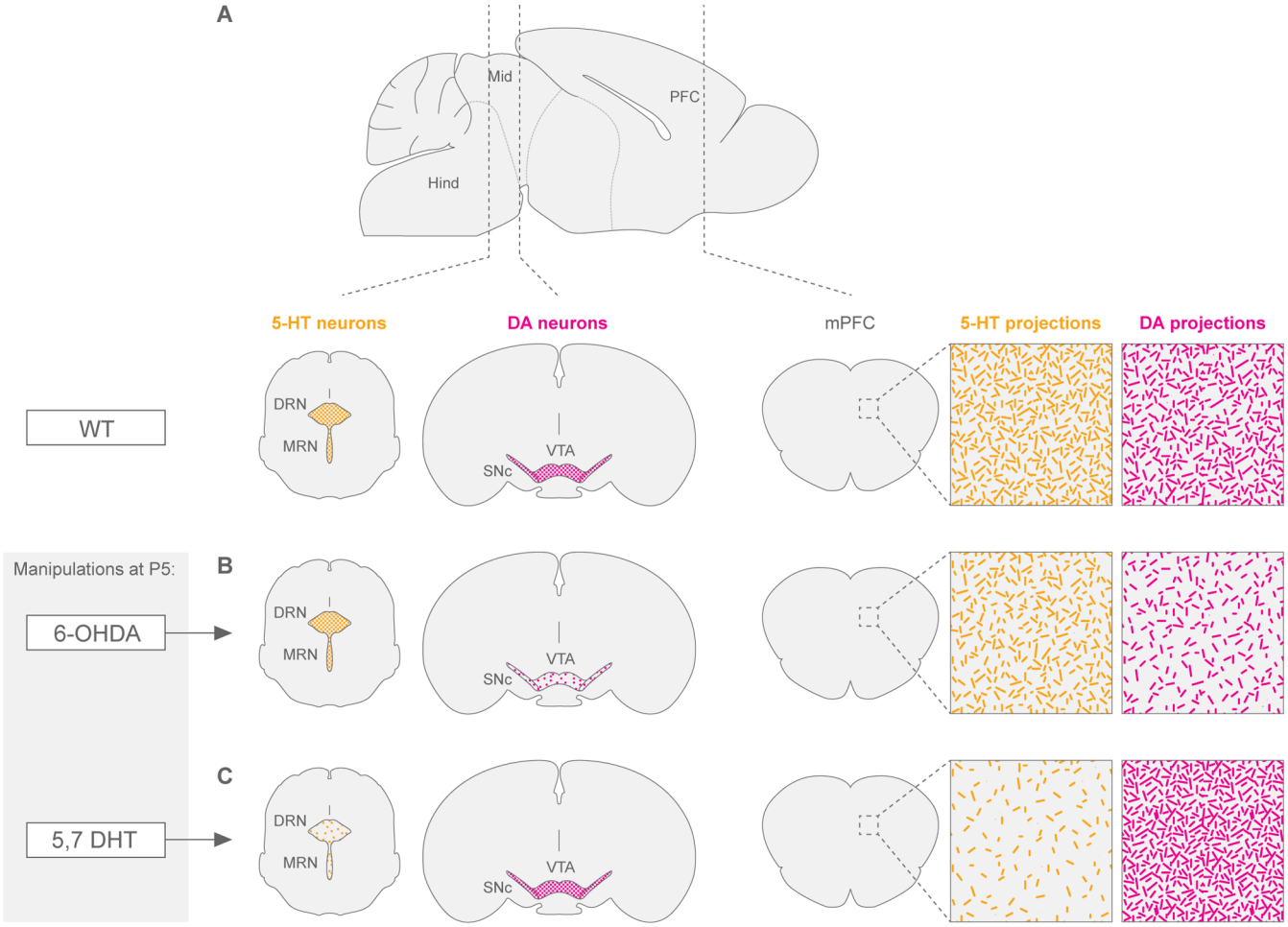

Figure 3. Early postnatal mDA and 5-HT neuronal ablation studies reveal bidirectional regulation of axonal development in the mPFC. (A) Sagittal schematic illustrates where cross sections are taken from the hindbrain, midbrain, and prefrontal cortex. Coronal schematics on the left illustrate wildtype 5-HT (orange) and mDA (magenta) cell bodies in the hindbrain and midbrain, respectively, while those on the right illustrate the 5-HT and mDA innervation patterns within the medial prefrontal cortex (mPFC). (B) Ablation of mDA neurons via 6-OHDA administration at P5 reduces the number of surviving mDA neurons, leaving 5-HT neurons in the raphe nuclei intact. 6-OHDA treatment diminishes mDA as well as 5-HT innervation of the mPFC. (C) Ablation of 5-HT neurons via 5,7-dihydroxytryptamine (5,7-DHT) at P5 reduces the number of surviving 5-HT neurons, leaving the mDA neurons in the SNc and VTA intact. 5,7-DHT treatment reduces 5-HT yet enhances mDA innervation of the mPFC.

