Review

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Mesencephalic GABA neuronal development: no more on the other side of oblivion

Abstract: Midbrain GABA neurons, endowed with multiple morphological, physiological and molecular characteristics as well as projection patterns are key players interacting with diverse regions of the brain and capable of modulating several aspects of behavior. The diversity of these GABA neuronal populations based on their location and function in the dorsal, medial or ventral midbrain has challenged efforts to rapidly uncover their developmental regulation. Here we review recent developments that are beginning to illuminate transcriptional control of GABA neurons in the embryonic midbrain (mesencephalon) and discuss its implications for understanding and treatment of neurological and psychiatric illnesses.

Keywords: brain; development; GABA.

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Introduction

The midbrain – an important relay center for sensory inputs and motor outputs networking with the forebrain, hindbrain and spinal cord – is a hub of constant activity (1–10). Gamma-aminobutyric acid (GABA) neurons are key players in midbrain neuronal circuitry, robustly networking with glutamatergic and dopaminergic (DA) neurons to shape multiple aspects of behavior. Midbrain GABA neurons function not only as local inhibitory interneurons but also as projection neurons with targets in various brain regions. Along their developmental path, midbrain GABA neurons acquire molecular and functional diversity and can be divided into three categories based on anatomy and function: (1) dorsally located GABA neurons in the superior colliculus (SC) and inferior colliculus (IC); (2) medially located GABA neurons in the midbrain reticular formation (MRF) and periaqueductal gray (PAG); (3) ventrally located GABA neurons in substantia nigra (SN), ventral tegmental area (VTA) and retrorubral field (RRF), (Figure 1A).

The functional diversity of midbrain GABA neurons based on their location is fascinating. Dorsal midbrain GABA neurons together with glutamatergic neurons are involved in processing and incorporation of sensorimotor, visual, auditory, and defensive behavior (11–16). Although GABA neurons are present in all of the seven layered SC, an abundance of GABA neurons is found in the superficial layers when compared to deep and intermediate layers (17–20). The SC is noteworthy for its high GABA content next only to the SN, globus pallidus and hypothalamus in the central nervous system (CNS) (21, 22). Medial midbrain GABA neurons of PAG regulate vocalization, endogenous control of pain sensation, fear, anxiety and aggression (23–25) while those in the MRF have been implicated with sleep-wake state control (26, 27). Ventral midbrain GABA neurons regulate DA neuron activity in the SN and VTA (6, 7, 28–31) and have projection targets similar to DA neurons, to the prefrontal cortex, basal ganglia and other limbic areas (6–10). Additionally, a considerable number of ascending projections from midbrain dopaminergic nuclei are GABAergic in nature (7). Ventral midbrain GABA neurons are thus critical for the function of dopaminergic pathways; have important functional roles in control of voluntary movements, emotion, mood, motivation, processing of appetite and aversive stimuli, addiction and reward behaviors (6, 29, 32–35) and may be involved in the etiology of several neurological and psychiatric disorders including schizophrenia, depression, mood disorders, addiction and Parkinson's disease. Midbrain GABA neurons can therefore serve as important targets for treatment of neuropsychiatric disorders and for drugs of abuse. Recent evidence reveals that midbrain SN and VTA DA neurons co-release GABA although they do not synthesize it, by using GABA reuptake transporters

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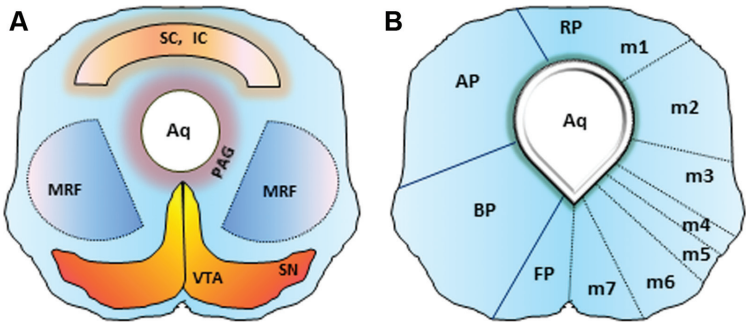

Figure 1
(A) Schema of location of mature midbrain GABA neuronal populations in the superior colliculus (SC), inferior colliculus (IC), midbrain reticular formation (MRF), periaqueductal gray (PAG), substantia nigra (SN) and ventral tegmental area (VTA). Midbrain GABA neuronal populations regulate several brain functions, for instance, they are involved in the SC and IC for sensory integration, in the MRF for sleep, PAG for nociception and defensive behavior, SN for motor activity and in the VTA for motivated behavior. (B) Coronal schematic section of the embryonic mesencephalon depicting its patterning along the dorso-ventral axis into roof plate (RP), alar plate (AP), basal plate (BP) and floor plate (FP) on the left side along with dorso-ventral subdivisions on the right (m1–m7). Aq, aqueduct.

(mGATs) to recycle extracellular GABA for release (36). Another study examining mechanisms of nicotine addiction shows that activation of midbrain GABA neurons in the VTA controls nicotine elicited burst firing of DA neurons and points to a concerted role for GABA neurons and DA neurons in mediating nicotine reinforcement (35). Thus, mechanisms adopted by DA neurons to locally regulate GABAergic transmission (36) or GABAergic regulation of DA neuron activity (35) illustrates the complexity of midbrain GABA-DA neuron interactions.

