



UvA-DARE (Digital Academic Repository)

Embryonic development of selectively vulnerable neurons in Parkinson's disease

Oliveira, M.A.P.; Balling, R.; Smidt, M.P.; Fleming, R.M.T.

DOI

[10.1038/s41531-017-0022-4](https://doi.org/10.1038/s41531-017-0022-4)

Publication date

2017

Document Version

Final published version

Published in

NPJ Parkinson's disease

License

CC BY

[Link to publication](#)

Citation for published version (APA):

Oliveira, M. A. P., Balling, R., Smidt, M. P., & Fleming, R. M. T. (2017). Embryonic development of selectively vulnerable neurons in Parkinson's disease. *NPJ Parkinson's disease*, 3, [21]. <https://doi.org/10.1038/s41531-017-0022-4>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

REVIEW ARTICLE OPEN

Embryonic development of selectively vulnerable neurons in Parkinson's disease

Miguel A. P. Oliveira¹, Rudi Balling¹, Marten P. Smidt² and Ronan M. T. Fleming¹

A specific set of brainstem nuclei are susceptible to degeneration in Parkinson's disease. We hypothesise that neuronal vulnerability reflects shared phenotypic characteristics that confer selective vulnerability to degeneration. Neuronal phenotypic specification is mainly the cumulative result of a transcriptional regulatory program that is active during the development. By manual curation of the developmental biology literature, we comprehensively reconstructed an anatomically resolved cellular developmental lineage for the adult neurons in five brainstem regions that are selectively vulnerable to degeneration in prodromal or early Parkinson's disease. We synthesised the literature on transcription factors that are required to be active, or required to be inactive, in the development of each of these five brainstem regions, and at least two differentially vulnerable nuclei within each region. Certain transcription factors, e.g., *Ascl1* and *Lmx1b*, seem to be required for specification of many brainstem regions that are susceptible to degeneration in early Parkinson's disease. Some transcription factors can even distinguish between differentially vulnerable nuclei within the same brain region, e.g., *Pitx3* is required for specification of the substantia nigra pars compacta, but not the ventral tegmental area. We do not suggest that Parkinson's disease is a developmental disorder. In contrast, we consider identification of shared developmental trajectories as part of a broader effort to identify the molecular mechanisms that underlie the phenotypic features that are shared by selectively vulnerable neurons. Systematic *in vivo* assessment of fate determining transcription factors should be completed for all neuronal populations vulnerable to degeneration in early Parkinson's disease.

npj Parkinson's Disease (2017)3:21; doi:10.1038/s41531-017-0022-4

INTRODUCTION

Parkinson's disease (PD), symptoms and pathology

PD is a clinical syndrome, identified by a combination of bradykinesia plus resting tremor or rigidity,¹ that is histopathologically confirmed by identification of both degeneration and loss of dopaminergic neurons (DN) within the substantia nigra pars compacta (SNc).² Neuronal degeneration is characterised by Lewy pathology, which consists of intracellular protein aggregates that co-identify with alpha-synuclein.³ The existence of a prodromal phase to PD is supported epidemiologically⁴ by clinical observation of early non-motor symptoms⁵ and by evidence of extranigral Lewy pathology associated with prodromal PD symptoms. The onset of PD is hypothesised to be up to 20 years before the occurrence of motor symptoms, with consistent and early cell loss in the substantia nigra.⁶ In the later stages of PD, cell loss and Lewy pathology is present in other brainstem nuclei^{7–10} but evidence of cell loss in prodromal PD has not yet been reported.^{7, 11–13} Based on the distribution of Lewy pathology in the brain, cardiac and cutaneous autonomic nerves,⁶ a neuropathological temporal staging scheme has been proposed for PD.^{14–17} Six sequential Braak stages of Lewy pathology generally seem to coincide with the onset or exacerbation of certain clinical symptoms¹⁸ (Fig. 1).

Lewy pathology may reflect a compensatory response to proteostatic stress,^{12, 19–25} but may also cause neuronal dysfunction,²⁶ e.g., by disruption of axonal organelle transport.¹³ Lewy pathology is also present in other synucleinopathies, e.g.,

dementia with Lewy Bodies and incidental Lewy Body disease.¹³ Despite variation in the association between Lewy pathology and onset of clinical signs, in the majority of PD patients, non-motor symptoms appear before motor symptoms in a manner consistent with Braak's neuropathological staging scheme. Prodromal (Braak stages 1, 2) and early PD (Braak stage 3) is characterised by Lewy pathology in a selective subset of brainstem nuclei (Fig. 1). This is consistent with the conclusions of multiple independent studies that have reported cell loss in many of the same brainstem nuclei, albeit in late PD.^{7–10, 27} An anatomically specific and consistent picture of cell loss combined with Lewy pathology provides evidence that certain neuronal populations are selectively vulnerable to degeneration in PD.^{7, 11, 13} (Fig. 1).

Selectively vulnerable neurons share some phenotypic characteristics, e.g., unmyelinated axons that have previously been hypothesised to increase the risk of degeneration in PD.¹³ A combination of anatomical, morphological, physiological and biochemical characteristics can be used to define the identity of a neuronal population. Even within a single brainstem nucleus, only certain neuronal populations, identifiable by detailed phenotypic characterisation, may be selectively vulnerable to degeneration. Therefore, comprehensive multimodal phenotypic characterisation of selectively vulnerable neurons in PD is required to further elucidate the relationship between selective vulnerability and shared neuronal phenotype.²⁸

From an embryological perspective, mature cellular phenotype is the cumulative result of a molecularly specified program that

¹Luxembourg Centre for Systems Biomedicine, University of Luxembourg, 6 Avenue du Swing, Belvaux L-4362, Luxembourg and ²Department of Molecular Neuroscience, Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Sciencepark 904, 1098 XH Amsterdam, The Netherlands
Correspondence: Ronan M. T. Fleming (ronan.mt.fleming@gmail.com)

Received: 6 July 2016 Revised: 24 May 2017 Accepted: 1 June 2017
Published online: 26 June 2017

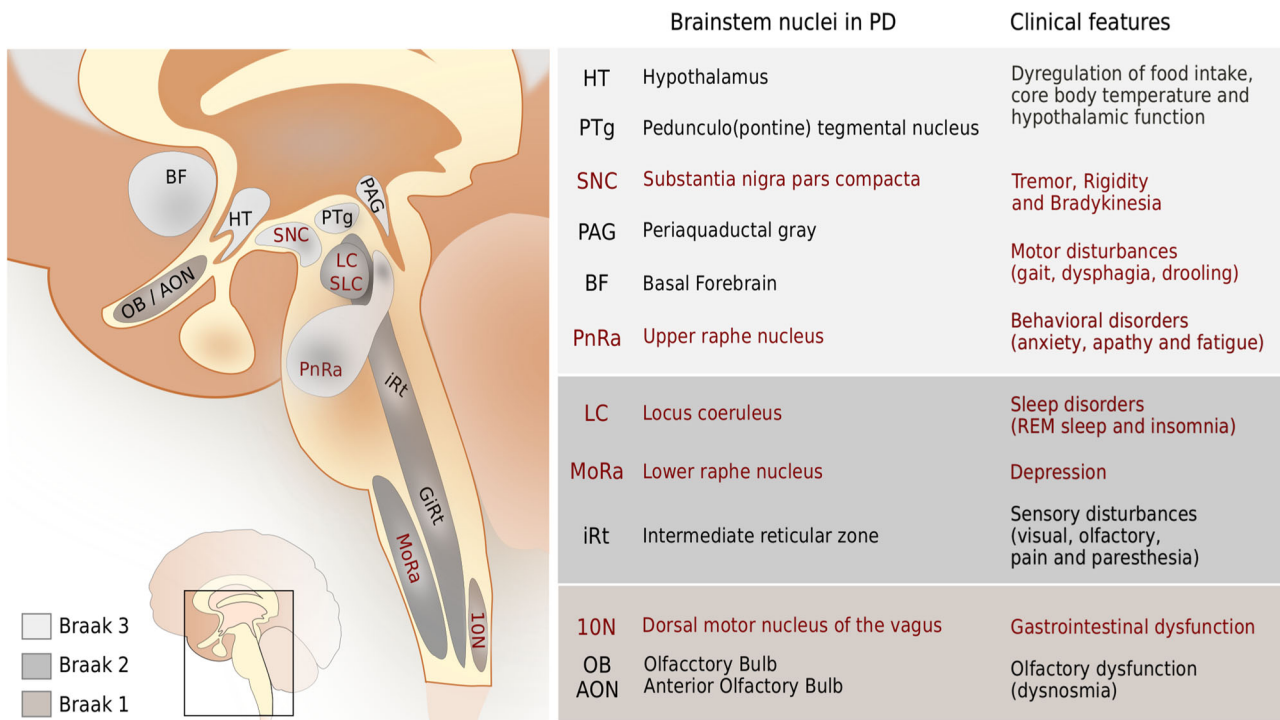


Fig. 1 Clinical manifestations,^{225–228} brainstem nuclei, Lewy pathology and cell loss in Parkinson's disease (PD). Nuclei with evidence cell loss in late PD (red) are distinguished from those with evidence for Lewy pathology alone (black). The dorsal motor nucleus of the vagus (10N)^{7, 8, 18, 229} and the intermediate reticular zone (IRt) of the medullary reticular formation^{18, 230} are among the earliest brainstem populations with Lewy pathology in PD (Braak stage 1). Located outside the brainstem, both olfactory bulb (OB) and the anterior olfactory nucleus (AON) may also show Lewy pathology at this early stage. Subsequently in Braak stage 2, Lewy pathology is found within three main nuclei: the locus coeruleus (LC),^{9, 10} the lower raphe nuclei (MoRa)^{18, 229} and the gigantically reticular nuclei (GiRt) of the medullary reticular formation.¹⁸ In Braak stage 3, together with the characteristic motor symptoms (extrapyramidal changes) and degeneration of the SNC,^{18, 27, 229} the upper raphe nuclei (PnRa), located in the pons, also presents with Lewy pathology. In Stage 3, multiple other nuclei of the midbrain tegmentum show Lewy pathology, including the pedunculopontine tegmental nucleus (PTg),¹⁸ the paranigral nucleus (PaN),¹⁸ the pigmented parabrachial nucleus (PBP)¹⁸ and the Edinger Westphal nucleus (EW).¹⁸ However, only neuronal cell loss of the SNC is widely considered specific for PD (Supplementary 1). (Figure adapted from ref. 231 with brain ontology according to the Human brain reference atlas of the Allen Brain Atlas.¹⁴²)

operates on a spatiotemporally evolving developmental lineage. Phenotypically similar neuronal populations share certain aspects of their developmental molecular specification program, spatiotemporal proximity, or both. Therefore, in this review we synthesise the literature on the spatiotemporal developmental lineage position and transcription factor specification of a set of neuronal populations with clear evidence of selective vulnerability to degeneration in prodromal or early PD (Fig. 2). Our objective is to assess whether selectively vulnerable neurons share similar developmental molecular specification programs, spatiotemporal proximity or both. We chose to restrict our focus to nuclei associated with prodromal evidence of Lewy pathology, evidence of neuronal cell loss in later stages of PD and sufficient developmental literature (e.g., genetic fate mapping). Specifically, we focus on neuronal populations of the dorsal motor nucleus of the vagus (10N), locus coeruleus (LC), upper raphe nuclei (PnRa), lower raphe nuclei (MoRa) and SNC. We summarise (and detail in Supplementary 1) the known phenotypic characteristics specifying the identity of each of the aforementioned mature neuronal populations. For each mature population, we review salient aspects of its developmental lineage and summarise the main transcription factors required for general specification of the corresponding mitotic progenitor, postmitotic progenitor and mature neuron. Where literature permits, we also distinguish between neuronal subtypes within each of these populations based on the origin of the corresponding progenitors and on variations to general specification programs, especially when

subtypes are associated with differential vulnerability. We conclude with a discussion of the developmental features that are shared between precursors of vulnerable neuronal populations. This developmental perspective compliments previous efforts to understand the phenotypic characteristics that are shared between selectively vulnerable mature neuronal populations¹³ (Fig. 2).