part of the telencephalon to different forebrain regions such as the striatum and cerebral cortex. They reach the ventral telencephalon at E14 in rat and mouse. Third, from here onward, mDA axons start to innervate their striatal, limbic and neocortical terminal target regions, a process that continues postnatally, with cortical mDA projection density continuing to increase until P60,[81] lending opportunities for postnatal events to modify the ongoing innervation. mDA axons appear to be guided by classical axon guidance molecules, including Netrin-1, Slits, Semaphorins, Wnts, and Ephrins, to reach their forebrain targets.[6]

Approximately half a day before the first mDA projections are observed, 5-HT neurons start extending axons toward their targets (Figure 2.4).[10,66,76] Rostral projections join the medial forebrain bundles and invade the diencephalon by E15 in rat and E13 in mouse.[10,82] Forty-eight hours later they have reached the telencephalon (E15 in mouse), a full day after mDA projections. Along the trajectory of the MFB 5-HT axons branch off to join several preexisting tracts including the fasciculus retroflexus, mammillotegmental tract, stria medullaris, supracallosal stria and external capsule.[83] Axon guidance molecules and mechanisms for the 5-HT system are only beginning to be explored, but appear to be at least partially overlapping with those necessary for mDA neuron pathfinding.[84]

mDA and 5-HT neurons start sending axons toward the forebrain shortly after they begin producing DA or 5-HT, respectively. Both neurons use the MFBs to target the forebrain, though 5-HT neurons project much more broadly than mDA neurons. Although both neurotransmitter systems start extending axons around the same time, mDA processes reach the forebrain earlier, perhaps due to their innate proximity to this target region. The implications of this temporally staggered innervation pattern are not yet known, but leave open the possibility that forebrain regions may be influenced by DA before 5-HT axons reach the area.

mDA AND 5-HT NEURONAL SYSTEMS INTERACT DURING DEVELOPMENT AND ADULTHOOD

We have described that in both the developing and adult mammalian brain the mDA and 5-HT neuronal systems reside in close proximity to one another, are influenced by an overlapping set of intercellular signaling factors, receive afferent input from many of the same brain regions, and send efferent

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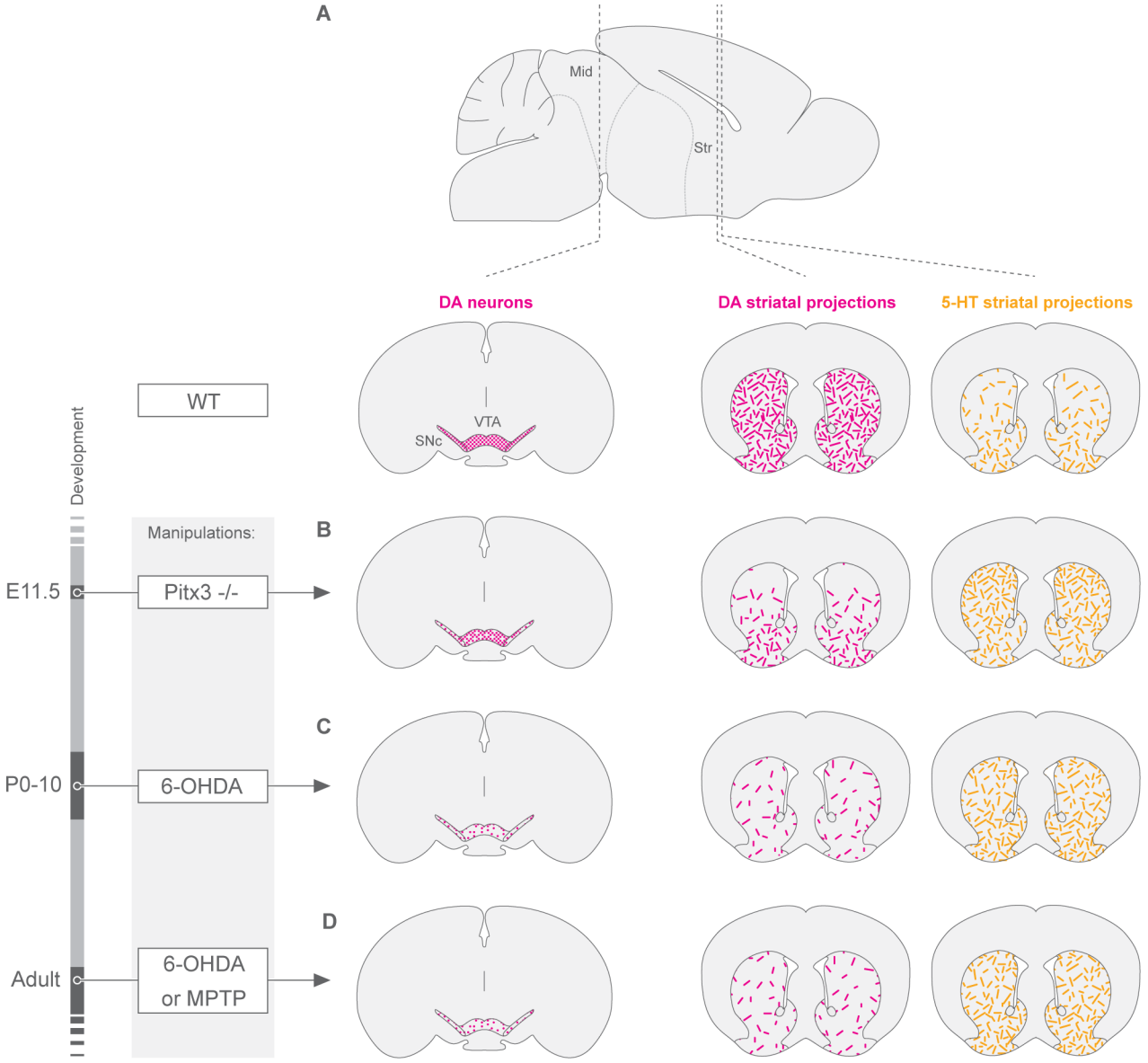