Despite the functional significance of GABA neurons in the adult midbrain, progress made in identifying the mechanisms underlying GABA neuron development has been relatively slow. Compared with the embryonic forebrain (37–40) and developing dorsal spinal cord (41–46) in which tremendous advances have been made in understanding GABA neuron development, in the embryonic midbrain, we are a decade behind. In the mesencephalon, DA neurons have been in the spotlight due to discovery of consequences of midbrain DA neurons degeneration in Parkinson's disease (PD) and dopamine related neuropsychiatric disorders (47–54). Therefore the molecular mechanisms that define DA neuron development – generation, specification and differentiation of DA neurons have been extensively studied to generate cell replacement and pharmacological intervention strategies to alleviate some of the disease symptoms. However, proper migration of GABA neurons to their final location in the ventral mesencephalon during embryonic development seems to be dependent on the complete DA neuron architecture in the ventral mesencephalon, signifying important interactions between them for final location and connectivity (55). Thus the embryonic developmental period offers a favorable milieu when mesencephalic GABA-DA neuron interactions form and establish. Clearly it is a very exciting time to bring mesencephalic GABA neuron development for study onto a common platform with DA neuron development. In this mini-review we discuss current knowledge about origin of mesencephalic GABA neurons and molecular mechanisms involved in their specification, proliferation, differentiation and migration and emphasize missing links where more work is needed. Generation of detailed maps of key regulators of mesencephalic GABA neuron fate and function will initiate new studies to screen for their failure in mouse and human pathology and help develop new therapeutic strategies that focus on co-ordinate rescue of GABA-DA neuron interactions and novel pharmacological intervention paradigms.

Origin of mesencephalic GABA neurons

The embryonic mesencephalon is patterned along the dorso-ventral axis into roof plate (RP), alar plate (AP), basal plate (BP) and floor plate (FP) by BMP/Wnt signals from the RP and Shh signals from the FP/BP (56–59) and further divided into seven dorso-ventral subdivisions (m1–m7) with specific gene expression codes (60–62) to ensure cellular diversity (Figure 1B). Domains m1–m3 are structured into layers and domains m4–m7 are organized into distinct nuclei. For spatial patterning at dorso-ventral levels of the mesencephalon, several GABAergic progenitor domains have been identified, defined by expression of transcription factors such as *Nkx6-1*, *Nkx2-2* and *Pax3/7* (63). GAD expression, starting at E10.5 has been reported in BP and AP (64, 65). Both ventro-lateral and dorsal

regions of the mesencephalon produce GABA neurons at E10.5–E14.5 (61, 66) and laminar organization develops during E13–E17 (67). The ventral mesencephalon at E13 is still completely devoid of GABA neurons, but by E17, GABA neurons are found robustly intermingled with DA neurons (55, 68).

GABA neurons originate from five of the seven mesencephalic progenitor domains (m1, m2, m3, dorsal half of m4 and m5). GABA neurons of the dorsal midbrain arise from the m1 and m2 domains. Domains m3–m5 may give rise to GABA neurons of the medial midbrain, MRF and PAG. GABA neurons in the ventral midbrain seem to have two developmental origins: 1) outside the midbrain and 2) from BP region. An early study has reported that all SN neurons in the rat brain originate from the midbrain-hindbrain border known as the isthmus (rostral most rhombomere 1) (69). More recent fate mapping studies have shown that midbrain VTA and SN pars reticulata (SNpr) GABA neurons originate from rhombomere 1 (r1), whereas the most anterior part of SNpr has a distinct origin outside the midbrain, possibly in the diencephalon (68). We have observed many GFP-positive GABA neurons in GAD65-GFP mesencephalon oriented ventrally in BP region at E13 and a stream of these neurons coursing from BP region to the ventral mesencephalon by E17. BrdU birthdating experiments additionally revealed that many E11-labeled neuronal progenitors migrated to contribute to GABA neurons of the ventral mesencephalon by E17 (55). The origin of these migrating cells remains to be better clarified.

Specification of GABAergic neuronal fate in the mesencephalon

Combinations of multiple transcription factors act as first selectors of neuronal fate (Figures 2 and 3) and contribute to neuronal diversity. In the mesencephalon, GABA neuronal fate determination is primarily associated with two basic helix-loop-helix (bHLH) genes: *Helt* (also known as *Megane* or *Heslike)* and *Ascl1* (also known as *Mash1*) (61, 65, 70–72).

Helt is co-expressed with *Ascl1* in the ventricular zone of the alar-basal mesencephalic boundary from E9.5 onwards. Subsequently the *Helt- Ascl1* co-expression domain expands dorsally as well. Gain of function studies in which transgenic mice were generated mis-expressing *Helt* from the nestin promoter-enhancer resulted in ectopic