Neuronal identity of vulnerable populations

Neuronal identity and its cellular and molecular phenotypic specification is mostly encoded by a profile of transcription factors, expressed by ancestral progenitors and by postmitotic neurons.^{29, 30} These transcription factors are expressed early in the developing brain, downstream of specific developmental inductions, and are responsible for the gradual fate restriction of the embryonic pool of pluripotent stem cells. The enormous variety of neuronal populations arises from combinatorial induction that is specific to each particular location within the brain, where subtle inductive differences generate different neuronal populations.²⁹ Lineage tracing studies provide a powerful means to understand the properties of mature populations, their development, homeostasis and disease vulnerability, especially when combined with experimental manipulation of signals regulating cell-fate decisions.³¹

Phenotypic characteristics are shared between vulnerable populations. It has been hypothesised that selectively vulnerable neurons share a set of common phenotypic characteristics leading to an increased risk of degeneration in PD.¹³ These characteristics,

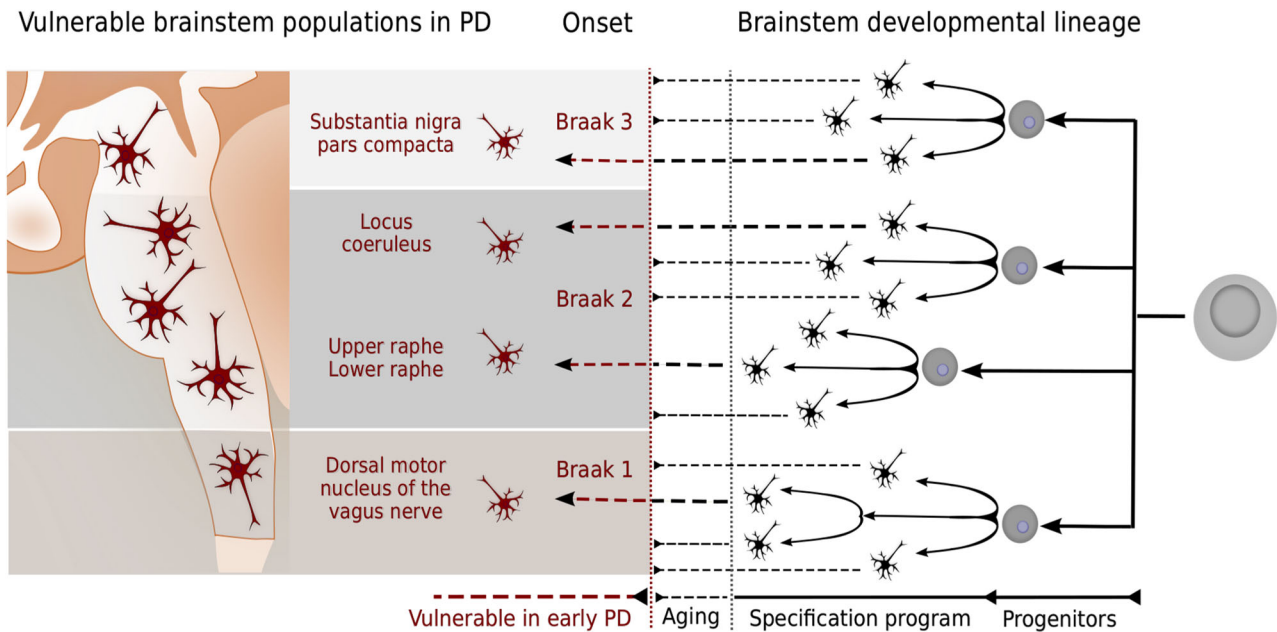


Fig. 2 Hypothesis: neurons selectively vulnerable to degeneration in PD share similarities in their cellular and molecular developmental programs. Vulnerable neuronal populations share certain identifiable phenotypic characteristics. Mature neuronal identity is largely the result of a developmental program, that is specific to each cell type. By comparing and contrasting the cellular developmental lineage and requirement for absence or presence of specific developmental transcription factors, we can discover shared developmental similarities of neurons selectively vulnerable in early PD

which include neurotransmission, electrophysiology, morphology and connectivity, do seem to be consistent with age being the single largest risk factor in PD.¹³ Regarding neurotransmission, the presence of high levels of cytosolic monoamines is hypothesised to underlie selective degeneration^{13, 32} since these populations also generally include a catecholamine-derived neuromelanin pigment in primates^{13, 33} (Fig. 3 and Supplementary 1 and 2). Electrophysiological characteristics associated with increased risk include autonomous activity, broad action potentials and a low intrinsic calcium buffering capacity.¹³ On morphology and connectivity, vulnerable populations are generally characterised as having long, poorly myelinated, highly branched axons and terminal fields.¹³

Currently, the majority of genes or expression signatures used in the biochemical characterisation of neuronal populations are related to their neurotransmitter identity (Fig. 3 and Supplementary 1 and 2). However, this characterisation only covers the ability to produce, secrete and re-uptake specific neurotransmitters,^{34, 35} which is not sufficient to completely specify the neuronal identity.³⁴ Some neurons co-release more than one neurotransmitter^{36, 37} and neuronal plasticity enables neurons to switch between neurotransmitters.^{34, 38–40} Therefore, a more inclusive characterisation of neuronal identity, besides neurotransmission alone, is required²⁸ (Supplementary 1).

Developmental programs that specify neurotransmitter phenotypes are well studied aspects of neuronal identity,^{28, 29, 41} particularly with respect to terminal differentiation of monoaminergic neurons (noradrenergic, dopaminergic and serotonergic).³⁴ Neuronal transcriptomic analysis is also a powerful way to characterise neuronal identity,^{42–44} e.g., transcriptome sequencing is quantitative, and highly reproducible.^{28, 45} However, the correlation between transcript and protein levels is generally too weak for accurate quantitative inference of one from the other.⁴⁶ Targeted quantification of key developmental proteins and confirmation of their role in specification of multiple nerve cell functions^{47, 48} can be used to compliment genome-scale measurements and lead to a more robust characterisation of neuronal identity.⁴⁹ By inferring cell-type-specific function from

developmental programs and expression profiles, one can also assess the cell-type specificity of functional attributes, derived from parallel morphological and electrophysiological studies.²⁸

RESULTS

Development of vulnerable brainstem populations

In the past two decades, substantial progress in developmental neuroscience has uncovered a large set of extracellular signals and transcriptional regulators that control the development and maturation of different types of neurons. However, the developmental program is not yet fully understood for each and every neuronal population. In order to better understand the generation of different neuronal populations, it is important to study the mechanisms behind the maintenance of infinite self-renewal capacity in stem cells (unrestricted fate potential) and the mechanisms responsible for lineage commitment during differentiation.⁵⁰ The final neuronal phenotype comprises generic pan neuronal characteristics and more specific characteristics, such as origin and termination of axonal projections.⁵¹

Neurons originate from multipotent stem cells in the neural plate (Supplementary 3.1) that continuously limit their fate and generate restricted mitotic progenitors that, in a sequential order, give rise to neuronal and glial progenitors.^{52–54} Neuronal differentiation occurs at different embryonic stages (E) and within different neuromeric segments of the early brain (prosomeres P3-1, mesomeres M1-2 and rhombomeres R1-8) (Fig. 4). For each neuromere-specific neuronal progenitor, the induction of a specific neuronal fate is controlled in a context-dependent manner by a combination of intrinsic factors and extrinsic signalling molecules, both of which act as regulators of neuronal differentiation^{52–55} (Supplementary 3.2). Specific intrinsic factors and inductive combinations result in the upregulation (or down-regulation) of certain genes, mostly transcription factors, which are required to be active (resp. inactive) to ensure lineage commitment and generation of specific neuronal fates.⁵⁰ Developmental transcription factors can either be transiently or

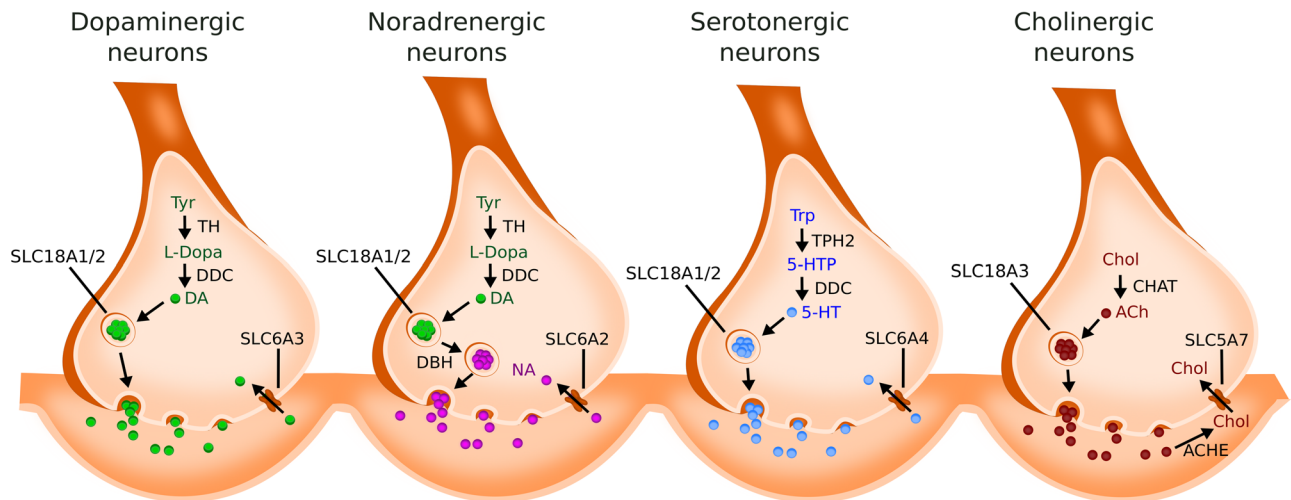


Fig. 3 Neurotransmitter identity (adapted from ref. 232). Multiple transmitters and transmitter-like substances have been studied in PD and it is clear that neurons releasing neurotransmitters other than dopamine (DA) are also susceptible to neurodegeneration.²²⁷ These transmitters include the monoamines serotonin (5-HT) and noradrenaline (NA) and the neurotransmitters acetylcholine (ACh). In catecholaminergic neurons (dopaminergic, noradrenergic and adrenergic), the neurotransmitter is synthesised from the amino acid tyrosine (Tyr) in a common biosynthetic pathway.²³² Mesodiencephalic DN of the SNC, together with the noradrenergic neurons of the LC, require the expression of tyrosine hydroxylase (TH) and amino acid decarboxylase (DDC). As opposed to DN, noradrenergic populations further require the expression of dopamine beta-hydroxylase (DBH), which converts DA into noradrenaline. Adrenergic neurons, in turn, also require the expression of phenylethanolamine-N-methyl-transferase (PNMT), which converts noradrenaline into adrenaline. On the other hand, although serotonergic neurons from the raphe nucleus synthesise 5-HT through the hydroxylation of tryptophan, a reaction catalysed by tryptophan hydroxylase (TPH), they also require the expression of DDC.²³² In neurotransmitter packing, both catecholaminergic and serotonergic neurons require the same vesicular monoamine transporters, SLC18A1/2.²³² The transporter responsible for the re-uptake of neurotransmitters from the synaptic cleft is specific for each population. Serotonergic neurons require the transporter SLC6A4, DN require SLC6A3 and noradrenergic neurons require SLC6A2. For neurotransmitter degradation, the catecholaminergic neurons require catechol-O-methyl transferase (COMT), monoamine oxidase (MAO) and aldehyde dehydrogenase (ALDH), while the serotonergic neurons only require MAO and ALDH.²³² Cholinergic neurons secrete acetylcholine (ACh), which is synthesised by the choline acetyltransferase (CHAT) from acetyl-CoA and choline (Chol). In cholinergic neurons from the 10N, ACh is packed into synaptic vesicles by an energy-dependent process that involves the SLC18A3. This vesicular ACh is released in the synaptic cleft and is rapidly converted into Chol, by the acetylcholinesterase (ACHE), which then is transported intracellularly by SLC5A7.^{227, 253} (also see Supplementary 2)

constitutively expressed during development, and still be individually required for fate restriction and the generation of specific mature populations.

Within each neuromere, an initial set of active genes, activated before neurogenesis, confers an intrinsic segmental identity to the corresponding progenitors (Fig. 4), which is inherited or diversified during neuronal induction by extrinsic signals, especially during terminal differentiation⁵⁶ (Fig. 4d). An anatomically defined nucleus may have different neuronal subtypes with differences that can be traced back to the neuromeric origin of the corresponding progenitor. For example, genetic fate mapping of raphe nuclei has established that different serotonergic neuronal subtypes arise from separate rhombomeres and from variations of the general serotonergic specification program.^{44, 48, 51, 57–62} The same developmental principle also applies to other types of neuron, e.g., noradrenergic⁶³ and visceromotor neurons,^{64, 65} where nuclear subtype specification has also been associated with neuromere-specific transcription factors and variations of the general specification program for each type of neuron. For mesodiencephalic dopaminergic populations^{66–78} mediolateral progenitor positioning has a similar effect.

Visceromotor populations of the dorsal motor nucleus of the vagus. Visceromotor neurons are a subset of cranial motor neurons that project from multiple brainstem nuclei, including the 10N, towards internal organs, like lungs, heart and viscera. The 10N visceromotor population project their axons to the viscera, via the vagus nerve (Supplementary 1.1).

Progenitors of visceromotor neurons Multiple subsets of visceromotor neurons are generated throughout brainstem, except

R1.^{64, 79} These subsets originate from neuromere-derived basal p3 progenitors (p3 or pMNV), which bilaterally flank the floor plate.^{64, 65, 80} These progenitors are also common to both branchiomotor⁸¹ and serotonergic populations.^{57, 82} The p3 pool of progenitors is generated after an anteroposterior (AP)-graded retinoic acid (RA) signalling, which confers multiple rhombomeric identities, specified by a combination of *Hox* genes⁶⁵ and a ventral SHH signalling, which is necessary to impose a specifically motor fate^{64, 83} (Fig. 4d and Supplementary tables 1, 2).