Figure 4. Ablation of mDA neurons alters intrastriatal 5-HT innervation patterns. (A) Dotted lines through the sagittal section of a wildtype adult mouse brain indicate the location of the ventral midbrain (Mid; left) and striatum (Str; right). In wildtype adult mice, the VTA and SNc (pink dots), project to the striatum, forming a vast network of DA axonal fibers (pink lines). In contrast, 5-HT neurons situated in the raphe nuclei (not shown) send sparse projections to the striatum (orange lines), which form a dorsal-ventral density gradient, with more fibers found in the ventral portion of the striatum than in the dorsal section. (B) Adult mice harboring the *Pitx3* null allele (expression of which begins at approximately E11.5) show significant loss of SNc and partial reduction in VTA mDA neurons as well as a corresponding decrease in striatal DA projections and increase in 5-HT projections. In this model, the dorsal aspect of the striatum, largely innervated by the SNc, is affected more than the ventral, as evidenced by fewer DA and more 5-HT axon terminals dorsally compared to ventrally. (C, D) Adult mice that underwent 6-OHDA mDA lesion perinatally (C) or 6-OHDA or MPTP mDA lesion during adulthood (D) show considerably reduced numbers of SNc and VTA neurons. This neuronal loss is accompanied by a significant reduction in striatal DA fibers and DA content as well as a concomitant increase in striatal 5-HT fiber density and content.

projections to common forebrain targets. Such characteristics suggest a capacity for cross-talk between these two monoaminergic systems. The remainder of this review focuses on the evidence for mDA regulation of 5-HT function throughout development. Evidence for 5-HT modulation of mDA function is reviewed elsewhere.[85,86]

Support for such an interaction stems from experiments modeling Parkinson's disease, in which rodent mDA neurons are chemically or genetically lesioned during the pre- or perinatal period. Indeed, the effects of such lesions on the rodent 5-HT system, as monitored at the molecular, cellular, and behavioral level, strongly support the notion that mDA neuron function is critical for the hodological and functional development of at least portions of the 5-HT neuronal system. Moreover, complementary experiments in which mDA neurons are lesioned during adulthood reflect that this mDA-dependence persists throughout the life span. Interestingly, although these models of Parkinson's disease reveal a 5-HT neuron dependence on mDA neuron function, there also appears to exist a DA-independent degeneration of 5-HT neurons in this disease. Specifically, medullary neuronal nuclei including some raphe nuclei demonstrate pathology even before mDA

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neurons.[87,88] In the following sections, we review data obtained from both perinatal and adult rodent mDA ablation studies and explore the implications they have for behaviors regulated by these monoaminergic systems.

mDA Perinatal Ablation Alters 5-HT Function in the Adult Brain. Chemical and genetic neuronal ablation strategies target mDA neurons via different mechanisms. The DA analogue 6-hydroxydopamine (6-OHDA) is lethal to all dopamine transporter (DAT) or norepinephrine transporter (NET)-expressing cells. However, when administered to neonatal rodents intracisternally or intracerebroventricularly along with a norepinephrine reuptake inhibitor, it specifically targets mDA neurons for destruction in a dose-dependent manner,[89−91] sparing noradrenergic neurons.[89,91,92] Importantly, this neurotoxin does not affect 5-HT neurons.

In a complementary fashion, the Aphakia mouse line constitutes a genetic approach to mDA neuron ablation. This line harbors deletions in the *Pitx3* gene: one spanning an upstream enhancer region, and another spanning the promoter, exon 1, and part of intron 1,[93−96] constituting a *Pitx3* null allele. Within the central nervous system (CNS), *Pitx3* is expressed exclusively by mDA neurons early in embryogenesis and is crucial for their survival.[97−99] Thus, the *Pitx3* homozygous null (Aphakia) mouse line serves as a genetic model of mDA neuron lesion.