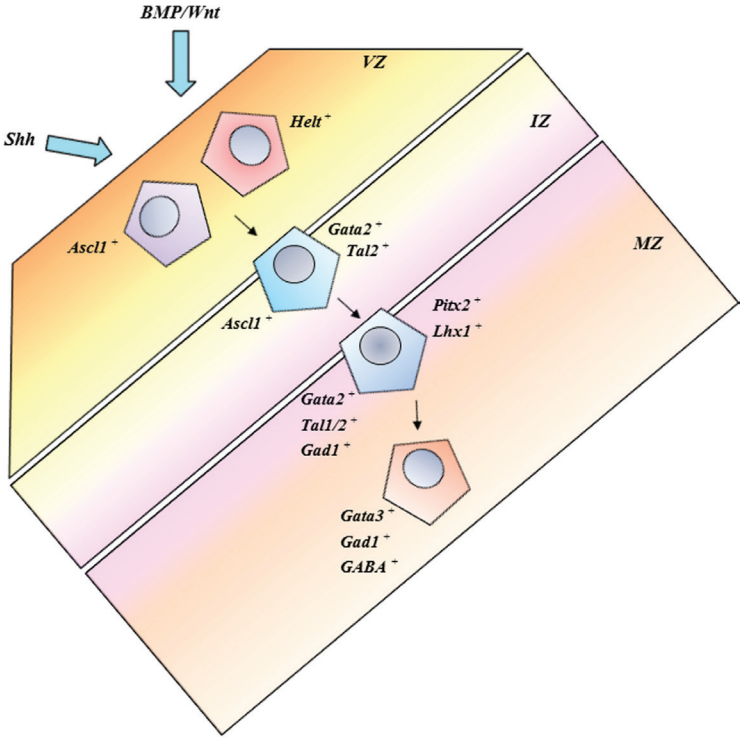

Figure 2 Overview of the molecular codes essential for generation of mesencephalic GABA neurons in relation to their specific position in the ventricular zone (VZ), intermediate zone (IZ) and mantle zone (MZ).
Beginning with inductive signals, a specific cascade of molecular instruction follows in the VZ, IZ and MZ to ensure GABAergic fate specification, neurogenesis, fate maintenance and differentiation into mature GABA neuronal populations.

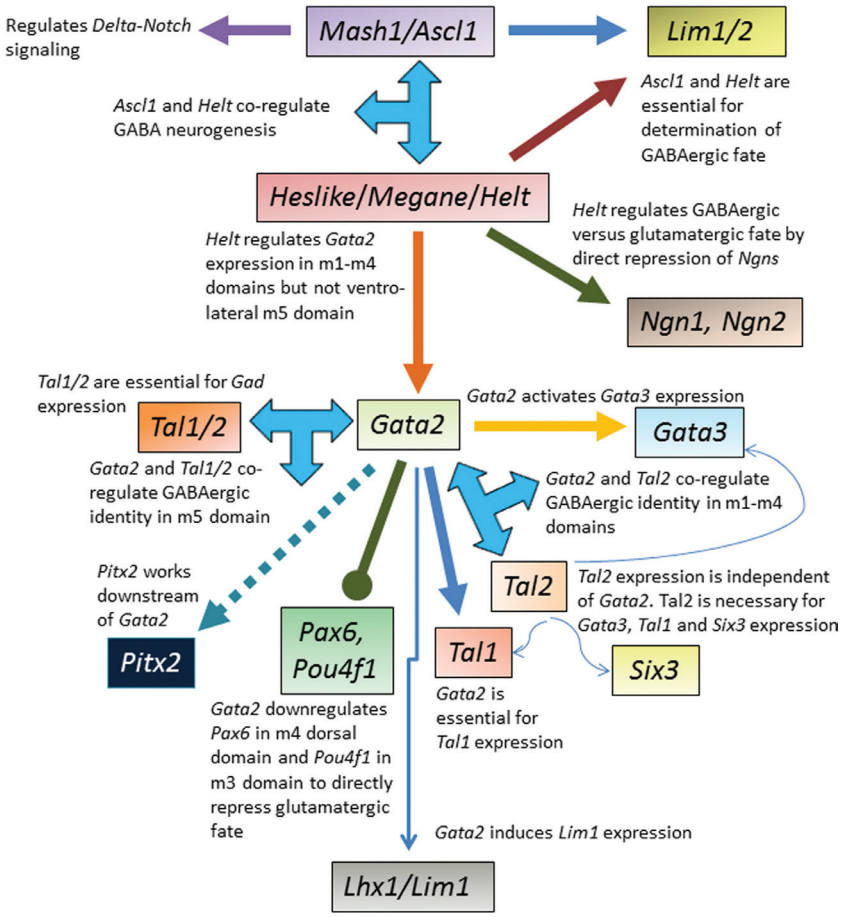

Figure 3 Summary of the signaling pathway of key molecular players identified up until now and their interplay that is essential for efficient generation of mesencephalic GABA neurons.

GABA neurons in the mantle layer outside the *Helt*/*Ascl1*$^+$ zone (70). As the effect of mis-expression of *Helt* was specific to the mesencephalon and no ectopic cells were observed in other brain regions (dorsal telencephalon and thalamus) which do not express *Ascl1*, *Helt* was initially believed to specify the GABA neuronal fate only when *Ascl1* was co-expressed (70). Interestingly, although ectopic *Helt* expression induced GABA neurons, it also suppressed the generation of glutamatergic neurons (61). Loss of function studies revealed that in homozygous *Helt*-null mice GABAergic progenitors were generated (61, 71) but they failed to become GABA neurons specifically in the dorsal mesencephalon. Ventral GABA neurons formed only from the m5 domain in *Helt*-null mesencephalon whereas in the dorsal domains there was a complete loss of GABA neurons and instead, glutamatergic neurons, induced by neurogenin (*Ngn*) genes emerged (61). *Helt* is now recognized as a key determinant of the GABAergic fate in dorsal mesencephalon by direct repression of *Ngn* genes (*Ngn1* and *Ngn2*) in GABAergic progenitors, and induction of *Lim1/2* and *Gata2* (60, 61). *Helt* is required for *Gata2* expression in the embryonic mesencephalon except in the ventro-lateral m5 domain (60). Loss of function of *Helt* did not affect dopaminergic and cholinergic neurons. Thus the essential role of *Helt* for regulating GABA neuron fate specification in the dorsal midbrain and for development of the SC was confirmed.