General specification program of visceromotor neurons The general specification program of visceromotor neurons (and branchiomotor neurons) begins with the acquisition of the correct p3 progenitor identity and neurogenesis around E9.5 (Embryonic day 9.5)^{57, 82} (Fig. 4d and Supplementary table 1–2). Visceromotor neurons require simultaneous expression of *Nkx2-2* and *Nkx2-9*.^{64, 65, 81, 84} *Nkx6-1* and *Nkx6-2* are also expressed at this stage but this expression is not necessary for specification, despite their importance in repressing alternative interneuronal fates and addressing migration and pathfinding.^{54, 65}

Phox2b is expressed earlier than *Phox2a* (not required) and *Phox2b* expression is required for visceromotor specification,⁵⁷ since *Phox2b*-mutant mice are depleted of all visceromotor and branchiomotor neurons.⁸⁵

In order to generate a motor neuron phenotype, p3 progenitors require low or absent *Foxa2* expression.⁵⁷ From E10.5 onwards, and within the rhombomeres R2–3 and R5–8, some p3 basal progenitors switch their visceromotor fate towards serotonergic, which coincides with the up-regulation of *Foxa2* and the down-regulation of *Nkx2-9* and *Phox2b*.^{57, 82}

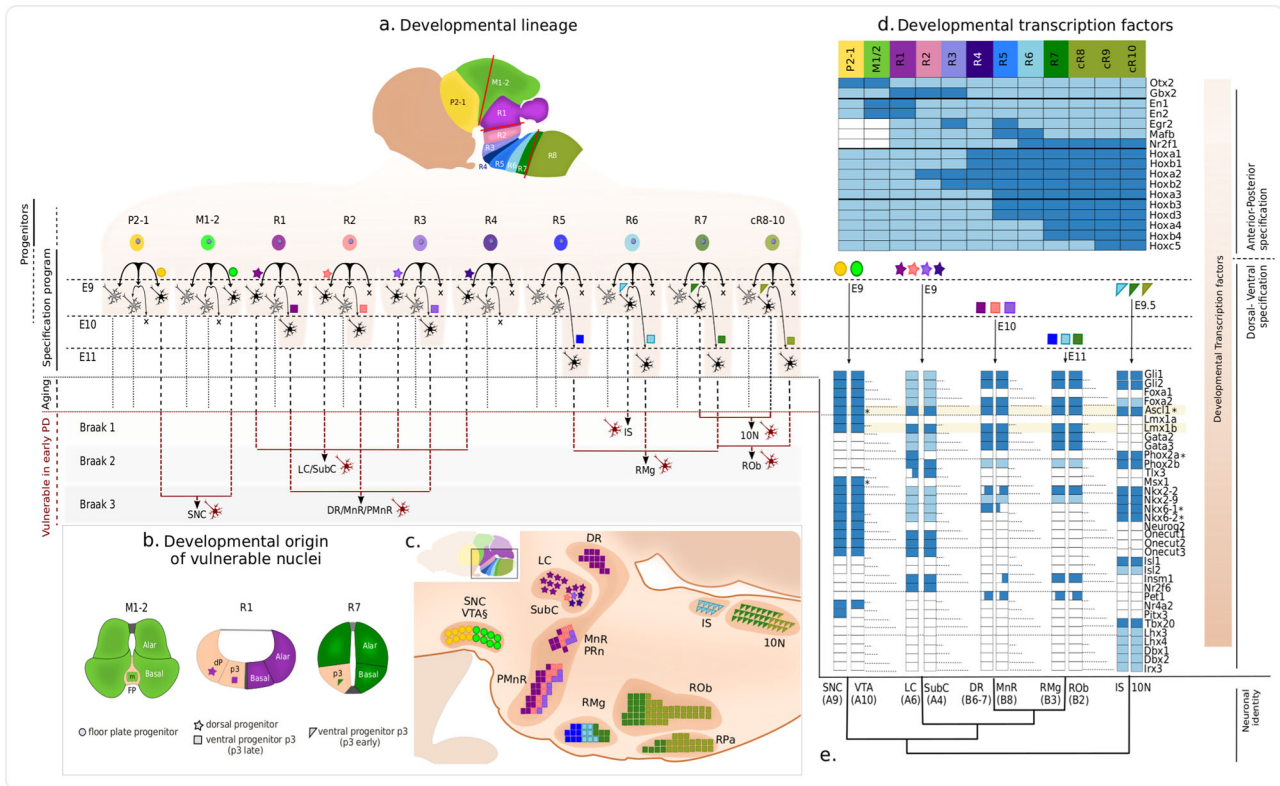


Fig. 4 Brainstem development and neuronal specification program of brainstem vulnerable populations in PD. **a** Each brainstem progenitor originates from one rostro-caudally segmented neuromere (colour coded) and gives rise to specific mature neurons via a developmental lineage. **b** Transverse view of three neuromeric segments (positions correspond to red lines in **a** with dorso-ventral progenitor origin (shape coded). **c** Mature neuronal populations after development, which may include migration, in vulnerable brainstem nuclei within a quasi-sagittal section of a juvenile brain (neuromeric segment is colour coded and dorso-ventral progenitor origin is shape coded, as before), adapted from^{58, 61, 63}. **d** A set of developmental transcription factors is associated with the identity of each segment (upper) and the specification program for each neuronal population (middle), whether required to be expressed (dark blue), or required not to be expressed (light blue) or an experimental knowledge gap (white). **e** Neuronal populations are clustered by their developmental requirements highlighting similarities. Dorsal motor nucleus of the vagus (10N), medullary/lower raphe nuclei [MoRa (RMg/B3, ROB/B2, RPa/B1)], pontine/upper raphe nuclei [PnRa (PRn/B5, DR/B6-7, MnR/B8, PMnR/B9)], locus coeruleus (LC/A6), subcoeruleus (SubC/A7), substantia nigra pars compacta (SNC/A9). Neuronal populations highlighted in this review are presented in black and red (vulnerability), the remaining neuronal populations are in light grey. The X sign represents the absence of neurogenesis from the corresponding progenitor. *involved but not required. **medial position. See Supplementary Information for details, including references to supporting literature

In the visceromotor specification program, *Ascl1* is expressed but not required.⁸⁶ Post-mitotic motor neurons require the expression of *Isl1*,^{65, 87, 88} and *Tbx20* is only expressed within branchiomotor and visceromotor neurons,^{65, 89} like the 10N visceromotor neurons. Both *Lhx3/4* are not expressed in the 10N visceral motor neurons, since they are key determinants of the ventral pathway in motor neurons and this population has a dorsal orientation of their axonal projections.^{65, 90} Developing visceromotor neurons do not express genes characteristic of dorsal progenitor sub-types, such as *Dbx1/2*⁹¹ and *Lrx3*.⁹² Also, their specification is not dependent on the somatic motor neuron markers *Pax6*,^{65, 93} *Mnx1*,^{65, 94} *Olig2*,^{65, 92} or *Isl2*.^{65, 87, 88}

Subtype specification program of visceromotor neurons In early PD, Lewy pathology,^{7, 11} but not cell loss, has been reported in the inferior salivatory nucleus (IS), which is adjacent to the 10N, and projects visceromotor neurons within the glossopharyngeal nerve. In contrast, Lewy pathology is more pronounced and is consistently observed in the 10N.¹¹ Visceromotor neurons from the IS and 10N nuclei both originate from basal p3 progenitors and during development they migrate dorsally into an alar position^{64, 65} (Fig. 4a, b). Although these subtypes share the same general specification program, they have different rhombomeric origins. The visceromotor neurons from the IS originate from R6,

while those from the 10N originate from R7-8.^{64, 65} IS progenitors require the *Mafb* gene to be present and the *Hoxa4* and *Hoxb4* genes to be absent, whereas 10N progenitors require the opposite (Fig. 4d). To our knowledge, no subtypes have been reported based on variations of the general specification program described above.

Serotonergic populations of raphe nuclei. Raphe nuclei contain the central serotonergic populations (B1–9),⁹⁵ totalling 20,000–30,000 neurons in rats,⁹⁵ which are distinguishable by their anatomical location, and can be divided into caudal (B1–B4) and rostral clusters (B5–B9). Raphe nuclei clusters^{61, 96} are positioned caudal and rostral to rhombomere R4, which is a neuromeric segment that is only populated by motor neurons.⁸²

Progenitors of serotonergic neurons of raphe nuclei Raphe nuclei contain serotonergic neurons generated from a mixture of rhombomere-derived basal p3 progenitors, which initially reside in the bilateral paramedian territories that flank the floor plate^{48, 51, 58, 61, 97} (Fig. 4a, b). These p3 progenitors are common to brainstem visceromotor neurons and branchio-motor neurons,^{57, 82} with the exception of those that are R4-derived.^{48, 58, 61}

A multipotent pool of hindbrain p3 progenitors are differentially induced along the AP axis, due to a gradient signalling by RA,

FGF8 signalling at rostral positions, FGF4 signalling at caudal positions and ventral SHH signalling.⁹⁸ Differential combinatorial induction across the hindbrain determines the neuronal sub-type specification program,^{51, 61} via a rhombomere-specific expression of *Hox* genes (Fig. 4d and Supplementary tables 1, 2). Differential expression of *Hox* genes, the *Hox code*, results in considerable differences between caudal and rostral serotonergic profiles. Caudal serotonergic populations, which localise within B1–B4 populations, result from progenitors expressing multiple *Hox* genes, while the rostral serotonergic populations within B1–B4 populations result from progenitors highly expressing *Hmx2/3* genes (Fig. 4d upper table). Both Shh signalling at ventral positions⁹⁹ and rhombencephalic absence of *Otx2* expression are required for the correct development of a serotonergic phenotype.^{61, 100}

General specification program of serotonergic neurons of raphe nuclei The general specification program for serotonergic neurons is known^{96, 101} although the details of the molecular mechanisms are still poorly understood.^{58, 60, 61, 102–105} In mice, the rostral and caudal cluster of serotonergic neurons start to differentiate at E10 and E11, respectively^{51, 57, 82} and their morphology is only defined after the P0 stage. The serotonergic developmental program mostly occurs through the activation of a pair of genetic cascades^{51, 57, 98, 106} (Fig. 4d and Supplementary tables 1, 2). The first cascade involves ventral SHH signalling and subsequent activation of both *Nkx* transcription factors (*Nkx2-2*) and *Foxa2* expression, which results in activation of *Gata* transcription factors (*Gata2/3*) and *Lmx1b*.^{51, 61, 100} This specification program requires the absence of *Otx2* expression and the presence of *Nkx2-2* expression.^{51, 84} Importantly, there is also a parallel secondary cascade, where *Ascl1* expression is activated around E11.5^{82, 107} and instructs a sequential expression of *Gata3*, *Lmx1b*, and *Insm1*.⁵¹ *Ascl1* and *Insm1* are both part of the genetic regulatory network that controls serotonergic identity,¹⁰⁸ where *Insm1* expression contributes with an additional control of THP2 expression.⁵¹ On the other hand, *Lmx1b*, which is expressed downstream from *Gata2/3* in both raphe clusters, is required for terminal differentiation and maintenance of all serotonergic populations. Like all aminergic neurons, *Lmx1b* has been suggested to regulate the expression of the vesicular monoamine transporter.^{51, 109} *Lmx1b* might also regulate SLC6A4 (SERT) and TPH2 expression¹¹⁰ in mature populations.

It has also been described that a complete neurochemical serotonergic phenotype is, to a certain extent, controlled by *Pet1*.⁵¹ Both *Lmx1b* and *Pet1* have been implicated in the regulation of SLC6A4^{110–112} and *Pet1* has been described as the only gene whose expression is limited to hindbrain serotonergic neurons. *Pet1* precedes the expression of serotonin by 12 h and acts on the binding sites closer to genes involved in the maturation of the serotonergic phenotype. Examples include TPH2, DDC, SLC6A4 and HTR1A (5-HT1a).^{111, 112} The developmental combination of *Nkx2-2*, *Lmx1b*, and *Pet1* seem to be sufficient for the generation of serotonergic neurons in ectopic expression studies.¹⁰⁹ However, across all serotonergic populations (B1–9), *Pet1* expression is not necessary for specification of ~30% of all serotonergic neurons, which have projections to highly selective targets in the brain and transcend classic anatomical subdivisions of the raphe.^{51, 59, 112}

Subtype specification program of serotonergic neurons of raphe nuclei Distinct subtypes of serotonergic populations (reviewed in ref. 105) have been defined in different raphe nuclei, either biochemically,^{44, 48, 60, 113, 114} based on distinct axonal trajectories and firing patterns,^{115, 116} or based on rhombomere-specific developmental programs.^{48, 51, 58–61, 101, 105} Both caudal and rostral clusters of the raphe, in the medulla and pons, respectively,

contain nuclei that tend to display differential vulnerability to degeneration, but further histopathological studies would be desirable.

In the medullary raphe (caudal cluster), the raphe obscurus (ROb/B2) is more vulnerable than the raphe magnus (RMg/B3, Supplementary 1.2). The raphe obscurus (ROb/B2) is derived from R7–8 progenitors, which do not require *Egr2* expression (Fig. 4d). The raphe magnus (RMg/B3) is thought to be derived partially from R5 progenitors, requiring early developmental expression of *Egr2*,⁶² and also partially from R6–7 progenitors, which do not require *Egr2* expression (Fig. 4d). In the pons (rostral cluster), the median raphe (MnR/B8) is especially vulnerable, while the dorsal raphe (DR/B6–7) is less vulnerable (Supplementary 1.2). The median raphe (MnR/B8) originates from a mixture of R1–3 progenitors, while the dorsal raphe (DR/B6–7) originates from R1 progenitors⁵⁸ (Fig. 4b).

Expression of *En1* and *En2* play an intrinsic role in the development of all R1-derived serotonergic neurons.⁴⁴ Unlike other serotonergic neurons, all R1-derived serotonergic neurons require the expression of *Nkx6-1*⁵¹ and do not require expression of *Insm1* for terminal differentiation.^{51, 108} In another deviation from the general specification program, a subset of R1-derived serotonergic neurons require expression of *Nkx2-2*.^{51, 84} The relative vulnerability of R1–3-derived neurons within the median raphe is not known.