Importantly, although both models target mDA neurons early in development, the exact timing of these manipulations bears on the effects observed later in adulthood. 6-OHDA lesions may be performed during the early postnatal period, allowing mDA circuitry to begin development prior to neuronal ablation in this model. By contrast, *Pitx3* null animals lose mDA neurons before axonal development begins, which means that the brain experiences compromised SNc, and to some extent VTA, derived DA signaling. Notably, as we shall see, both approaches yield comparable effects on the 5-HT system in the striatum, which may indicate that it is DA signaling later in development, rather than perinatally, that influences 5-HT axonal development in this region. Together, these chemical and genetic mDA lesioning strategies offer tools with which to probe the role that mDA neurons play in regulating 5-HT neuron development and function in various neuronal nuclei throughout the rodent brain at different times during development.

mDA Lesion-Induced Changes in 5-HT Function Are Region-Specific: The mPFC. The available data reflect that mDA lesions result in significant changes in 5-HT neuron function in regions of the brain important for both cognitive and motor function. As a critical regulator of executive function, the medial prefrontal cortex (mPFC) constitutes a key center of neural processing and receives considerable innervation from both mDA and 5-HT neurons.[100] The balance between these innervations may, then, play a key role in establishing and maintaining a functional executive system. Yet, the question stands as to whether DA and 5-HT axonal wiring in this region is interdependent. Cunningham et al. demonstrated that 6-OHDA-mediated mDA neuron ablation at P5 resulted in severe DA *and 5-HT* denervation of the mPFC (Figure 3B).[100] Such results suggest that the absence of mDA neurons and/or DA projections within the mPFC precipitated loss of 5-HT axons in the mPFC, and raise the notion that 5-HT mPFC innervation may in some way be facilitated by the presence of DA signaling. By contrast, it appears that mPFC DA innervation is *suppressed* by 5-HT signaling, as neonatal chemical lesion of DRN 5-HT neurons led to an increase in mPFC DA fibers (Figure 3C).[101] Interestingly, this developmental interdependence appears to be restricted to 5-HT and DA, as lesion of NE neurons drove no significant change in 5-HT innervation of shared targets.[102] Thus, in the mPFC, there exists an interdependent relationship between mDA and 5-HT axonal innervation patterns such that mDA neuron ablation reduces 5-HT mPFC innervation while 5-HT neuronal lesion increases DA mPFC innervation (Figure 3).

mDA Lesion-Induced Changes in 5-HT Function Are Region-Specific: The Striatum. Another key region demonstrating an mDA-5-HT interaction is the striatum, with its vast network of DA innervation constituting the direct and indirect pathways that regulate movement and motor activity. In wildtype mice, the extensive striatal DA innervation is manifest as dense TH+ fibers, while the limited 5-HT innervation is visible as sparse Sert (Slc6a4 serotonin transporter)+ fibers (Figure 4A). DA axons emanating from the SNc largely target the dorsal striatum, forming the nigrostriatal pathway, while those from the VTA innervate the ventral striatum (nucleus accumbens), as part of the mesocortical pathway. The 5-HT fibers, on the other hand, are thought to project from cell bodies situated in the DRN, and form density gradients both from rostral to caudal and dorsal to ventral within the striatum, with the caudal and ventral areas more densely innervated by 5-HT axons.[103,104]

Both models of mDA ablation (pharmacological and genetic) showed loss of TH+ axons in the striatum (Figure 4B,C). HPLC,[103,105−108] microdialysis,[109] immunohistochemistry,[110] and radioligand binding[111] studies all demonstrated a decrease in DA levels and TH+ fibers in the striatum, with the most severe reduction seen dorsally.[97,112−114] In parallel to such changes in mDA circuitry, pronounced changes in striatal 5-HT circuitry and function are observed in both *Pitx3*−/− and 6-OHDA neonatally lesioned mice (Figure 4B,C). HPLC measurements in adult mice lesioned as neonates show increased levels of striatal 5-HT,[115,116] while immunohistochemical analysis demonstrates an increased density of 5-HT projections within the striatum compared to control animals.[103,104,117] Furthermore, the usual rostral-caudal density gradient observed in the striatum is disrupted, as there is a significant increase in the rostral 5HT axon density, which is greater than that observed in the caudal sector.[103,104] Snyder et al. further demonstrated that this enhancement in striatal innervation originates from 5-HT neurons located in the rostral DRN, the same region from which they found the caudal striatum to be innervated, suggesting that those 5-HT neurons projecting to the caudal striatum sprout collaterals to the rostral striatum upon depletion of mDA neurons.

A similar phenomenon of 5-HT striatal hyperinnervation has been documented in the *Pitx3* null (Aphakia) line. Radioligand binding[114] and immunohistochemical[112] studies have revealed increased Sert density in the dorsal striatum—the very same region demonstrating decreased TH+ fibers in Aphakia mice (Figure 4B). These studies also detected an increase in extracellular 5-HT via cyclic voltammetry,[114] suggesting that the absence of DA neuronal innervation in the dorsal striatum was associated with an increase not only in 5-HT innervation but also activity in this region. It is possible that such changes in 5-HT circuitry and activity are spurred by the increased striatal "free-space" created by mDA neuron ablation and the resulting striatal DA denervation. According to this hypothesis, changes in the function of other (non-5-HT) neuronal systems should

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also be evident in mDA lesioned animals. However, importantly, striatal NE innervation patterns were unchanged in 6-OHDA-lesioned neonatal rats,[90,103] suggesting that the documented changes in 5-HT function were likely not merely induced by axon-free space.