Ascl1, expressed by neural progenitors in all mesencephalic progenitor domains is another GABAergic fate determinant that is not regulated by *Helt* or other proneural genes such as *Ngns*. *Ascl1* was unaffected in the *Helt*-null mice and may have also compensated for loss of *Helt* in ventral but not dorsal mesencephalon (70). In *Ascl1*-null mice, virtually no GABA neurons formed in the mesencephalon up until E11.5 while *Helt* continued to be expressed and other neurons were generated. From E13.5 onwards, GABA neurons were produced in the ventromedial mesencephalon (m3–m5), but not dorsally (m1–m2). By E18.5, GABA neurons were completely lost in *Ascl1*-null dorsal mesencephalon including SC, IC and dorsal PAG. Although GABA neurons were reduced in medial mesencephalon including ventral PAG and MRF, GABA neurons

of the ventral mesencephalon in SN and VTA were not affected and do not seem to require *Ascl1* (72).

Thus ventral mesencephalic GABA neurons represent an intriguing population of GABA neurons that develop independently of regulation by *Helt* and *Ascl1*.

Mesencephalic GABA neurogenesis

GABAergic neurogenesis is completed first in the MRF by E12.5, next in the SNpr by E13.5, followed by SC at E14.5 (68) and has been associated mostly with transcription factor combinations *Helt* and *Ascl1* (Figures 2 and 3). The *Helt-Ascl1* co-expression domain in the mesencephalon is co-ordinate with GABA neurogenesis, decreasing rapidly as neuronal differentiation proceeds (70). However, *Ascl1's* requirement for neurogenesis seems to be highly region-specific. In *Ascl1*-null mesencephalon, a loss of neurogenic and neural stem cell specific expression with markers *Delta1* (Notch ligand) and *Hes5* (Notch target) was observed at early embryonic stages. This delay at the start of neurogenesis is believed to cause reduced GABA neuron numbers in the ventromedial midbrain of *Ascl1*-null mice (72). *Ascl1* has been reported to promote GABAergic neurogenesis in *in vitro* cultured mesencephalic neural progenitors (73). Co-expression of both *Ascl1* and *Helt* seems to significantly promote GABA neurogenesis in neural precursor cell cultures (70).

Mesencephalic GABA neuron differentiation and fate maintenance

After the cell cycle exit, the GABA neuron precursors activate genes typical for functional GABA neuron precursors. This role is assigned to C4 zinc-finger transcription factor *Gata2*, bHLH transcription factors *Tal1/2*, paired-like homeodomain transcription factor *Pitx2* and homeobox transcription factor *Lhx1* (*Lim1*) (Figures 2 and 3). *Gata2* is expressed in m1–m5 domains in the embryonic mesencephalon and expression is activated in GABAergic progenitors as they exit the cell cycle, become postmitotic precursor cells and begin to differentiate (60). Selective loss of *Gata2* in the mouse midbrain and rhombomere 1 (*Gata2*^{cko} mutant) resulted in a specific loss of GABA neuron precursors at early embryonic stages and transformation from GABAergic to glutamatergic phenotype in all mesencephalic GABA neuron subpopulations in the *Gata2*^{cko} mutant during embryonic development except for the GABA neurons associated with DA neurons in the SNpr and VTA that were unaffected (60, 63). *Tal1/2* are expressed in m1–m4 dorsal half and m5 mesencephalic domains and co-operatively activate genes necessary for GABA neuronal differentiation (74). *Tal2* is co-expressed with *Gata2* in the ventricular zone (VZ) and intermediate zone (IZ) and regulates selection of GABAergic over glutamatergic neuronal fate. An ectopic upregulation of glutamatergic gene expression was observed in *Tal2*^{cko} mutants in m3 and m4 dorsal domains (74). Similar to *Gata2*^{cko} mutant, in *Tal2*^{cko} mutant, GABAergic markers were completely lost in the dorsal mesencephalon including SC. Again, both *Gata2* and *Tal2* are dispensable for ventral mesencephalic GABA neurons (74).

Pitx2, is expressed in m1–m4 and m6 domains with highest expression in the intermediate layer of the SC (75) and works downstream of *Gata2* in the transcription factor cascade (60, 75). Loss of *Pitx2* disrupts GABAergic neuronal differentiation and axonal outgrowth in the dorsal mesencephalon, specifically SC (76). In the ventral mesencephalon *Pitx2* lineage neurons are mostly glutamatergic (75). *Lhx1* (or *Lim1*) is expressed in postmitotic precursors of all mesencephalic GABA neurons in domains m1–m5 (60) and is an important marker of GABAergic differentiation.