Noradrenergic populations of the coeruleus complex. Central noradrenergic neurons are found in the medulla (A1, A2) and the pons (A4–7). Within the coeruleus complex of the pons, we focus on the LC (A6), which is the largest central noradrenergic population and the SubC (A4). Coeruleus complex noradrenergic neurons are generated from a mixture of specific rhombomere-derived neuronal progenitors located within R1–6.⁶³

Progenitors of coeruleus complex noradrenergic neurons During development, multipotent rostral alar progenitors are induced by FGF8 and WNT signalling, from the anteriorly adjacent isthmus organiser, enabling the expression of *En1/2*, and the AP-graded RA signalling influences all rhombomere-derived progenitor pools, which result in the expression of specific combinations of *Hox* genes (Fig. 4d and Supplementary table 1). BMPs (BMP5, 7), which are produced in the dorsal ectoderm and roof plate, establish a dorsoventral signalling gradient that specifies the identity of caudodorsal progenitors¹¹⁷ (Fig. 4d and Supplementary table 2). In mice, LC development requires NOTCH-RBPJ signalling and its direct regulation of *Ascl1* expression, as well as its indirect regulation through the target gene *Hes1*.¹¹⁸ In zebrafish, Shh signalling may play an indirect role in the maintenance of LC noradrenergic populations.¹¹⁹

General specification program of coeruleus complex noradrenergic neurons In mice, the noradrenergic specification program begins with the acquisition of the correct alar progenitor identity and is followed by neurogenesis around E9 (Fig. 4d and Supplementary tables 1, 2). At least four transcription factors, *Ascl1*, *Phox2a/b* and *Tlx3*,^{34, 120, 121} are required for this program. Dorsal BMP signalling is required for the downstream expression of *Ascl1*, *Phox2a/b*^{34, 117, 122} and analysis of knock-out phenotypes for these genes suggest that they act according to a linear cascade. *Ascl1* expression is essential for dopamine beta-hydroxylase (DBH) expression in all noradrenergic populations and it induces the expression of both *Phox2a* and *Phox2b* in the LC.^{120, 123–126} *Phox2a/b* expression is required for correct specification and differentiation of LC noradrenergic populations,^{124, 127–129} and mid- to hind-brain motor neurons.¹²¹ *Phox2a* is required for the activation of *Phox2b*, and the latter is also necessary for the expression of DBH, which is a key enzyme in

noradrenaline synthesis. *Lmx1b* seems to be required for noradrenergic development, since knockout mice present no vesicular monoamine transporter immunoreactivity in the brainstem.^{51, 109, 130}

While *Insm1* expression is necessary for the timely onset of TH expression, the expression of *Nr2f6*¹³¹ and transient expression of *Onecut1/2/3* are both required for full development of LC noradrenergic neurons.¹³² On the contrary, *Gata2* and *Gata3* are not expressed in LC and there is no strict correlation between expression of these genes and noradrenergic differentiation.¹³³

Coeruleus complex noradrenergic neuronal subtype specification programs Within the coeruleus complex, the LC is more vulnerable to degeneration than the SubC (Supplementary 1.3). The LC (A6) mainly originates from a dorso-alar R1 progenitor pool^{63, 134} (Fig. 4a, b). Alar progenitor pools from R1 to R6 each contribute to the SubC (A4)⁶³ (Fig. 4b), which can be subdivided into dorsal and ventral parts. Like the LC, the dorsal SubC mainly derives from R1 progenitors, but can also include some R2-4 derived neurons. The ventral SubC is mainly derived from alar R4 progenitors, but can also include R2-3 derived neurons.⁶³ A few R2-derived neurons are consistently observed within both the LC and the SubC, and can be identified by the expression of *Hoxa2*.⁶³

Almost all of the LC (A6) and the dorsal part of the SubC (A4) arise from the aforementioned dorso-alar R1 progenitor pool. These progenitors require the expression of *En1* and during development they migrate ventrocaudally to a basolateral location within R1.^{63, 134, 135} A particular subset of R1-derived neurons can be further distinguished within the caudal LC as they require transient expression of *Tlx3* to induce expression of DBH.¹³⁶ In contrast to noradrenergic LC neurons, the noradrenergic neurons of the SubC and other CNS populations (A1/2/5/7) do not require the developmental expression of *Phox2a*.¹²⁴ Within each of these anatomically defined nuclei, especially in the C2/A2 and C1/A1 medullary nuclei, there is a subset of neurons that have not yet been associated with any particular rhombomere.⁶³

Mesodiencephalic dopaminergic populations. Mesodiencephalic DN are organised ventrally in a continuum along the mesencephalon and diencephalon.¹³⁷⁻¹⁴² In the mouse ventral midbrain, the retrorubral field (A8), SNC (A9), and VTA (A10) populations together contain 20,000–30,000 DN, representing almost 75% of all central DN.^{68, 143, 144}

Progenitors of mesodiencephalic DN In the mesodiencephalon, a competent pool of multipotent floor plate progenitors is generated and maintained once this region has been defined (Fig. 4d and Supplementary table 1). The correct positioning of the isthmus requires *Gbx2/Otx2*^{98, 145} and subsequent interaction between floor plate-produced SHH and isthmus-produced FGF8 is required for a ventral mesodiencephalic dopaminergic phenotype (Fig. 4d and Supplementary table 2). WNT1 is expressed in both dorsal (roof plate) and medioventral (floor and basal plate) midbrain and, like FGF8, WNT1 is also produced within the isthmus and required for the development of bilaterally flanking mesodiencephalic dopaminergic populations.¹⁴⁶ TGF β and other members of TGF β superfamily¹⁴⁷ are essential for the proper development of these populations.¹⁴⁸ It has been described that RA signalling is involved in the terminal differentiation program where it is suggested to be essential for a SNc subset of DA neurons.¹⁴⁹

Combinatorial induction diversifies genetic regulation⁶⁸ and generates multiple heterogeneous subsets of mesodiencephalic progenitors. Along the anteroposterior axis FGF8, WNT1 and BMP are sensed differently due to variable distance to organisational centres¹⁵⁰ (Fig. 4d and Supplementary tables 1, 2). At least eight

different subsets of ventral mesodiencephalic progenitors have already been proposed¹⁵¹ arising from spatiotemporal inductive differences, including floor plate mediolateral differences in SHH signalling.^{151, 152} In contrast to continuous *Shh* expression within the hindbrain floor plate, mesodiencephalic precursors transiently express *Shh* due to suppression via WNT signalling, which causes a unique neurogenic response within brainstem floor plate precursors and has been suggested to be a prerequisite for differentiation of DN.¹⁵³⁻¹⁵⁷ Regional *Otx2* expression within the mesodiencephalon is essential for the unique neurogenic potential of mesodiencephalic floor plate cells¹⁵⁸ (through the expression of *Lmx1a*⁶⁸), since hindbrain and spinal cord floor plate precursors do not appear to undergo neurogenesis.¹⁵⁸ In mesodiencephalic floor plate cells, the absence of intrinsic *Otx2* expression shifts them towards a serotonergic neuronal fate.¹⁵⁹⁻¹⁶¹

General specification program of mesodiencephalic DN In mice, the first sign of a dopaminergic phenotype appears around E9, with the expression of *Lmx1a* and *Msx1*,^{67, 150, 162} while the corresponding mature mesodiencephalic DN are only first detectable around E10 by the expression of TH, in the absence of DBH expression.¹⁶³ Multiple intrinsic factors and extrinsic inducers are required to activate the correct differentiation program (Fig. 4d and Supplementary table 2), which consists of many inter-dependent downstream genetic cascades.^{68, 144}

The mesodiencephalic DN general specification program occurs once the corresponding progenitor markers are expressed together with *Foxa1/2*, *Lmx1b*, *Msx2* and *Neurog2*.¹⁶⁴ In chick, *Foxa2* is necessary and sufficient for specification of the entire floor plate into a dopaminergic phenotype,^{165, 166} and its expression can occur via a SHH-dependent or SHH-independent pathway.¹⁶⁷ In this program, both *Foxa1*^{168, 169} and *Foxa2*¹⁶⁸⁻¹⁷¹ are necessary to promote neurogenesis by maintaining *Lmx1a* and *Lmx1b* expression,¹⁶⁹ regulating the expression of *Neurog2* and *Ascl1*¹⁶⁸ and inhibiting *Nkx2-2* expression.¹⁶⁹ *Ascl1* has no detected function in the development of normal mesodiencephalic DN, although it can partially rescue the generation of their precursors in the absence of *Neurog2*.

While *Lmx1a*^{172, 173} is required for early differentiation, *Lmx1b*^{172, 174} is an essential regulator³⁴ that is co-expressed with *Lmx1a* and the transcriptional repressor *Msx1*. *Lmx1a* expression appears to be directly induced by SHH and it ultimately induces multiple proneural factors, such as *Neurog2*, and then *Msx1*.^{158, 164, 175} *Neurog2* expression starts neurogenesis, and is required for neuronal differentiation of mitotic precursors and is maintained after neuronal maturation.^{150, 176, 177} *Msx1* inhibits the expression of neurogenesis regulators, such as *Nkx6-1*,¹⁷⁵ nevertheless its expression is neither necessary nor sufficient for the generation of mesodiencephalic DNs.¹⁵⁰ At E10.5-E11.5 in mice, both *Neurog2* and *Msx1* are responsible for proliferative cascades that allow cells to become postmitotic and to migrate radially from the initial ventricular surface into an intermediate zone of the floor plate mantle. Furthermore, loss of *Onecut1/2/3* expression results in a diminished generation of ventral mesencephalic DN.¹⁷⁸

Expression of *Nr4a2*^{158, 175, 179, 180} (*Nurr1*) is crucial for the generation and maintenance of mesodiencephalic dopaminergic populations and is downregulated in PD patients.¹⁸¹ Its expression occurs around E10.5 in mice when the corresponding mitotic precursors exit the cell cycle. This expression marks the developmental stages of both young and fully differentiated neurons, and regulates the expression of proteins involved in dopamine synthesis¹⁸² and transport.¹⁸³⁻¹⁸⁵ *Nr4a2* represses *Neurog2* expression and its expression can be observed across the mesencephalic flexure, diencephalon and posterior hypothalamus, although it is not exclusively present in mesodiencephalic DN.^{140, 180, 182}

At later stages of development, immature postmitotic cells derived from the mesodiencephalic floor plate also express *Pitx3*,^{149, 186} which is dependent on correct regional specification by *Lmx1b*¹⁸⁷ and modulated by *En1*.⁶⁹

Subtype specification program of mesodiencephalic DN In the mesodiencephalon, the SNC (A9) and the VTA (A10) are vulnerable to degeneration in PD, but SNC DN are considerably more affected than those from the VTA (Supplementary 1.4). Despite their shared origin and general specification program, subtypes of mature mesodiencephalic DN display clear phenotypic diversity.^{70–73, 78} Multiple vertebrate studies,^{139, 188–193} including in mice,^{139, 194–196} suggest that the SNC and VTA both contain mesodiencephalic DN from multiple mesodiencephalic neuromeres (M1-2 and P2-1)^{68, 158, 197} (Fig. 4a, b).

After neurogenesis and during the subsequent radial and tangential migration towards their final locations, post-mitotic mesodiencephalic cells differentiate into mature neurons. However, the developmental programs for VTA and SNC DN are distinct.^{70, 78, 187} The SNC and VTA both contain different subtypes of mesodiencephalic DN.^{143, 144} VTA DN arise from paramedian floor plate progenitors, whereas SNC DN, located lateral to the VTA, arise from median floor plate progenitors.^{152, 155, 156, 198–200}

Later, during migration, the intrinsic expression of *Otx2* becomes restricted to VTA subtypes,^{140, 201, 202} while *Sox6* expression becomes restricted to SNC subtypes.^{72, 202–205}

Although both SNC and VTA subtypes express *Pitx3* during terminal differentiation^{143, 144} and in aged humans,²⁰⁶ only the SNC, in terms of survival, requires expression of *Pitx3*.^{68, 186, 207} *Pitx3* suppresses the intrinsic expression of *En1* and thereby of posterior markers such as CCK in SNC DN.^{69, 70, 78} Different patterns of gene expression during development permit the identification of two foetal dopaminergic neuronal subtypes that diversify into five adult mouse DN subtypes.^{47, 144} The relationship between molecular subtypes and selective vulnerability needs to be established further through advanced gene function analysis. A first sign of such a relationship has been described through the selective requirement of SNC neurons towards RA (RA) signalling and detoxification machinery in the breakdown pathway of DA. The aldehyde dehydrogenase enzyme, *Ahd2*, depends on the activity of *Pitx3* and *En1*, and the selective dependence of the SNC to this gene has been described.^{149, 208} This suggests that the SNC has specific requirements in term of genetic programming and metabolic demand that require the neurons to be equipped with high enough levels of *Ahd2*. This requirement is an inherent vulnerability in terms of function and distinguishes the SNC DN from VTA DN. Additional evidence towards this concept might be discovered through using the subset transcriptome data as described above. In addition to this, nigral neurons have a higher rate of oxidative phosphorylation and a more complex axonal arborisation.²⁰⁹ Such phenotypic differences rely on specific molecular programming as discussed above and highlight the importance of understanding the molecular machinery behind SNC programming.