Together, such findings point to a developmental interaction between the 5-HT and DA neurons in which the wiring of each respective system is influenced by the presence (or absence) of the other. It is important, however, to bear in mind that the effect of removing one or the other of these monoamines is dependent on the neural anatomical context: as we have seen, for example, while mDA ablation leads to 5-HT hyperinnervation of the striatum, it leads to 5-HT hypoinnervation of the mPFC. This result suggests that the DA and 5-HT axons in various brain regions may be qualitatively distinct in their receptivity to modulation by the other. It remains to be shown whether the axons innervating these various neural nuclei emanate from distinct neurons, or whether collateral arms of a single neuron may be molecularly specified for a given region. Nevertheless, the data are indeed suggestive of an interdependence between the DA and 5-HT systems during pre- and perinatal development, one that impacts upon 5-HT cellular function, circuit formation, and as discussed next, associated organismal behavior.

mDA Lesion-Induced Changes in 5-HT Neuron Function Associate with Behavioral Modification. Both models of mDA ablation result in a hyperactive behavioral phenotype. In the context of the *Pitx3* null mouse line, this increase in activity occurs exclusively during the light (sleep) phase, suggestive of a dysfunctional sleep pattern.[114] Indeed, mDA neurons have been shown to be important for normative sleep patterns, as has the 5-HT system,[86] which as described above, shows striking alterations in this model.

In the case of both pharmacological and genetic developmental mDA ablation, it has been shown that the resulting alterations in 5-HT innervation patterns mediate the observed hyperactivity. Reduction of 5-HT synthesis via Tph2 inhibition by administration of *para*-chlorophenylalanine (PCPA) during adulthood rescued the hyperactivity phenotype in adult animals whose mDA neurons had been ablated perinatally.[92,114] Importantly, PCPA treatment had no effect on the behavior of unlesioned or wildtype control animals (who maintained an intact mDA system). These results indicate that under conditions of normal DA function, the 5-HT system does not play a significant role in regulating locomotor activity. However, upon neonatal ablation of mDA neurons and their associated (striatal) projections, the functional role of 5-HT signaling changes such that 5-HT becomes a factor sufficient for modulation of motor behavior. This change reflects a qualitative shift in the function of the 5-HT inputs, which we know to undergo substantial rewiring upon abrogation of DA signaling.

mDA Ablation during Adulthood Mimics Effects of Perinatal Ablation. It is clear that significant reduction of mDA neurons (and thus striatal DA content) during the embryonic or neonatal time frame leads to alterations in striatal 5-HT levels, 5-HT axonal innervation density, and associated behavioral changes. The effect on 5-HT neurons of mDA neuron ablation during adulthood, however, remains more elusive. Yet the data appear to support the notion that the mDA and 5-HT systems are inextricably linked during the adult period as well as developmentally.

In contrast to methods of neonatal ablation, studies of adult mDA neuron destruction employ local or systemic administration of one of two neurotoxins, 6-OHDA or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), respectively. The evidence suggests that these two neurotoxins, while both targeting mDA neurons, differ in the potency and specificity of the induced lesion. In the studies referenced here, 6-OHDA administration to adult rodents is local, injected into either the SNc, the striatum, or the medial-forebrain bundle connecting the two and thus targets the nigrostriatal system known for its role in motor control, although it is possible that some VTA neurons are also affected. Importantly, in all such studies, lesioned rats were pretreated with a noradrenergic transporter blocker in order to restrict the lesion to mDA neurons. In contrast, MPTP is generally administered systemically, and thus holds the potential to target both the SNc and VTA, the latter known for its regulation of motivated behaviors. However, it has been demonstrated that MPTP lesions are somewhat less severe than those induced by 6-OHDA.[118]

The extent of toxin-induced mDA lesion is evident on the chemical (changes in striatal DA levels), cellular (changes in TH+ cell count in the ventral midbrain or changes in the density of striatal TH+ fibers) (Figure 4D), and behavioral level (changes in measures of motor control). Indeed, it has been established that the more severe the mDA ablation, the worse an animal performs on motor tasks.[119−121] As such, these behavioral metrics are often used as proxy measures of successful mDA neuron ablation as an alternative to histological or histochemical measures. It remains unclear, however, whether the changes in motor control reflect a lesion severe enough to induce a chemical phenotype in the 5-HT system. Thus, there remains a certain degree of ambiguity about the effects of mDA neuron ablation on 5-HT function in adult rodents.

The majority of studies report an increase in measures of striatal 5-HT innervation and activity following toxin administration (Figure 4D). These changes take the form of increased 5-HT fiber density,[120,122−124] increased levels of extracellular 5-HT neurotransmitter,[125] and increased 5-HT turnover.[126] Importantly, the degree of increased 5-HT observed in the striatum and SNc correlated positively with the extent of SNc TH+ neuron destruction.[122] That is, the fewer mDA neurons remaining in the SNc, the more dense the striatal 5-HT innervation. This correlative observation, made by Gaspar and colleagues in nonhuman primates, suggests that DA-dependent changes in 5-HT striatal innervation, largely characterized in rodent models, also apply to primate neurophysiology and behavior.