Migration of mesencephalic GABA neurons

Neuronal migration is a key event during brain development as neurons and/or neuronal progenitors originating in VZs navigate along diverse courses to eventually find their destination and integrate into specific brain circuits. In the pallial telencephalon, pyramidal neurons follow radial migratory routes using radial glial guides to the cortical plate (40, 77). GABA neurons of the subpallial telencephalon, conversely, take long tangential paths into the cortex (78, 79) along vascular guides (80) to become intermingled with excitatory neurons. Unlike in the embryonic telencephalon where neuronal migration has been well elucidated, this vital event has been little explored in the embryonic mesencephalon. In the dorsal mesencephalon, majority of GABA neurons have been reported to migrate radially from the dorsal VZ to their final location in superficial layers through deep and intermediate layers in an inside-out manner (81) followed by slight tangential dispersion within the superficial layer (Figure 4A). Few cells here (81) show direct tangential migration from VZ to the superficial layer of the SC (Figure 4A). Three types of inhibitory neurons – stellate cells, pyriform cells and horizontal cells with tangentially oriented dendrites – thus come to reside in the multilayered SC (20).

In the ventral mesencephalon, GABA neuronal migration seems to be more complex with contributions from both outside and within. Recent genetic fate mapping studies have uncovered a separate origin of VTA and SNpr GABA neurons outside the mesencephalon (68). The findings unveil a new migratory pathway of GABA neurons originating in r1 compartment and crossing the r1-mesencephlon boundary to migrate to the VTA and SNpr as postmitotic neuronal precursors at E14.5–E15.5 (Figure 4B). Guidance mechanisms for this form of migration remain to be elucidated. The anterior SNpr GABA neurons in the diencephalon however do not arrive from the r1 region; they are speculated to come from the diencephalon although contribution from the mesencephalon is not excluded (68). These results were further supported by analysis of *Tal1* mutant mice in which GABAergic precursors are normal in the mesencephalon, but failure in GABA neuron production in r1 correlated with loss of mature VTA and SNpr GABA neurons (68). Additionally, as *Gata2* regulates GABA neuronal differentiation in the mesencephalon but not r1, in *Gata2* mutants, although mesencephalic precursors failed to activate GABAergic neuron-specific gene expression, the VTA and SNpr GABA neurons appeared largely unaffected (60), supporting their outside origin and border migration across r1-mesencephalon compartments.

Our studies have shown that both DA and GABA neurons occupy ventral mesencephalon in a temporally sequential manner during embryonic development and depicted GABA neuronal silhouette oriented from BP region to ventral mesencephalon in GAD65-GFP embryos (55). It emphasized the importance of perpendicular migration, a novel mode of neuronal migration that seems to be unique to the mesencephalon (Figure 4A) and essential for proper set up of the anatomical architecture of ventral mesencephalic structures (55). BrdU birthdating experiments revealed that E10 and E11 labeled neuronal progenitors migrated ventrally (from FP) as well as perpendicular (from BP) to the aqueduct to form the distinct anatomical architecture of the boat shaped SN and VTA by E17 (55). *Pitx3* represents a critical regulator of mesencephalic DA neuron development (49, 51, 82–85) and there is selective and early loss of A9 DA neurons in the SN of *ak/ak* mice (49, 84–86). The cells affected in the *ak/ak* mice are known to be very sensitive to neurotoxins such as 6-OHDA and MPTP (54, 87) and are the same cells that degenerate first in Parkinson's disease (PD) patients (83–85). Our findings have revealed that loss of SN DA neurons in *ak/ak* mesencephalon is a result of defective perpendicular migration that resulted in cells which were stuck or trailing in the middle of their migratory trajectory and distributed abnormally in the red nucleus area. Interestingly, coordinate with this loss of DA neurons there was also a significant loss of ventral mesencephalic GABA neurons that persisted in the adult *ak/ak* midbrain (55). Pre-existing DA neurons seem to modulate the migration of BP GABA neurons to ventral mesencephalon along perpendicular migration routes suggestive of important interactions between both neuronal populations for final location and connectivity and the earliest signs of interdependence that arises during embryonic development (55). Identification of the anatomical/cellular substrate for perpendicular neuronal migration will help model future investigations to induce this intriguing population of GABA neurons to migrate successfully in cell transplantation experiments coordinate with DA neurons.

At a mechanistic level, neuronal migration overall in the mesencephalon suffers greatly from lack of insights.

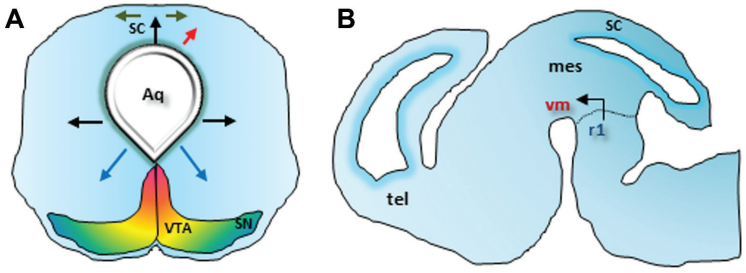

Figure 4

(A) Coronal view of embryonic mesencephalon showing different patterns of neuronal migration. Black arrows depict radial migration of GABA neurons from the VZ to their final location. Green arrows indicate slight tangential dispersion within the superficial layer of the SC. Red arrow depicts that few cells show direct tangential migration from VZ to the superficial layer of the SC. Blue arrows highlight perpendicular migration of GABA neurons from basal plate region to ventral mesencephalon. (B) Sagittal view of embryonic mesencephalon depicting migration of postmitotic GABA neuronal precursors that cross the specific mesencephalon-rhombomere1 (r1) boundary to enter from r1 into the ventral mesencephalon (vm). The mode of migration of these neurons with origins outside the mesencephalon is currently unknown. tel, Telencephalon; mes, Mesencephalon; SC, superior colliculus.