DISCUSSION

A shared requirement for a specific set of developmental transcription factors can be used to infer functional similarity as well as proximity within a cellular developmental lineage (Fig. 4d). In PD, vulnerable neuronal populations do share certain functional similarities, so we synthesised the literature on required activity of or inactivity of a set of 51 developmental transcription factors across five brainstem regions with clear evidence of vulnerability to degeneration in PD. Within each region, we also refine our analysis to include more and less vulnerable nuclei and neuronal subtypes. Variations in the types, amount and duration of developmental induction results in different sets of required active (or inactive) developmental transcription factors, thereby shaping the landscape

of lineage commitment possibilities, e.g., rhombencephalic p3 progenitors receiving a longer duration of developmental induction commit to a serotonergic fate, which correlates with an increase in *Foxa2* expression and a decrease of *Nkx2-9* and *Phox2b* expression, in a switch from an otherwise motor fate.^{57, 82}

Our synthesis suggests that some developmental requirements are shared between vulnerable brainstem regions. We find that vulnerable neuronal populations often share a common requirement for Shh signalling, but this induction alone is not sufficient to predict neuronal vulnerability in PD, since many other ventral brainstem nuclei do not seem to be especially vulnerable to degeneration. The activity of some transcription factors is similar in each of the five studied brainstem populations that are vulnerable to degeneration in early PD. For example, *Ascl1* is expressed in all five populations, although it is only required for the development of LC noradrenergic and raphe serotonergic populations. Together with *Phox2b*, *Ascl1* co-regulates catecholamine synthesising enzymes in noradrenergic populations.²¹⁰ During the specification of neuronal fate, the requirement for *Ascl1* activity varies depending on the lineage of an individual cell.²¹¹ Absence of *Ascl1* results in loss of olfactory and autonomic neurons as well as delayed differentiation of retinal neurons.^{212, 213}

Phox2b is required for development of noradrenergic neurons, and visceromotor neurons of the dorsal motor nucleus of the vagus (10N), while its paralogue *Phox2a* is also required for LC, but not subcoeruleus (SubC) noradrenergic neurons. Trochlear motor and oculomotor neurons share the same *Phox2a* and *Phox2b* developmental requirements as LC noradrenergic neurons,^{121, 128} however these neurons do not seem to be vulnerable in PD. In most of the brainstem, *Phox2b* represses serotonergic differentiation and therefore it is required to be absent for specification of raphe serotonergic neurons. Although *Phox2b* is expressed in caudal midbrain dopaminergic populations, it does not seem to be required for the specification of substantia nigra, pars compacta (SNC) DN.²¹⁴

In mice, *Lmx1b* expression is required for the expression of monoamine vesicular transporters in all brainstem aminergic neurons (dopaminergic, noradrenergic and serotonergic),^{51, 109, 130, 174} despite not being required for zebrafish LC noradrenergic populations.¹⁸⁴ Visceromotor neurons of the 10N do not express vesicular monoamine transporters,³³ but it is not known if *Lmx1b* is required for the development of these neurons. Importantly, *Lmx1a/b* is required to control autophagic-lysosomal function, integrity of nerve terminals, long-term survival of midbrain DN²¹⁵ and recently has been implicated in regulation of mitochondrial function.²¹⁶ *Lmx1a/b* conditional ablation, after neuronal specification, results in abnormalities that show striking resemblance to early cellular abnormalities seen in PD. Moreover, a decrease in *Lmx1b* expression has been reported in midbrain DN of PD patients.²¹⁵ Finally, it has been shown that a specific subset of SNC neurons is absent in *Lmx1a* mutants.²¹⁷

Within each of the five vulnerable brainstem regions we considered, different nuclei are more or less vulnerable to degeneration in PD. Of the visceromotor neurons, those in the 10N are more vulnerable to degeneration than those in the inferior salivatory (IS) nucleus.^{7, 11} Even though these nuclei share the same general specification program, the IS originates from the R6 rhombomere, while the 10N originates from the R7-8 rhombomeres.^{64, 65}

Differential vulnerability within mesodiencephalic dopaminergic nuclei is well established and there also exist differences in their developmental specification programs. Medial and paramedian mesodiencephalic floor plate progenitors generate DN in the SNC and VTA, respectively. Both express *Pitx3*,^{143, 144, 206} but knock-out of *Pitx3* results in selective loss of SNC neurons,^{68, 186, 207} so *Pitx3* expression is required for the development of the SNC but not required for the VTA.

Within some vulnerable nuclei, the existence of different developmental programs that generate different neuronal subtypes are known, e.g., three dopaminergic neuronal subtypes can

be distinguished in human VTA.¹⁴⁴ Within other vulnerable nuclei, differential vulnerability of anatomically distinct areas is known in PD, e.g., there is an increasing fraction of cell loss from medial to dorsolateral SNC.²¹⁸ However, to our knowledge, a simultaneous analysis of developmental subtype-specific markers and anatomically resolved quantification of cell loss in PD have not been reported. We suggest that tissue samples from previous cell loss studies should be immunohistopathologically revisited to check if there is a relationship between the relative degree of neuroprotection of neuronal subtypes that are defined by differential expression of known developmental transcription factors responsible for adult maintenance.

The developmental origins of selectively vulnerable neurons needs further clarification. Further experimental work is required to assess the temporal requirement for certain transcription factors (e.g., *Onecut*, *Gata2/3*, *Isl1*, *Insm1*, *Pet*, *Tbx20*, *Nr2f6*, *Nr4a2*, *Pitx3*, *Lmx1a*) that seem to be necessary for specification of a subset of nuclei but are not yet known to be required for other vulnerable nuclei that we have considered. The need for data on the timing of requirements is supported by evidence that *Nr4a2* is required for maintenance of mesencephalic DN in adult mice.²¹⁹

This review presents a comprehensive manual curation of the development of ten vulnerable brainstem nuclei in five different brainstem regions. Our compendium of transcription factor requirements is accurate but not yet comprehensive at genome scale. This limitation can be partially overcome by complementing our compendium with developmental omics data, e.g., the Allen developmental primate atlas.²²⁰ In turn, the noise in such large scale datasets can be mitigated by using our manually curated transcription factor requirements as an anchor to benchmark data integration algorithms. Ultimately, cell fate mapping and gene inactivation studies are required to establish the combination of genes required for developmental specification of each neuronal subtype.

CONCLUSIONS

In early PD, mature neurons that are selectively vulnerable to degeneration can be identified by some shared biochemical, morphological and functional characteristics. However, the molecular basis for selective vulnerability in PD remains to be fully elucidated. As mature neuronal identity is largely the result of a developmental program that is specific to each cell type (Fig. 4e), for five brainstem regions, each with at least two nuclei with varying degrees of vulnerability, we compared and contrasted their cellular lineage and their requirement for absence or presence of 51 transcription factors (Fig. 4d). Certain combinations of transcription factors seem to be required for development of many vulnerable brainstem regions, e.g., *Asc1l* coregulates catecholamine-synthesising enzymes in noradrenergic populations.

Within vulnerable brainstem regions, certain nuclei are more vulnerable to degeneration than others and this correlates with important differences in the developmental transcription factor requirements for their lineage, e.g., *Pitx3* is expressed in all mesodiencephalic DN but it is only required for development of SNC, but not ventral tegmental area, DN. Of the vulnerable visceromotor neurons, those from the inferior salivary nucleus are less vulnerable than those from the dorsal motor nucleus of the vagus, yet they both have almost the same developmental program, except that they originate from separate rhombencephalic neuromeres. Tracing the molecular consequences of developmental specification programs in more and less vulnerable brainstem nuclei, e.g., with experimental determination of the genomic targets of key transcription factors would help to identify the molecular species that participate in the biochemical pathways that could be associated with differential vulnerability. The development of a comprehensive molecular basis for the shared characteristics of vulnerable neurons is an essential pre-requisite for development of drugs targeted towards the causes of PD.

METHODS

To completely reconstruct an anatomically resolved cellular developmental lineage of adult human neurons as well as the corresponding developmental transcription factors would require human experimental data, which is not available. Fortunately, the brainstem and its development is highly homologous between mammalian species. It also contains the most archaic neuronal networks in the brain, which may be related to susceptibility degeneration in PD.²²¹ Therefore, we relied on manual curation of developmental studies in model organisms to obtain the details of neuronal progenitor patterning, neurogenesis and cell fate specification^{222, 223} as well as the details on genoarchitecture and neuromere-related lineage mapping.²²⁴ Unless indicated otherwise, all statements refer to murine studies. Differentiation and fate restriction requirements were studied by considering multiple loss-of-function studies, which describe the dependence of each neuronal population on a specific set of gene products. In this regard, when possible, we highlight whether a particular gene is necessary to be active, or necessary to be inactive, for each lineage (Fig. 4d). Furthermore, migration patterns were selectively reconstructed by curating lineage tracing studies (Fig. 4c). We used the same neuromere scheme and ontology as the Allen Developing Mouse Brain reference atlas.¹⁴²

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files.

ACKNOWLEDGEMENTS

We would like to thank Jens Schwamborn and Diana El Assal for critical comments on early versions of this manuscript. We would also like to thank the reviewers for critical comments that significantly improved the manuscript. This project has received funding from the the Fonds national de la recherche, Luxembourg under grant #6669348, the European Union's Horizon 2020 research and innovation programme under grant agreement #668738, from the Fonds national de la recherche under the aegis of the EU Joint Programme—Neurodegenerative Disease Research, grant agreement INTER/JPND/ 14/02/SynSpread, and support for international scientific exchange from the Fondation du Pélican.

AUTHOR CONTRIBUTIONS

R.F. and R.B. conceived the project, M.O. and M.S. reviewed and synthesised the literature. M.O., R.B., M.S. and R.F. wrote the manuscript.

ADDITIONAL INFORMATION

Supplementary Information accompanies the paper on the *npj Parkinson's Disease* website (doi:10.1038/s41531-017-0022-4).

Competing interests: The authors declare that they have no competing financial interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Postuma, R. B. et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* **30**, 1591–1601 (2015).
2. Dickson, D. W. et al. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol.* **8**, 1150–1157 (2009).
3. Fahn, S. & Sulzer, D. Neurodegeneration and neuroprotection in Parkinson disease. *NeuroRx* **1**, 139–154 (2004).
4. Hawkes, C. H. The prodromal phase of sporadic Parkinson's disease: does it exist and if so how long is it? *Mov. Disord.* **23**, 1799–1807 (2008).