Other studies, however, failed to document an increase in striatal 5-HT content or innervation upon lesion of SNc DA neurons. Some observed marked decreases in striatal 5-HT innervation[127] and neurotransmitter levels,[128] while others failed to observe any change at all.[129] Possible explanations for such disparities in the effect of mDA ablation may relate to the extent of mDA neuron destruction and the associated decrease in TH+ fibers in the striatum. Some studies report as low as 50% SNc neuron destruction following lesioning, which was accompanied by a 30% decrease in absolute striatal 5-HT levels.[128] In contrast, others have shown that a 90% reduction in SNc DA levels is required to induce striatal 5-HT hyperinnervation.[130] Thus, a moderate degree of mDA denervation may in fact lead to decreased 5-HT activity and

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Table 1. Dopaminergic Drugs and Their Effects on 5-HT Neurons[a]

drug	drug target	route of administration	drug effect on 5-HT levels or 5-HT neurons	conclusions drawn	ref
dopamine	nonspecific DA receptor agonist	local mPFC	increased mPFC 5-HT levels	5-HT axon terminals in mPFC are directly or indirectly sensitive to DA receptor stimulation	142
apomorphine	nonspecific DA receptor agonist	systemic	increased DRN 5-HT release; increased 5-HT neuron firing rate	Effect blocked by D1-like or D2-like antagonists, thus both receptors influence 5-HT activity	132, 133, 134, 137, 138
		local mPFC	increased mPFC 5-HT levels	5-HT axon terminals in mPFC are directly or indirectly sensitive to DA receptor stimulation	142
		local hippocampus	increased hippocampal 5-HT levels	5-HT axon terminals in the hippocampus are directly or indirectly senstive to DA receptor stimulation	141
		local SNc	increased DRN 5-HT levels	mDA activity specifically modulates DRN 5-HT function	133, 134
		local DRN	no effect on 5-HT levels	DRN neurons may not be somatodendritically sensitive to DA receptor stimulation	133, 134
quinpirole	D2-like receptor agonist	systemic	increased DRN 5-HT neuron firing rate and 5-HT levels	stimulation of D2-like receptors systemically induces DRN 5-HT neuron activity	134, 137, 138
		local DRN	no effect on 5-HT neuron activity	DRN neurons may not be somatodendritically sensitive to D2-like receptor stimulation	134
haloperidol	D2-like receptor antagonist	systemic	decrease in basal DRN 5-HT levels	systemic blockade of D2-like receptors suppresses DRN 5-HT neuron activity	136
		local mPFC	increased mPFC 5-HT levels	5-HT axon terminals in mPFC are directly or indirectly sensitive to DA receptor stimulation	142

[a]mPFC, medial prefrontal cortex; SNc, substantia nigra pars compacta; DRN, dorsal raphe nucleus.

innervation, while an ablation of greater magnitude may induce the opposite effect.

The behavioral effects of efficacious mDA lesion and associated changes in 5-HT function are revealed by pharmacological interventions, which demonstrate that the interaction between the mDA and 5-HT systems is involved in modulating both motor and nonmotor behaviors. Inden et al. demonstrated that the amphetamine-induced rotational behavior in rodents observed only following mDA neuron ablation was suppressed by systemic administration of either SSRIs (selective serotonin reuptake inhibitors) or 5-HT1a receptor agonists,[129] both of which act to increase 5-HT signaling postsynaptically. This phenotypic suppression was abrogated if SSRI administration was accompanied by systemic 5-HT1a antagonism. These results suggest that, in the context of mDA neuron ablation, the amphetamine-induced rotation is modulated by (likely postsynaptic) 5HT1a stimulation. Winter et al. documented a similar interaction in the context of nonmotor behaviors. They observed a positive correlation between increases in learned helplessness and the extent of SNc and VTA neuron ablation.[121] This behavioral phenotype was rescued by increases in either DA (via L-DOPA administration) or 5-HT (via SSRI administration), suggesting that both systems converge to modulate this behavior.

Thus, the available data from mDA ablation studies in both the developing and adult rodent suggest that the mDA and 5-HT systems do, in fact, converge in various neural regions to modulate both neurochemistry and behavior. Phenotypic variability is likely attributable to differential efficacy of mDA lesions, with moderate and extensive effects on mDA neuron viability leading to different effects on the 5-HT system.

There exists, then, a certain degree of plasticity in the 5-HT system, seemingly kept in check by mDA signaling. Importantly, this plastic ability of 5-HT neurons to adapt to the lesion of the mDA system is not confined to a developmental critical window, but rather persists throughout life. Perhaps such phenomena will help us to better understand and harness the inherent capacity of the brain to rewire itself in the face of physiological and experiential challenge.

PHARMACOLOGICAL INTERVENTIONS IN ANIMAL MODELS AND THE CLINIC UNVEIL EVIDENCE FOR A BEHAVIORALLY RELEVANT mDA−5-HT NEURON INTERACTION

mDA and 5-HT neurons are known to project to common target regions throughout the brain,[131] as well as to send reciprocal axonal projections between the ventral midbrain and DRN.[20,21] Thus, these two neurotransmitter systems have at least two methods of directly influencing one another's function: (1) presynaptic interactions between mDA and 5-HT axon terminals at target regions, and (2) axonal input onto cell somas in the mDA and 5-HT nuclei. Pharmacological manipulation of either population in rodent and clinical studies aids in localizing the cross-talk between these systems and deciphering the relevance of each type of interaction to behavioral regulation.