Mesencephalic axon guidance and/or ventral migration of DA neurons has been linked only to a small number of molecules [netrin 1 (88) and its receptor DCC (89), reelin (90), proteoglycan phosphacan 6B4 (91), L1CAM (92), neural cell adhesion molecule (93) and polysialic acid (93)]. Transcription factors, primary mechanisms controlling neuronal migration, remain unidentified in the embryonic mesencephalon. For perpendicular migration to occur from the BP to the ventral mesencephalon, neurons need to perfectly synchronize multiple actions and respond appropriately to guidance cues encountered during their trajectory. Transcriptional regulation is inevitably a key determinant of this process. In the vertebrate spinal cord, for instance, expression of specific combinations of transcription factors in postmitotic motorneurons encodes axon trajectories (94–96) and similar transcription factors carry on different functions depending on the cellular context (97–99). Another example is that of postmitotic *Nkx2.1* expression, which has to be down-regulated for interneurons to migrate to the cortex, whereas *Nkx2.1* expression is required for interneuron migration to the striatum (100). Similar possibilities lie in the embryonic mesencephalon and much work lies ahead with respect to elucidating mechanisms of neuronal migration.

Conclusion and perspectives

The generation of several mouse models with abnormal development and function of cortical GABA interneurons, which recapitulated defective behavior similar to those seen in conditions like schizophrenia, autism, epilepsy, mood and anxiety disorders, was a major step that associated forebrain GABA neurons with the pathobiology of several neuropsychiatric illnesses (101–107). Thus, the identification of the molecular components involved in forebrain GABA neuron development in both mouse and human triggered efficient generation of GABA neuron populations based on ES cell engineering. ES-derived GABA interneurons today have remarkable potential – they are functional, survive well, migrate and integrate into desired regions in both embryonic and adult brains post-transplantation and are attractive candidates for cell-based therapies (108–112). In the midbrain, ES cell technology today focuses selectively to generate DA neurons (113–116). This is possible because the molecular cascade of DA neuron development has been well studied. A missing link in the midbrain field is our current incapability for efficient generation of GABA neurons from ES cells, as the molecular mechanisms underlying mesencephalic GABA neuron fate and function are just beginning to be elucidated and furthermore the transcriptional machinery identified here, except for *Ascl1* is different from that of the forebrain.

The quest to unravel the transcriptional cascade in the embryonic midbrain is however no easy task. It is particularly daunting given the intricate diversity of GABA neurons found in the mesencephalon based on location and function and transcriptional factor networks, with overlapping function for GABAergic vs. glutamatergic fate, or GABAergic vs. serotonergic/dopaminergic fate depending on their domain. Table 1 summarizes expression stages, domains of expression and function of key molecular players regulating mesencephalic GABA neuron development. As most of these candidates are broadly expressed in several brain regions as well as other organs, systemic knockouts die prenatally or at birth (Table 1). There is a great need for gain and loss of function assays, including generation of more mouse mutants with deletion of GABA neuron-specific genes selectively in the embryonic mesencephalon and subsequent analysis of brain development and postnatal behavior to be able to fully understand the developmental significance of mesencephalic GABA neurons in the CNS. *Helt*, *Gata2* and *Tal1/2* are transcription factors that have been best studied in this regard (Table 1). Given *Helt*'s unique expression pattern in the mesencephalon and prosomere1-2 in the diencephalon, isolation of *Helt* served as a vital key to unlock the mysteries of mesencephalic GABA neuronal development. *Helt* deletion resulted in loss of weight and postnatal lethality in mice around 3–5 weeks of age with behavioral defects such as fore/hindlimb clenching and seizure-like phenotype from P14 onward (71). Elegant work demonstrating conditional inactivation of *Gata2* and *Tal1/2* in mouse midbrain and r1 have provided several new insights into the specific role of these transcription factors for GABA neuron development in the embryonic midbrain, although postnatal phenotypes have not yet been reported (60, 74).

The VTA and SNpr GABA neurons are particularly distinct and interesting because they develop independently of the known regulators of midbrain GABAergic fate specification and maintenance: *Helt*, *Ascl1*, *Gata2* and *Tal2* (60, 61, 65, 71, 72, 74). Given the functional importance of GABA-DA neuron associations in the adult midbrain, it has become critical to decode transcriptional regulation of ventral mesencephalic GABA neurons as well as delineate development of GABA-DA neuron interactions and connectivity. Additionally, as GABA neurons were also affected in addition to DA neurons as a result of loss of *Pitx3* (55), a crucial transcriptional regulator of DA neuron development, there lies the possibility of overlapping

Table 1 Key molecular players regulating mesencephalic GABA neuronal development.