5. Goetz, C. G. et al. Movement Disorder Society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* **23**, 2129–2170 (2008).
6. Hawkes, C. H., Del Tredici, K. & Braak, H. A timeline for Parkinson's disease. *Parkinsonism Relat. Disord.* **16**, 79–84 (2010).
7. Halliday, G. M. et al. Neuropathology of immunohistochemically identified brainstem neurons in Parkinson's disease. *Ann. Neurol.* **27**, 373–385 (1990).
8. Gai, W. P., Blumbergs, P. C., Geffen, L. B. & Blessing, W. W. Age-related loss of dorsal vagal neurons in Parkinson's disease. *Neurology* **42**, 2106–2111 (1992).
9. Zweig, R. M., Cardillo, J. E., Cohen, M., Giere, S. & Hedreen, J. C. The locus ceruleus and dementia in Parkinson's disease. *Neurology* **43**, 986–991 (1993).
10. Zarow, C., Lyness, S. A., Mortimer, J. A. & Chui, H. C. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in alzheimer and parkinson diseases. *Arch. Neurol.* **60**, 337–341 (2003).
11. Del Tredici, K., Rüb, U., De Vos, R. A. I., Bohl, J. R. E. & Braak, H. Where does parkinson disease pathology begin in the brain? *J. Neuropathol. Exp. Neurol.* **61**, 413–426 (2002).
12. Halliday, G. M., Del Tredici, K. & Braak, H. Critical appraisal of brain pathology staging related to presymptomatic and symptomatic cases of sporadic Parkinson's disease. *J. Neural Transm. Suppl.* **70**, 99–103 (2006).
13. Sulzer, D. & Surmeier, D. J. Neuronal vulnerability, pathogenesis, and Parkinson's disease: neuronal vulnerability, pathogenesis, and PD. *Mov. Disord.* **28**, 715–724 (2013).
14. Braak, H. et al. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* **24**, 197–211 (2003).
15. Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H. & Tredici, K. D. Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res.* **318**, 121–134 (2004).
16. Braak, H. et al. Stanley fahn lecture 2005: the staging procedure for the inclusion body pathology associated with sporadic Parkinson's disease reconsidered. *Mov. Disord.* **21**, 2042–2051 (2006).
17. Braak, H. & Tredici, K. D. Invited article: nervous system pathology in sporadic Parkinson disease. *Neurology* **70**, 1916–1925 (2008).
18. Braak, H. & Tredici, K. D. *Neuroanatomy and Pathology of Sporadic Parkinson's Disease* (Springer, 2008).
19. Conway, K. A., Rochet, J.-C., Bieganski, R. M. & Lansbury, P. T. Kinetic stabilization of the α -synuclein protofibril by a dopamine- α -synuclein adduct. *Science* **294**, 1346–1349 (2001).
20. Müller, C. M. et al. Staging of sporadic Parkinson disease-related alpha-synuclein pathology: inter- and intra-rater reliability. *J. Neuropathol. Exp. Neurol.* **64**, 623–628 (2005).
21. Parkkinen, L., Kauppinen, T., Pirttilä, T., Autere, J. M. & Alafuzoff, I. α -synuclein pathology does not predict extrapyramidal symptoms or dementia. *Ann. Neurol.* **57**, 82–91 (2005).
22. Burke, R. E., Dauer, W. T. & Vonsattel, J. P. G. A critical evaluation of the braak staging scheme for Parkinson's disease. *Ann. Neurol.* **64**, 485–491 (2008).
23. Halliday, G., Hely, M., Reid, W. & Morris, J. The progression of pathology in longitudinally followed patients with Parkinson's disease. *Acta Neuropathol.* **115**, 409–415 (2008).
24. Parkkinen, L., Pirttilä, T. & Alafuzoff, I. Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance. *Acta Neuropathol.* **115**, 399–407 (2008).
25. Jellinger, K. A. A critical evaluation of current staging of α -synuclein pathology in Lewy body disorders. *Biochim. Biophys. Acta* **1792**, 730–740 (2009).
26. Dugger, B. N. & Dickson, D. W. Cell type specific sequestration of choline acetyltransferase and tyrosine hydroxylase within lewy bodies. *Acta Neuropathol.* **120**, 633–639 (2010).
27. Damier, P., Hirsch, E. C., Agid, Y. & Graybiel, A. M. The substantia nigra of the human brain II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain* **122**, 1437–1448 (1999).
28. Wichterle, H., Gifford, D. & Mazzoni, E. Mapping neuronal diversity one cell at a time. *Science* **341**, 726–727 (2013).
29. Pearson, B. J. & Doe, C. Q. Specification of temporal identity in the developing nervous system. *Annu. Rev. Cell Dev. Biol.* **20**, 619–647 (2004).
30. Dasen, J. S., Tice, B. C., Brenner-Morton, S. & Jessell, T. M. A hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. *Cell* **123**, 477–491 (2005).
31. Kretschmar, K. & Watt, F. M. Lineage tracing. *Cell* **148**, 33–45 (2012).
32. Chen, L. et al. Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. *J. Neurosci.* **28**, 425–433 (2008).
33. Weihe, E., Depboylu, C., Schütz, B., Schäfer, M. K.-H. & Eiden, L. E. Three types of tyrosine hydroxylase-positive CNS neurons distinguished by dopa decarboxylase and VMAT2 Co-expression. *Cell. Mol. Neurobiol.* **26**, 659–678 (2006).
34. Goridis, C. & Rohrer, H. Specification of catecholaminergic and serotonergic neurons. *Nat. Rev. Neurosci.* **3**, 531–541 (2002).
35. Lajtha, A. & Sylvestre, V. *Handbook of Neurochemistry and Molecular Neurobiology* (Springer, 2008).
36. Hnasko, T. S. et al. Vesicular glutamate transport promotes dopamine storage and glutamate corelease in vivo. *Neuron* **65**, 643–656 (2010).
37. Stuber, G. D., Hnasko, T. S., Britt, J. P., Edwards, R. H. & Bonci, A. Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J. Neurosci.* **30**, 8229–8233 (2010).
38. Landis, S. C. Target regulation of neurotransmitter phenotype. *Trends Neurosci.* **13**, 344–350 (1990).
39. Ernsberger, U. & Rohrer, H. Development of the cholinergic neurotransmitter phenotype in postganglionic sympathetic neurons. *Cell Tissue Res.* **297**, 339–361 (1999).
40. Francis, N. J. & Landis, S. C. Cellular and molecular determinants of sympathetic neuron development. *Annu. Rev. Neurosci.* **22**, 541–566 (1999).
41. Yu, H.-H., Chen, C.-H., Shi, L., Huang, Y. & Lee, T. Twin-spot MARCM to reveal the developmental origin and identity of neurons. *Nat. Neurosci.* **12**, 947–953 (2009).
42. Sugino, K. et al. Molecular taxonomy of major neuronal classes in the adult mouse forebrain. *Nat. Neurosci.* **9**, 99–107 (2005).
43. Cahoy, J. D. et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci.* **28**, 264–278 (2008).
44. Wylie, C. J. et al. Distinct transcriptomes define rostral and caudal serotonin neurons. *J. Neurosci.* **30**, 670–684 (2010).
45. Wang, Z., Gerstein, M. & Snyder, M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* **10**, 57–63 (2009).
46. Sharma, K. et al. Cell type- and brain region-resolved mouse brain proteome. *Nat. Neurosci. advance online publication*, November (2015).
47. Poulin, J.-F. et al. Defining midbrain dopaminergic neuron diversity by single-cell gene profiling. *Cell Rep.* **9**, 930–943 (2014).
48. Okaty, B. W. et al. Multi-scale molecular deconstruction of the serotonin Neuron System. *Neuron* **88**, 774–791 (2015).
49. Park, J. et al. Inputs drive cell phenotype variability. *Genome Res.* **24**, 930–941 (2014).
50. Lefebvre, V., Dumitriu, B., Penzo-Mendez, A., Han, Y. & Pallavi, B. Control of cell fate and differentiation by sry-related high-mobility-group box (Sox) transcription factors. *Int. J. Biochem. Cell Biol.* **39**, 2195–2214 (2007).
51. Kiyasova, V. & Gaspar, P. Development of raphe serotonin neurons from specification to guidance. *Eur. J. Neurosci.* **34**, 1553–1562 (2011).
52. McKay, R. Stem cells in the central nervous system. *Science* **276**, 66–71 (1997).
53. Rao, M. S. Multipotent and restricted precursors in the central nervous system. *Anat. Rec.* **257**, 137–148 (1999).
54. Anderson, D. J. Stem cells and pattern formation in the nervous system: the possible versus the actual. *Neuron* **30**, 19–35 (2001).
55. Leone, D. P. et al. Regulation of neural progenitor proliferation and survival by $\beta 1$ integrins. *J. Cell Sci.* **118**, 2589–2599 (2005).
56. Ferran, J. et al. Genoarchitectonic profile of developing nuclear groups in the chicken pretectum. *J. Comp. Neurol.* **517**, 405–451 (2009).
57. Jacob, J. et al. Transcriptional repression coordinates the temporal switch from motor to serotonergic neurogenesis. *Nat. Neurosci.* **10**, 1433–1439 (2007).
58. Jensen, P. et al. Redefining the serotonergic system by genetic lineage. *Nat. Neurosci.* **11**, 417–419 (2008).
59. Kiyasova, V. et al. A genetically defined morphologically and functionally unique subset of 5-HT neurons in the mouse raphe nuclei. *J. Neurosci.* **31**, 2756–2768 (2011).
60. Gaspar, P. & Lillesaar, C. Probing the diversity of serotonin neurons. *Philos. Trans. R. Soc. Lond. B* **367**, 2382–2394 (2012).
61. Alonso, A. et al. Development of the serotonergic cells in murine raphe nuclei and their relations with rhombomeric domains. *Brain Struct. Funct.* **218**, 1229–1277 (2013).
62. Brust, R. D., Corcoran, A. E., Richerson, G. B., Nattie, E. & Dymecki, S. M. Functional and developmental identification of a molecular subtype of brain serotonergic neuron specialized to regulate breathing dynamics. *Cell Rep.* **9**, 2152–2165 (2014).
63. Robertson, S. D., Plummer, N. W., de Marchena, J. & Jensen, P. Developmental origins of central norepinephrine neuron diversity. *Nat. Neurosci.* **16**, 1016–1023 (2013).
64. Pattyn, A., Vallstedt, A., Dias, J. M., Sander, M. & Ericson, J. Complementary roles for Nkx6 and Nkx2 class proteins in the establishment of motoneuron identity in the hindbrain. *Development* **130**, 4149–4159 (2003).
65. Guthrie, S. Patterning and axon guidance of cranial motor neurons. *Nat. Rev. Neurosci.* **8**, 859–871 (2007).
66. Simeone, A. Genetic control of dopaminergic neuron differentiation. *Trends Neurosci.* **28**, 62–65 (2005).

67. Prakash, N. & Wurst, W. Development of dopaminergic neurons in the mammalian brain. *Cell. Mol. Life Sci.* **63**, 187–206 (2006).
68. Hegarty, S. V., Sullivan, A. M. & O'Keefe, G. W. Midbrain dopaminergic neurons: a review of the molecular circuitry that regulates their development. *Dev. Biol.* **379**, 123–138 (2013).
69. Veenvliet, J. V. et al. Specification of dopaminergic subsets involves interplay of En1 and Pitx3. *Development* **140**, 3373–3384 (2013).
70. Veenvliet, J. V. & Smidt, M. P. Molecular mechanisms of dopaminergic subset specification: fundamental aspects and clinical perspectives. *Cell. Mol. Life Sci.* **71**, 4703–4727 (2014).
71. Bodea, G. O. & Blaess, S. Establishing diversity in the dopaminergic system. *FEBS Lett.* **589**, 3773–3785 (2015).
72. Anderegg, A., Poulin, J.-F. & Awatramani, R. Molecular heterogeneity of midbrain dopaminergic neurons—moving toward single cell resolution. *FEBS Lett.* **589**, 3714–3726 (2015). (24PartA).
73. Blaess, S. & Ang, S.-L. Genetic control of midbrain dopaminergic neuron development. *Wiley Interdiscip. Rev. Dev. Biol.* **4**, 113–134 (2015).
74. Rekaik, H., Blaudin de Thé, F.-X., Prochiantz, A., Fuchs, J. & Joshi, R. L. Dissecting the role of engrailed in adult dopaminergic neurons – insights into Parkinson disease pathogenesis. *FEBS Lett.* **589**, 3786–3794 (2015). (24PartA).
75. Doucet-Beaupré, H., Ang, S.-L. & Lévesque, M. Cell fate determination, neuronal maintenance and disease state: the emerging role of transcription factors Lmx1a and Lmx1b. *FEBS Lett.* **589**, 3727–3738 (2015).
76. Huang, E. J. Ventral midbrain dopaminergic neurons: from neurogenesis to neurodegeneration. *FEBS Lett.* **589**, 3691–3692 (2015).
77. Kramer, E. R. & Liss, B. GDNF–Ret signaling in midbrain dopaminergic neurons and its implication for Parkinson disease. *FEBS Lett.* **589**, 3760–3772 (2015).
78. Arenas, E., Denham, M. & Villaescusa, J. C. How to make a midbrain dopaminergic neuron. *Development* **142**, 1918–1936 (2015).
79. Cordes, S. P. Molecular genetics of cranial nerve development in mouse. *Nat. Rev. Neurosci.* **2**, 611–623 (2001).
80. Briscoe, J. & Ericson, J. The specification of neuronal identity by graded sonic hedgehog signalling. *Semin. Cell Dev. Biol.* **10**, 353–362 (1999).
81. Jacob, J., Hacker, A. & Guthrie, S. Mechanisms and molecules in motor neuron specification and axon pathfinding. *BioEssays* **23**, 582–595 (2001).
82. Pattyn, A. et al. Coordinated temporal and spatial control of motor neuron and serotonergic neuron generation from a common pool of CNS progenitors. *Genes Dev.* **17**, 729–737 (2003).
83. Ericson, J., Morton, S., Kawakami, A., Roelink, H. & Jessell, T. M. Two critical periods of sonic hedgehog signaling required for the specification of motor neuron identity. *Cell* **87**, 661–673 (1996).
84. Briscoe, J. et al. Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. *Nature* **398**, 622–627 (1999).
85. Pattyn, A., Hirsch, M., Goridis, C. & Brunet, J. F. Control of hindbrain motor neuron differentiation by the homeobox gene Phox2b. *Development* **127**, 1349–1358 (2000).
86. Pattyn, A., Guillemot, F. & Brunet, J.-F. Delays in neuronal differentiation in Mash1/Ascl1 mutants. *Dev. Biol.* **295**, 67–75 (2006).
87. Thor, S., Andersson, S. G. E., Tomlinson, A. & Thomas, J. B. A LIM-homeodomain combinatorial code for motor-neuron pathway selection. *Nature* **397**, 76–80 (1999).
88. Tsuchida, T. et al. Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* **79**, 957–970 (1994).
89. Kraus, F., Haenig, B. & Kispert, A. Cloning and expression analysis of the mouse T-box gene Tbx20. *Mech. Dev.* **100**, 87–91 (2001).
90. Sharma, K. et al. LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. *Cell* **95**, 817–828 (1998).
91. Vallstedt, A. et al. Different levels of repressor activity assign redundant and specific roles to Nkx6 genes in motor neuron and interneuron specification. *Neuron* **31**, 743–755 (2001).
92. Novitsch, B. G., Chen, A. I. & Jessell, T. M. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the Bhlh repressor Olig2. *Neuron* **31**, 773–789 (2001).
93. Ericson, J. et al. Pax6 controls progenitor cell identity and neuronal fate in response to graded shh signaling. *Cell* **90**, 169–180 (1997).
94. William, C. M., Tanabe, Y. & Jessell, T. M. Regulation of motor neuron subtype identity by repressor activity of Mnx class homeodomain proteins. *Development* **130**, 1523–1536 (2003).
95. Jacobs, B. L. & Azmitia, E. C. Structure and function of the brain serotonin system. *Physiol. Rev.* **72**, 165–229 (1992).
96. Lidov, H. G. & Molliver, M. E. Immunohistochemical study of the development of serotonergic neurons in the rat CNS. *Brain Res. Bull.* **9**, 559–604 (1982).
97. Watson, C. Hindbrain. In *The Mouse Nervous System* (eds Watson, C., Paxinos, G. & Puelles, L.) Ch. 12, 398–423 (Academic, 2012).
98. Ye, W., Shimamura, K., Rubenstein, J. L. R., Hynes, M. A. & Rosenthal, A. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **93**, 755–766 (1998).
99. Matisse, M. P., Epstein, D. J., Park, H. L., Platt, K. A. & Joyner, A. L. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. *Development* **125**, 2759–2770 (1998).
100. Lumsden, A. & Krumlauf, R. Patterning the vertebrate neuraxis. *Science* **274**, 1109–1115 (1996).
101. Wallace, J. A. & Lauder, J. M. Development of the serotonergic system in the rat embryo: an immunocytochemical study. *Brain Res. Bull.* **10**, 459–479 (1983).
102. Gaspar, P., Cases, O. & Maroteaux, L. The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* **4**, 1002–1012 (2003).
103. Cordes, S. Molecular genetics of the early development of hindbrain serotonergic neurons. *Clin. Genet.* **68**, 487–494 (2005).
104. Deneris, E. S. Molecular genetics of mouse serotonin neurons across the lifespan. *Neuroscience* **197**, 17–27 (2011).
105. Smidt, M. P. & van Hooft, J. A. Subset specification of central serotonergic neurons. *Front. Cell. Neurosci.* **7**, 200–207 (2013).
106. Lillesaar, C., Tannhäuser, B., Stigloher, C., Kremmer, E. & Bally-Cuif, L. The serotonergic phenotype is acquired by converging genetic mechanisms within the zebrafish central nervous system. *Dev. Dyn.* **236**, 1072–1084 (2007).
107. Pattyn, A. et al. Ascl1/Mash1 is required for the development of central serotonergic neurons. *Nat. Neurosci.* **7**, 589–595 (2004).
108. Jacob, J. et al. Insm1 (IA-1) is an essential component of the regulatory network that specifies monoaminergic neuronal phenotypes in the vertebrate hindbrain. *Development* **136**, 2477–2485 (2009).
109. Cheng, L. et al. Lmx1b, Pet-1, and Nkx2.2 coordinately specify serotonergic neurotransmitter phenotype. *J. Neurosci.* **23**, 9961–9967 (2003).
110. Zhao, Z.-Q. et al. Lmx1b is required for maintenance of central serotonergic neurons and mice lacking central serotonergic system exhibit normal locomotor activity. *J. Neurosci.* **26**, 12781–12788 (2006).
111. Hendricks, T., Francis, N., Fyodorov, D. & Deneris, E. S. The ets domain factor pet-1 is an early and precise marker of central serotonin neurons and interacts with a conserved element in serotonergic genes. *J. Neurosci.* **19**, 10348–10356 (1999).
112. Hendricks, T. J. et al. Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. *Neuron* **37**, 233–247 (2003).
113. Lowry, C. A. & Hale, M. W. Serotonin and the neurobiology of anxious states. In *Handbook of Behavioral Neuroscience*, Vol. 21 (eds Müller, C. P. & Jacobs, B. L.) Ch. 3.6, 379–397 (Elsevier, 2010).
114. Calizo, L. H. et al. Raphe serotonin neurons are not homogenous: electrophysiological, morphological and neurochemical evidence. *Neuropharmacology* **61**, 524–543 (2011).
115. Beck, S. G., Pan, Y.-Z., Akanwa, A. C. & Kirby, L. G. Median and dorsal raphe neurons are not electrophysiologically identical. *J. Neurophysiol.* **91**, 994–1005 (2004).
116. Kocsis, B., Varga, V., Dahan, L. & Sik, A. Serotonergic neuron diversity: identification of raphe neurons with discharges time-locked to the hippocampal theta rhythm. *Proc. Natl. Acad. Sci. USA* **103**, 1059–1064 (2006).
117. Tilleman, H. et al. Bmp5/7 in concert with the mid-hindbrain organizer control development of noradrenergic locus coeruleus neurons. *Mol. Cell. Neurosci.* **45**, 1–11 (2010).
118. Shi, M. et al. Notch–Rbpj signaling is required for the development of noradrenergic neurons in the mouse locus coeruleus. *J. Cell. Sci.* **125**, 4320–4332 (2012).
119. Lam, C. S., Sleptsova-Friedrich, I., Munro, A. D. & Korzh, V. SHH and FGF8 play distinct roles during development of noradrenergic neurons in the locus coeruleus of the zebrafish. *Mol. Cell. Neurosci.* **22**, 501–515 (2003).
120. Brunet, J.-F. & Pattyn, A. Phox2 genes — from patterning to connectivity. *Curr. Opin. Genet. Dev.* **12**, 435–440 (2002).
121. Mong, J. et al. Transcription factor-induced lineage programming of noradrenergic and motor neurons from embryonic stem cells. *Stem Cells* **32**, 609–622 (2014).
122. Vogel-Höpker, A. & Rohrer, H. The specification of noradrenergic locus coeruleus (LC) neurons depends on bone morphogenetic proteins (BMPs). *Development* **129**, 983–991 (2002).
123. Hirsch, M. R., Tiveron, M. C., Guillemot, F., Brunet, J. F. & Goridis, C. Control of noradrenergic differentiation and Phox2a expression by MASH1 in the central and peripheral nervous system. *Development* **125**, 599–608 (1998).
124. Pattyn, A., Goridis, C. & Brunet, J.-F. Specification of the central noradrenergic phenotype by the homeobox gene phox2b. *Mol. Cell. Neurosci.* **15**, 235–243 (2000).
125. Lo, L., Tiveron, M. C. & Anderson, D. J. MASH1 activates expression of the paired homeodomain transcription factor Phox2a, and couples pan-neuronal and