Systemic DA Receptor Stimulation Excites DRN 5-HT Neurons. The available data from animal studies robustly reflect that DA neurotransmission does impact upon the function of 5-HT neurons—be it a direct or indirect effect. Many studies have demonstrated that systemic application of DA or DA receptor agonists lead to altered 5-HT activity in rodents. For example, the nonspecific DA receptor agonist, apomorphine, causes an in vivo increase in 5-HT release in the DRN (Table 1).[132−134] This effect is abrogated by antagonism of the D1 or D2 receptors or by toxin-mediated (6-OHDA) lesioning of mDA neurons,[134,135] indicating that it is DA activity per se catalyzing the increase in 5-HT levels. A corollary of this DA-mediated excitation of 5-HT neurons is that antagonism of DA receptors should decrease 5-HT activity. This was indeed observed to be the case, as the in vivo application of the D2 antagonist haloperidol decreased basal DRN 5-HT levels (Table 1).[136] In line with these neurochemical findings, systemic administration of apomorphine or the D2 receptor agonist quinpirole increases firing rates of DRN putative 5-HT neurons (neurons were identified as serotonergic by virtue of electrophysiological properties) in vivo and ex vivo (Table 1).[134,137,138] Thus, both neurochemical

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Table 2. Psychotropic Clinical Drugs and Their Effects on 5-HT Neurons

drug	drug effect	conclusions drawn	ref
SSRI in combination with methylphenidate	increase in PFC DA release compared to methylphenidate alone; no such effect in the nigrostriatal pathway	drug combinations yield increased specificity and efficacy	154
dopamine reuptake inhibitor (DRI)	increased extracellular DA levels; increased 5-HT neuron firing rate	systemically increased DA levels lead to activation of 5-HT neurons	151
triple reuptake inhibitor (TRI)	increased extracellular levels of all monoamines; incomplete suppression of 5-HT neuron activity, in contrast to SSRIs alone	SSRI treatment alone typically cause a *complete* cessation of 5-HT activity while TRIs do not; thus, TRI-induced increases in DA and/or NE may *increase* 5-HT neuron activity	151

and electrophysiological measures implicate DA in exerting an excitatory influenced on DRN 5-HT neurons.

Evidence for 5-HT Neuron Receptivity to DA Signaling Is Ambiguous. The anatomical evidence for mDA innervation of the DRN is rather robust. TH+ fibers are visible in the DRN and DA neurons in the SNc and VTA are labeled by retrograde tracing from the DRN.[16,21,23−25,133] However, whether this DA input *directly* affects 5-HT neurons remains unclear, as in situ hybridization and radio-ligand binding studies suggest that few 5-HT neurons of the DRN actually express DA receptors.[134,139] The pharmacological evidence for a direct mDA-5-HT circuit in the raphe nuclei is similarly ambiguous. Local application of apomorphine in the DRN failed to stimulate 5-HT neurons[133] and, in one case, was found to decrease DRN 5-HT levels (Table 1).[134] To further complicate the picture, ex vivo electrophysiological studies demonstrate an excitatory response of putative 5-HT neurons mediated by D2 stimulation of a Trp channel nonspecific cation current (Table 1).[137,138] Collectively, these results implicate mDA neurons in the modulation of DRN 5-HT neuron activity, directly or indirectly. However, as we discuss in the subsequent section, there is also evidence to suggest that DA may exert influence on 5-HT release in some forebrain targets located distal to the DRN.

Local mDA Modulation of 5-HT Function in Forebrain Targets Is Region-Specific. Just as the effects of mDA neuron ablation on 5-HT activity are target-region specific (see discussion above), the effect of DA receptor stimulation within a specified target region may be a function of which neural region is targeted. In vitro and in vivo evidence suggests that intrahippocampal DA stimulation enhances hippocampal 5-HT levels, an effect blocked by DA receptor antagonism in vitro or mDA lesion in vivo (Table 1).[140,141] In a similar fashion, infusion of DA, apomorphine, or the D2 receptor antagonist haloperidol into the mPFC led to an increase in mPFC 5-HT levels (Table 1).[142] While the first two substances directly increase DA levels within the mPFC, haloperidol likely leads to increased DA through abrogation of mDA neuron autoinhibition via blockade of presynaptic D2 receptors on mDA neuron terminals. Such an increase in mPFC 5-HT was previously shown to be associated with an enhancement of learned helplessness,[142] suggesting that DA control over 5-HT cortical release may influence social behaviors, and underscoring the importance of the evident mDA-5-HT neuron interaction for understanding pathological affect. We next review evidence obtained from the clinical and preclinical literature pertaining to treatments of psychiatric illnesses in which social behavior is significantly disturbed.

Clinical Treatment with Monoaminergic Psychotherapeutic Drugs Illustrates Interactions between the mDA and 5-HT Neurotransmitter Systems. Some of the most prevalent and disruptive psychiatric disorders have documented etiologies in the mDA and 5-HT neurotransmitter systems. Attention deficit hyperactivity disorder (ADHD) and major depressive disorder (MDD) are disorders affecting individuals throughout development that have documented etiologies centered on monoaminergic dysfunction. Examining the effects on mDA and 5-HT systemic function of pharmacological therapeutics aimed at ameliorating the devastating symptoms of such illnesses can lend insight into how these two neuronal populations work together, directly and indirectly, to influence clinically relevant behaviors. In the sections to follow, we review the clinical literature pertaining to the monoamine-targeted pharmacological treatments for these two psychopathologies, and attempt to glean information about how such interventions affect mDA and 5-HT neuronal systems and, consequently, behavior and affective state.