Gene	Earliest stage of expression and expression domains in the embryonic mesencephalon	Specific function in the mesencephalon	All brain regions where genes are expressed	Phenotypes of homozygous null mutants/ transgenic mice
Heslike/Megane/Helt	E9.5: alar-basal boundary E9.5–E12.5 onward: further dorsal expansion towards roof plate. Mostly absent from ventral mesencephalon. Expression in VZ, not detected in mantle zone. E13.5–E17.5: expression is down-regulated and disappears by 17.5	1) Specifies GABA neuron fate in dorsal mesencephalon 2) Possible role in GABA neurogenesis 3) Essential for GABA neuronal development in SC	1) Diencephalon (p1–2) 2) Mesencephalon	Homozygous *Helt*-null mice show behavioral defects, seizure-like activity and postnatal lethality at 3–5 weeks after birth Transgenic mice mis-expressing *Helt* in the *Ascl1* region die at E12.5
Mash1/Ascl1	E9.5: alar-basal boundary and dorsal mesencephalon, E10.5: both domains connect E11.5–E12.5 onward: further dorsal (m1–m2) and ventral (m3–m5) expansion of the domain Expression in VZ; overlaps with *Helt*, also expressed in the MZ	1) Specification of GABA neuronal fate in dorsal mesencephalon 2) GABA neurogenesis specifically in the dorsal mesencephalon and partially in the ventro-medial mesencephalon	1) Telencephalon 2) Diencephalon 3) Mesencephalon 4) R1 5) Developing spinal cord	As *Ascl1* shows broad expression in the CNS, PNS and in neuroendocrine cells in lung and kidney, homozygous *Ascl1*-null mice die soon after birth with severe disruptions in several aspects of neuronal development
Gata2	E10.5–12.5: expression in basal plate region in IZ and MZ E12.5 onward: expression appears in dorsal mesencephalon. Expression coincides with midbrain regions producing *Gad1* positive neurons throughout development	Functions as an essential postmitotic selector selector gene for GABAergic over glutamatergic identity	1) Diencephalon (p1–2) 2) Mesencephalon 3) Ventral spinal cord	*Gata2* homozygous null mutant embryos die at E10.5 during embryogenesis. Conditional inactivation of *Gata2* in midbrain and r1 depicts its specific role in GABA neuronal development in midbrain and not r1. Postnatal phenotype not reported
Tal1/2	E10.5–E12.5 onward: *Tal1* expression confined to IZ and MZ; co-expressed with *Gata2* in the IZ; co-expressed with *Gata3* in the MZ. *Tal2* expressed in VZ and IZ and co-expressed with *Gata2*. Expressed in m1-m4 dorsal half and m5 domains	1) *Tal1/2* co-operatively guides GABA neuron differentiation 2) *Tal2* is important for sub-type specification in postmitotic neurons; selection of GABAergic over glutamatergic neuron fate	1) Diencephalon 2) Mesencephalon 3) Ventral R1 4) Ventral spinal cord	*Tal1* gene knockout results in mid-gestational lethality due to lack of yolk sac erythropoiesis. Conditional inactivation of *Tal1/2* in the embryonic midbrain highlights its role in postmitotic GABA neuronal differentiation. Postnatal phenotype not reported
Pitx2	E10.5 onward: expression limited to postmitotic neurons of the mantle zone, in domains m1–m4 and m6. Highest expression in intermediate layer of SC	Essential for GABA neuronal differentiation and axonal outgrowth in the dorsal midbrain, specially SC	1) Mesencephalon 2) Ventral R1	*Pitx2* is essential for normal development of several organs including pituitary and eyes. Complete loss of *Pitx2* in mice results in embryonic lethality by E15 due to cardiac defects

molecular pathways and shared mechanisms mediating GABA-DA neuron development in the ventral mesencephalon. Many vital goals remain to be achieved before we can explore and combine the distinctive features of both GABA neurons and DA neurons so as to understand complex neurological and psychiatric illnesses and to test novel therapies that can ultimately become a clinical reality. Clearly, the field of mesencephalic GABA neuron development is poised for several exciting discoveries in the future.