- subtype-specific components of autonomic neuronal identity. *Development* **125**, 609–620 (1998).
126. Goridis, C. & Brunet, J.-F. Transcriptional control of neurotransmitter phenotype. *Curr. Opin. Neurobiol.* **9**, 47–53 (1999).
 127. Tiveron, M.-C., Hirsch, M.-R. & Brunet, J.-F. The expression pattern of the transcription factor *phox2* delineates synaptic pathways of the autonomic nervous system. *J. Neurosci.* **16**, 7649–7660 (1996).
 128. Pattyn, A., Morin, X., Cremer, H., Goridis, C. & Brunet, J. F. Expression and interactions of the two closely related homeobox genes *Phox2a* and *Phox2b* during neurogenesis. *Development* **124**, 4065–4075 (1997).
 129. Morin, X. et al. Defects in sensory and autonomic ganglia and absence of locus coeruleus in mice deficient for the homeobox gene *Phox2a*. *Neuron* **18**, 411–423 (1997).
 130. Ding, Y.-Q. et al. *Lmx1b* is essential for the development of serotonergic neurons. *Nat. Neurosci.* **6**, 933–938 (2003).
 131. Warnecke, M., Oster, H., Revelli, J.-P., Alvarez-Bolado, G. & Eichele, G. Abnormal development of the locus coeruleus in *Ear2(Nr2f6)*-deficient mice impairs the functionality of the forebrain clock and affects nociception. *Genes Dev.* **19**, 614–625 (2005).
 132. Espana, A. & Clotman, F. *Onecut* factors control development of the locus coeruleus and of the mesencephalic trigeminal nucleus. *Mol. Cell. Neurosci.* **50**, 93–102 (2012).
 133. Tsarovina, K. et al. Essential role of *Gata* transcription factors in sympathetic neuron. *Development* **131**, 4775–4786 (2004).
 134. Aroca, P., Lorente-Cánovas, B., Mateos, F. R. & Puelles, L. Locus coeruleus neurons originate in alar rhombomere 1 and migrate into the basal plate: studies in chick and mouse embryos. *J. Comp. Neurol.* **496**, 802–818 (2006).
 135. Shi, M., Guo, C., Dai, J.-X. & Ding, Y.-Q. *DCC* is required for the tangential migration of noradrenergic neurons in locus coeruleus of mouse brain. *Mol. Cell. Neurosci.* **39**, 529–538 (2008).
 136. Qian, Y. et al. Formation of brainstem (nor)adrenergic centers and first-order relay visceral sensory neurons is dependent on homeodomain protein *Rnx/Tlx3*. *Genes Dev.* **15**, 2533–2545 (2001).
 137. Puelles, L. & Rubenstein, J. L. R. Forebrain gene expression domains and the evolving prosomeric model. *Trends Neurosci.* **26**, 469–476 (2003).
 138. Puelles, E. et al. *Otx* dose-dependent integrated control of antero-posterior and dorso-ventral patterning of midbrain. *Nat. Neurosci.* **6**, 453–460 (2003).
 139. Marín, F., Herrero, M.-T., Vyas, S. & Puelles, L. Ontogeny of tyrosine hydroxylase mRNA expression in mid- and forebrain: neuromeric pattern and novel positive regions. *Dev. Dyn.* **234**, 709–717 (2005).
 140. Simeone, A. et al. The role of *otx2* in adult mesencephalic-diencephalic dopaminergic neurons. *Mol. Neurobiol.* **43**, 107–113 (2011).
 141. Smits, S. M., von Oerthel, L., Hoekstra, E. J., Burbach, J. P. H. & Smidt, M. P. Molecular marker differences relate to developmental position and subsets of mesodiencephalic dopaminergic neurons. *PLoS One* **8**, e76037 (2013).
 142. Allen Developing Mouse Brain Atlas. Allen Institute for Brain Science. <http://developingmouse.brainmap.org/>. (2013)
 143. Fu, Y., Paxinos, G., Watson, C. & Halliday, G. M. The substantia nigra and ventral tegmental dopaminergic neurons from development to degeneration. *J. Chem. Neuroanat.* **76**, 98–107 (2016).
 144. La Manno, G. et al. Molecular diversity of midbrain development in mouse, human, and stem cells. *Cell* **167**, 566–580.e19 (2016).
 145. Joyner, A. L., Liu, A. & Millet, S. *Otx2*, *Gbx2* and *Fgf8* interact to position and maintain a mid-hindbrain organizer. *Curr. Opin. Cell Biol.* **12**, 736–741 (2000).
 146. Prakash, N. et al. A *Wnt1*-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors in vivo. *Development* **133**, 89–98 (2006).
 147. Farkas, L. M., Dünker, N., Roussa, E., Unsicker, K. & Kriegelstein, K. transforming growth factor- β s are essential for the development of midbrain dopaminergic neurons in vitro and in vivo. *J. Neurosci.* **23**, 5178–5186 (2003).
 148. Roussa, E. & Kriegelstein, K. Induction and specification of midbrain dopaminergic cells: focus on SHH, FGF8, and TGF- β . *Cell Tissue Res.* **318**, 23–33 (2004).
 149. Jacobs, F. M. J. et al. Retinoic acid counteracts developmental defects in the substantia nigra caused by *Pitx3* deficiency. *Development* **134**, 2673–2684 (2007).
 150. Abeliovich, A. & Hammond, R. Midbrain dopamine neuron differentiation: factors and fates. *Dev. Biol.* **304**, 447–454 (2007).
 151. Smits, S. M., Burbach, J. P. H. & Smidt, M. P. Developmental origin and fate of meso-diencephalic dopamine neurons. *Prog. Neurobiol.* **78**, 1–16 (2006).
 152. Joksimovic, M. et al. Spatiotemporally separable *Shh* domains in the midbrain define distinct dopaminergic progenitor pools. *Proc. Natl. Acad. Sci.* **106**, 19185–19190 (2009).
 153. Fasano, C. A. & Studer, L. Too much sonic, too few neurons. *Nat. Neurosci.* **12**, 107–108 (2009).
 154. Joksimovic, M. et al. *Wnt* antagonism of *Shh* facilitates midbrain floor plate neurogenesis. *Nat. Neurosci.* **12**, 125–131 (2009).
 155. Hayes, L., Zhang, Z., Albert, P., Zervas, M. & Ahn, S. The timing of sonic Hedgehog and *Gli1* expression segregates midbrain dopamine neurons. *J. Comp. Neurol.* **519**, 3001–3018 (2011).
 156. Blaess, S. et al. Temporal-spatial changes in Sonic Hedgehog expression and signaling reveal different potentials of ventral mesencephalic progenitors to populate distinct ventral midbrain nuclei. *Neural Dev.* **6**, 29 (2011).
 157. Mesman, S., von Oerthel, L. & Smidt, M. P. Mesodiencephalic dopaminergic neuronal differentiation does not involve *GLI2a*-mediated *shh*-signaling and is under the direct influence of canonical *wnt* signaling. *PLoS One* **9**, e97926 (2014).
 158. Ono, Y. et al. Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. *Development* **134**, 3213–3225 (2007).
 159. Simeone, A., Puelles, E. & Acampora, D. The *Otx* family. *Curr. Opin. Genet. Dev.* **12**, 409–415 (2002).
 160. Puelles, E. et al. *Otx2* regulates the extent, identity and fate of neuronal progenitor domains in the ventral midbrain. *Development* **131**, 2037–2048 (2004).
 161. Simeone, A. et al. *Otx* genes in neurogenesis of mesencephalic dopaminergic neurons. *Dev. Neurobiol.* **71**, 665–679 (2011).
 162. Alavian, K. N., Scholz, C. & Simon, H. H. Transcriptional regulation of mesencephalic dopaminergic neurons: the full circle of life and death. *Mov. Disord.* **23**, 319–328 (2008).
 163. Di Porzio, U., Zuddas, A., Cosenza-Murphy, D. B. & Barker, J. L. Early appearance of tyrosine hydroxylase immunoreactive cells in the mesencephalon of mouse embryos. *Int. J. Dev. Neurosci.* **8**, 523–532 (1990).
 164. Gale, E. & Li, M. Midbrain dopaminergic neuron fate specification: of mice and embryonic stem cells. *Mol. Brain* **1**, 8 (2008).
 165. Ang, S.-L. & Rossant, J. *HNF-3 β* is essential for node and notochord formation in mouse development. *Cell* **78**, 561–574 (1994).
 166. Sasaki, H. & Hogan, B. L. M. *HNF-3 β* as a regulator of floor plate development. *Cell* **76**, 103–115 (1994).
 167. Bayly, R. D., Brown, C. Y. & Agarwala, S. A novel role for *FOXA2* and *SHH* in organizing midbrain signaling centers. *Dev. Biol.* **369**, 32–42 (2012).
 168. Ferri, A. L. M. et al. *Foxa1* and *Foxa2* regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. *Development* **134**, 2761–2769 (2007).
 169. Lin, W. et al. *Foxa1* and *Foxa2* function both upstream of and cooperatively with *Lmx1a* and *Lmx1b* in a feedforward loop promoting mesodiencephalic dopaminergic neuron development. *Dev. Biol.* **333**, 386–396 (2009).
 170. Metzakopian, E. et al. Genome-wide characterization of *Foxa2* targets reveals upregulation of floor plate genes and repression of ventrolateral genes in midbrain dopaminergic progenitors. *Development* **139**, 2625–2634 (2012).
 171. Kittappa, R., Chang, W. W., Awatramani, R. B. & McKay, R. D. G. The *foxa2* gene controls the birth and spontaneous degeneration of dopamine neurons in old age. *PLoS Biol.* **5**, e325 (2007).
 172. Deng, Q. et al. Specific and integrated roles of *Lmx1a*, *Lmx1b* and *Phox2a* in ventral midbrain. *Development* **138**, 3399–3408 (2011).
 173. Nakatani, T., Kumai, M., Mizuhara, E., Minaki, Y. & Ono, Y. *Lmx1a* and *Lmx1b* cooperate with *Foxa2* to coordinate the specification of dopaminergic neurons and control of floor plate cell differentiation in the developing mesencephalon. *Dev. Biol.* **339**, 101–113 (2010).
 174. Smidt, M. P. et al. A second independent pathway for development of mesencephalic dopaminergic neurons requires *Lmx1b*. *Nat. Neurosci.* **3**, 337–341 (2000).
 175. Andersson, E. et al. Identification of intrinsic determinants of midbrain dopamine neurons. *Cell* **124**, 393–405 (2006).
 176. Kele, J. et al. Neurogenin 2 is required for the development of ventral midbrain dopaminergic neurons. *Development* **133**, 495–505 (2006).
 177. Andersson, E., Jensen, J. B., Parmar, M., Guillemot, F. & Björklund, A. Development of the mesencephalic dopaminergic neuron system is compromised in the absence of neurogenin 2. *Development* **133**, 507–516 (2006).
 178. Chakrabarty, K. et al. Genome wide expression profiling of the mesodiencephalic region identifies novel factors involved in early and late dopaminergic development. *Biol. Open* **1**, 693–704 (2012).
 179. Zetterström, R. H. et al. Dopamine neuron agenesis in *nurr1*-deficient mice. *Science* **276**, 248–250 (1997).
 180. Saucedo-Cardenas, O. et al. *Nurr1* is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc. Natl. Acad. Sci.* **95**, 4013–4018 (1998).
 181. Glaab, E. & Schneider, R. Comparative pathway and network analysis of brain transcriptome changes during adult aging and in Parkinson's disease. *Neurobiol. Dis.* **74**, 1–13 (2015).
 182. Jacobs, F. M. J. et al. Identification of *Dlk1*, *Ptpu* and *Klhl1* as novel *Nurr1* target genes in meso-diencephalic dopamine neurons. *Development* **136**, 2363–2373 (2009).