The Etiology of ADHD and MDD Can Involve mDA Dysfunction Modulated by 5-HT Activity. The etiology of ADHD is thought to stem from mDA dysfunction, which appears to be inextricably linked to symptoms of inattentiveness, hyperactivity, and impulsivity.[143] Individuals with ADHD show decreased cerebrospinal fluid (CSF) and urine levels of the DA metabolites.[144] They further show a blunted response to the DAT-blocker, methylphenidate,[145] which serves as a primary treatment of ADHD, implicating low basal levels of DA in the psychopathology of ADHD.

MDD, which has traditionally been viewed as a product of 5-HT dysfunction in the mammalian brain, is increasingly understood to also stem from considerable mDA hypofunctionality.[146] It has become apparent that part of the efficacy of antidepressant therapeutics targeting the 5-HT system is due to effects on mDA neurons. Indeed, MDD is characterized by hypoactivity in the mesolimbic (reward-processing) mDA system, as evidenced by the fact that MDD patients are less willing to exert effort to attain rewards[147] and display relatively low levels of CSF and plasma DA metabolites.[146] Furthermore, MDD symptom severity in humans inversely correlates with the extent of DA binding to D2 receptors in the striatum; the more DA−D2 binding, the fewer symptoms. This measure is elevated in SSRI responders,[148] indicating that SSRIs somehow potentiate DA release in this system. Accordingly, SSRIs have been shown to activate reward networks and to increase NE and DA levels in the nucleus accumbens.[149,150] Thus, lessons learned from 5-HT-based antidepressant treatments reflect the importance of DA function in MDD as well as of the interactive effects between 5-HT and DA.

The mDA−5-HT Interplay Is Central to Novel Treatments for ADHD and MDD-Associated Hypodopaminergic Function. Newer drug therapies for both ADHD and MDD target multiple monoaminergic systems, underscoring the importance of the mDA-5-HT interplay in the clinical setting.[146,151,152] As both disorders include hypodopaminergic function, a goal of pharmacotherapies is to enhance DA activity

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in brain regions whose function underlies the disordered symptoms. A common treatment for both MDD and ADHD is the combination of DA and 5-HT reuptake inhibitors.[153] Though SSRIs alone show moderate efficacy in MDD, and have little to no effect in ADHD treatment, they in fact potentiate the methylphenidate-induced DA increase in regions of the brain highly relevant to ADHD and MDD psychopathology: when administered together, these drugs increase PFC DA levels above that which is seen with methylphenidate alone (Table 2).[154] Interestingly, this synergistic effect is not observed in the nigrostriatal pathway. The implications of this finding are twofold: (1) Enhanced 5-HT levels *in combination* with DAT blockade have a more robust effect on PFC DA function than DAT blockade alone. (2) It suggests that different mDA circuits may be differentially modulated by pharmacological treatments targeting the 5-HT system. This latter result is of key importance, as many of the therapeutic drugs targeting single neurotransmitter systems result in considerable and undesirable off-target effects due to their broad activity throughout the brain. This finding, however, suggests that it is possible to refine the effects of pharmacotherapies by targeting multiple neural systems in combination.

This synergistic relationship between the mDA and 5-HT systems appears to be bidirectional, with increases in DA signaling also resulting in enhanced 5-HT activity. It has been observed, for example, that extended treatment with dopamine reuptake inhibitors (DRIs), and thus elevated DA levels, results in increased 5-HT neuron firing (Table 2).[151] Triple reuptake inhibitors (TRIs), which block reuptake of DA, 5-HT, and NE, are also thought to increase 5-HT neuronal excitation via enhanced DA and NE signaling, which counteracts the acute indirect autoinhibitory effects of 5-HT transporter blockade (Table 2). Thus, several lines of evidence suggest the existence of a synergistic relationship between enhancement of DA and 5-HT levels in the treatment of MDD and ADHD, which may have critical implications for the development of therapeutics for other psychiatric ailments.

A ROLE FOR MODERN MOUSE GENETICS IN DELINEATING THE INTERPLAY OF mDA AND 5-HT IN THE DEVELOPING AND ADULT BRAIN

As we have seen, most of the evidence for a DA-5-HT interaction in various neural regions emanates from pharmacological manipulations of mDA activity and chemical or electrical readouts of the induced effects on the 5-HT system. Little work has been done employing modern genetic tools in the mouse, which would allow for a nuanced dissection of the mDA-5-HT interplay. It would be informative to observe the effects of conditional or virally induced DA receptor knockouts or expression of activating or inhibiting effector molecules (e.g., DREADDs, channelrhodopsins, etc.) on the mDA-5-HT interplay in different neural nuclei. Such studies would, for example, facilitate a comprehensive understanding of whether DA receptors are expressed and active on 5-HT neuron cell bodies and/or axon terminals in the striatum. The results of these experiments hold the potential to greatly inform the rational design of novel therapeutics aimed at alleviating the pathology underlying psychiatric illnesses.

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