183. Castillo, S. O. et al. Dopamine biosynthesis is selectively abolished in substantia nigra/Ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the *nurr1* gene. *Mol. Cell. Neurosci.* **11**, 36–46 (1998).
184. Filippi, A. et al. Expression and function of *nr4a2*, *lmx1b*, and *pitx3* in zebrafish dopaminergic and noradrenergic neuronal development. *BMC Dev. Biol.* **7**, 135 (2007).
185. Smits, S. M., Ponnio, T., Conneely, O. M., Burbach, J. P. H. & Smidt, M. P. Involvement of *Nurr1* in specifying the neurotransmitter identity of ventral midbrain dopaminergic neurons. *Eur. J. Neurosci.* **18**, 1731–1738 (2003).
186. Smidt, M. P. et al. Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene *Pitx3*. *Development* **131**, 1145–1155 (2004).
187. Smidt, M. P. & Burbach, J. P. H. How to make a mesodiencephalic dopaminergic neuron. *Nat. Rev. Neurosci.* **8**, 21–32 (2007).
188. Puellas, L. & Verney, C. Early neuromeric distribution of tyrosine-hydroxylase-immunoreactive neurons in human embryos. *J. Comp. Neurol.* **394**, 283–308 (1998).
189. Verney, C. Distribution of the catecholaminergic neurons in the central nervous system of human embryos and fetuses. *Microsc. Res. Tech.* **46**, 24–47 (1999).
190. Verney, C., Zecevic, N. & Puellas, L. Structure of longitudinal brain zones that provide the origin for the substantia nigra and ventral tegmental area in human embryos, as revealed by cytoarchitecture and tyrosine hydroxylase, calretinin, calbindin, and GABA immunoreactions. *J. Comp. Neurol.* **429**, 22–44 (2001).
191. González, A., Smeets, W. J. & Marín, O. Evidences for shared features in the organization of the basal ganglia in tetrapods: studies in amphibians. *Eur. J. Morphol.* **37**, 151–154 (1999).
192. Smeets, W. Ja. J., Marín, O. & González, A. Evolution of the basal ganglia: new perspectives through a comparative approach. *J. Anat.* **196**, 501–517 (2000).
193. Medina, L., Puellas, L. & Smeets, W. J. Development of catecholamine systems in the brain of the lizard *Gallotia galloti*. *J. Comp. Neurol.* **350**, 41–62 (1994).
194. Vitalis, T., Cases, O., Engelkamp, D., Verney, C. & Price, D. J. Defects of tyrosine hydroxylase-immunoreactive neurons in the brains of mice lacking the transcription factor *Pax6*. *J. Neurosci.* **20**, 6501–6516 (2000).
195. Puellas, E., Martínez-de-la Torre, M., Watson, C. & Puellas, L. Midbrain. In *The Mouse Nervous System* (eds Watson, C., Paxinos, G. & Puellas, L.) Ch. 10, 337–359 (Academic, 2012).
196. Puellas, L., Martínez-de-la Torre, M., Ferran, J.-L. & Watson, C. Diencephalon. In *The Mouse Nervous System* (eds Watson, C., Paxinos, G. & Puellas, L.) Ch. 9, 313–336 (Academic, 2012).
197. Bonilla, S. et al. Identification of midbrain floor plate radial glia-like cells as dopaminergic progenitors. *Glia* **56**, 809–820 (2008).
198. Puellas, L., Amat, J. A. & Martínez-de-la Torre, M. Segment-related, mosaic neurogenetic pattern in the forebrain and mesencephalon of early chick embryos: I. Topography of AChE-positive neuroblasts up to stage HH18. *J. Comp. Neurol.* **266**, 247–268 (1987).
199. Placzek, M. & Briscoe, J. The floor plate: multiple cells, multiple signals. *Nat. Rev. Neurosci.* **6**, 230–240 (2005).
200. Rubenstein, J. & Rakic, P. *Patterning and Cell Type Specification in the Developing CNS and PNS: Comprehensive Developmental Neuroscience* (Academic, 2013).
201. Di Salvio, M. et al. *Otx2* controls neuron subtype identity in ventral tegmental area and antagonizes vulnerability to MPTP. *Nat. Neurosci.* **13**, 1481–1488 (2010).
202. Di Giovannantonio, L. G. et al. *Otx2* selectively controls the neurogenesis of specific neuronal subtypes of the ventral tegmental area and compensates *En1*-dependent neuronal loss and MPTP vulnerability. *Dev. Biol.* **373**, 176–183 (2013).
203. Di Salvio, M., Di Giovannantonio, L. G., Omodei, D., Acampora, D. & Simeone, A. *Otx2* expression is restricted to dopaminergic neurons of the ventral tegmental area in the adult brain. *Int. J. Dev. Biol.* **54**, 939–945 (2010).
204. Chung, C. Y. et al. The transcription factor orthodenticle homeobox 2 influences axonal projections and vulnerability of midbrain dopaminergic neurons. *Brain* **133**, 2022–2031 (2010).
205. Panman, L. et al. *Sox6* and *Otx2* control the specification of substantia nigra and ventral tegmental area dopamine neurons. *Cell Rep.* **8**, 1018–1025 (2014).
206. Reyes, S. et al. Trophic factors differentiate dopamine neurons vulnerable to Parkinson's disease. *Neurobiol. Aging* **34**, 873–886 (2013).
207. Hwang, D.-Y., Ardayfio, P., Kang, U. J., Semina, E. V. & Kim, K.-S. Selective loss of dopaminergic neurons in the substantia nigra of *Pitx3*-deficient alparkia mice. *Mol. Brain Res.* **114**, 123–131 (2003).
208. Jacobs, F. M. J. et al. Retinoic acid-dependent and -independent gene-regulatory pathways of *Pitx3* in meso-diencephalic dopaminergic neurons. *Development* **138**, 5213–5222 (2011).
209. Pacelli, C. et al. Elevated mitochondrial bioenergetics and axonal arborization size are key contributors to the vulnerability of dopamine neurons. *Curr. Biol.* **25**, 2349–2360 (2015).
210. Pontual, L. D., et al. Noradrenergic neuronal development is impaired by mutation of the proneural *HASH-1* gene in congenital central hypoventilation syndrome (Ondine's curse). *Hum. Mol. Genet.* **12**, 3173–3180 (2003).
211. Perras, C. M. et al. Divergent functions of the proneural genes *Mash1* and *Ngn2* in the specification of neuronal subtype identity. *Genes Dev.* **16**, 324–338 (2002).
212. Park, C.-H. et al. Acquisition of in vitro and in vivo functionality of *Nurr1*-induced dopamine neurons. *FASEB J.* **20**, 2553–2555 (2006).
213. Kageyama, R., Ishibashi, M., Takebayashi, K. & Tomita, K. bHLH transcription factors and mammalian neuronal differentiation. *Int. J. Biochem. Cell Biol.* **29**, 1389–1399 (1997).
214. Hoekstra, E. J., von Oerthel, L., van der Linden, A. J. A. & Smidt, M. P. *Phox2b* influences the development of a caudal dopaminergic subset. *PLoS One* **7**, e52118 (2012).
215. Laguna, A. et al. Dopaminergic control of autophagic-lysosomal function implicates *Lmx1b* in Parkinson's disease. *Nat. Neurosci.* **18**, 826–835 (2015). April.
216. Doucet-Beaupré, H. et al. *Lmx1a* and *Lmx1b* regulate mitochondrial functions and survival of adult midbrain dopaminergic neurons. *Proc. Natl. Acad. Sci.* **113**, E4387–E4396 (2016).
217. Hoekstra, E. J. et al. *Lmx1a* encodes a rostral set of mesodiencephalic dopaminergic neurons marked by the *Wnt/B-catenin* signaling activator *R-spondin 2*. *PLoS One* **8**, e74049 (2013).
218. Rinne, J. O., Mlic, J. R., Paljärvi, L. & Rinne, U. K. Dementia in Parkinson's disease is related to neuronal loss in the medial substantia nigra. *Ann. Neurol.* **26**, 47–50 (1989).
219. Alavian, K. N. et al. The lifelong maintenance of mesencephalic dopaminergic neurons by *Nurr1* and engrailed. *J. Biomed. Sci.* **21**, 27 (2014).
220. Bakken, T. E. et al. A comprehensive transcriptional map of primate brain development. *Nature* **535**, 367–375 (2016).
221. Diederich, N. J. & Parent, A. Parkinson's disease: acquired frailty of archaic neural networks? *J. Neurosci.* **314**, 143–151 (2012).
222. Briscoe, J. & Novitsch, B. G. Regulatory pathways linking progenitor patterning, cell fates and neurogenesis in the ventral neural tube. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 57–70 (2008).
223. Jessell, T. M. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* **1**, 20–29 (2000).
224. Donkelaar, H. J. t., Cruysberg, J. R. M., Pennings, R. & Lammens, M. Clinical Neuroembryology: Development and developmental disorders of the Human Central Nervous System. 321–370 (Springer, Berlin Heidelberg 2014).
225. Hoehn, M. M. & Yahr, M. D. Parkinsonism: onset, progression and mortality. *Neurology* **17**, 427–442 (1967).
226. Olanow, C. W., Stocchi, F. & Lang, A. E., (eds) *Parkinson's Disease: Non-motor and Non-dopaminergic Features* (Wiley-Blackwell, 2011).
227. Pfeiffer, R. F., Wszolek, Z. K. & Ebad, M. *Parkinson's Disease*, 2nd edn (CRC Press, 2012).
228. Gallagher, D. A., Goetz, C. G., Stebbins, G., Lees, A. J. & Schrag, A. Validation of the MDS-UPDRS Part I for nonmotor symptoms in Parkinson's disease. *Mov. Disord.* **27**, 79–83 (2012).
229. Jellinger, K. A. Neuropathology of sporadic Parkinson's disease: evaluation and changes of concepts. *Mov. Disord.* **27**, 8–30 (2012).
230. Travers, J. B., Yoo, J.-E., Chandran, R., Herman, K. & Travers, S. P. Neurotransmitter phenotypes of intermediate zone reticular formation projections to the motor trigeminal and hypoglossal nuclei in the rat. *J. Comp. Neurol.* **488**, 28–47 (2005).
231. Boeve, B. F. Idiopathic REM sleep behaviour disorder in the development of Parkinson's disease. *Lancet Neurol.* **12**, 469–482 (2013).
232. Flames, N. & Hobert, O. Transcriptional control of the terminal fate of monoaminergic neurons. *Annu. Rev. Neurosci.* **34**, 153–184 (2011).
233. Webster, R. Acetylcholine (ACh). In *Neurotransmitters, Drugs and Brain Function* (ed. Webster, R. A.) 117–136 (Wiley, 2001).



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.