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References

1. Mehler WR, Feferman ME, Nauta WJ. Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. Brain 1960; 83: 718–50.
2. Garey LJ, Jones EG, Powell TP. Interrelationships of striate and extrastriate cortex with the primary relay sites of the visual pathway. J Neurol Neurosurg Psychiatry 1968; 31: 135–57.
3. Valverde F. The neuropil in superficial layers of the superior colliculus of the mouse. A correlated Golgi and electron microscopic study. Z Anat Entwicklungsgesch 1973; 142: 117–47.
4. Meredith MA, Stein BE. Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. J Neurophysiol 1986; 56: 640–62.
5. Wickelgren BG. Superior colliculus: some receptive field properties of bimodally responsive cells. Science 1971; 173: 69–72.
6. Laviolette SR, van der Kooy D. The neurobiology of nicotine addiction: bridging the gap from molecules to behaviour. Nat Rev Neurosci 2004; 5: 55–65.
7. Fields HL, Hjelmstad GO, Margolis EB, Nicola SM. Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annu Rev Neurosci 2007; 30: 289–316.
8. Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 1982; 9: 321–53.
9. Oades RD, Halliday GM. Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res 1987; 434: 117–65.
10. Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989; 12: 366–75.
11. Dean P, Redgrave P, Westby GW. Event or emergency? Two response systems in the mammalian superior colliculus. Trends Neurosci 1989; 12: 137–47.
12. Westby GW, Keay KA, Redgrave P, Dean P, Bannister M. Output pathways from the rat superior colliculus mediating approach and avoidance have different sensory properties. Exp Brain Res 1990; 81: 626–38.
13. Oliver DL, Winer JA, Beckius GE, Saint Marie RL. Morphology of GABAergic neurons in the inferior colliculus of the cat. J Comp Neurol 1994; 340: 27–42.
14. Merchan M, Aguilar LA, Lopez-Poveda EA, Malmierca MS. The inferior colliculus of the rat: quantitative immunocytochemical study of GABA and glycine. Neuroscience 2005; 136: 907–25.
15. Kaneda K, Phongphanphanee P, Katoh T, Isa K, Yanagawa Y, Obata K, Isa T. Regulation of burst activity through presynaptic and postsynaptic $GABA_B$ receptors in mouse superior colliculus. J Neurosci 2008; 28: 816–27.
16. Ito T, Oliver DL. The basic circuit of the IC: tectothalamic neurons with different patterns of synaptic organization send different messages to the thalamus. Front Neural Circuits 2012; 6: 48.
17. Harvey AR, Heavens RP, Yellachich LA, Sirinathsinghji DJ. Expression of messenger RNAs for glutamic acid decarboxylase, preprotachykinin, cholecystokinin, somatostatin, proenkephalin and neuropeptide Y in the adult rat superior colliculus. Neuroscience 2001; 103: 443–55.
18. Binns KE. The synaptic pharmacology underlying sensory processing in the superior colliculus. Prog Neurobiol 1999; 59: 129–59.
19. Jeon CJ, Gurski MR, Mize RR. Glutamate containing neurons in the cat superior colliculus revealed by immunocytochemistry. Vis Neurosci 1997; 14: 387–93.
20. Mize RR. The organization of GABAergic neurons in the mammalian superior colliculus. Progr Brain Res 1992; 90: 219–48.
21. Okada Y, Nitsch-Hassler C, Kim JS, Bak IJ, Hassler R. Role of aminobutyric acid (GABA) in the extrapyramidal motor system. 1. Regional distribution of GABA in rabbit, rat, guinea pig and baboon CNS. Exp Brain Res 1971; 13: 514–8.
22. Fahn S, Cote LJ. Regional distribution of gamma-aminobutyric acid (GABA) in brain of the rhesus monkey. J Neurochem 1968; 15: 209–13.
23. Bandler R, Keay KA, Floyd N, Price J. Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. Brain Res Bull 2000; 53: 95–104.
24. Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. Prog Neurobiol 1995; 46: 575–605.
25. Barbaresi P. Postnatal development of GABA-immunoreactive neurons and terminals in rat periaqueductal gray matter: a light and electron microscopic study. J Comp Neurol 2010; 518: 2240–60.
26. Luppi PH, Clement O, Sapin E, Gervasoni D, Peyron C, Leger L, Salvert D, Fort P. The neuronal network responsible for paradoxical sleep and its dysfunctions causing narcolepsy and rapid eye movement (REM) behavior disorder. Sleep Med Rev 2011; 15: 153–63.
27. Brischoux F, Mainville L, Jones BE. Muscarinic-2 and orexin-2 receptors on GABAergic and other neurons in the rat mesopontine tegmentum and their potential role in sleep-wake state control. J Comp Neurol 2008; 510: 607–30.
28. Omelchenko N, Sesack SR. Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. Synapse 2009; 63: 895–906.
29. Tepper JM, Lee CR. GABAergic control of substantia nigra dopaminergic neurons. Progr Bain Res 2007; 160: 189–208.
30. Margolis EB, Toy B, Himmels P, Morales M, Fields HL. Identification of rat ventral tegmental area GABAergic neurons. PLoS One 2012; 7: e42365.
31. Nair-Roberts RG, Chatelain-Badie SD, Benson E, White-Cooper H, Bolam JP, Ungless MA. Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral

tegmental area, substantia nigra and retrorubral field in the rat. Neuroscience 2008; 152: 1024–31.
32. Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature 2012; 482: 85–8.
33. Jhou TC, Fields HL, Baxter MG, Saper CB, Holland PC. The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. Neuron 2009; 61: 786–800.
34. Vargas-Perez H, Ting AKR, Walton CH, Hansen DM, Razavi R, Clarke L, Bufalino MR, Allison DW, Steffensen SC, van der Kooy D. Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats. Science 2009; 324: 1732–4.
35. Tolu S, Eddine R, Marti F, David V, Graupner M, Pons S, Baudonnat M, Husson M, Besson M, Reperant C, Zemdegs J, Pages C, Hay YA, Lambolez B, Caboche J, Gutkin B, Gardier AM, Changeux JP, Faure P, Maskos U. Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. Mol Psychiatry 2013; 18: 382–93.
36. Tritsch NX, Oh WJ, Gu C, Sabatini BL. Midbrain dopamine neurons sustain inhibitory transmission using plasma membrane uptake of GABA, not synthesis. Elife 2014; 3: e01936.
37. DeFelipe J. Cortical interneurons: from Cajal to 2001. Progr Brain Res 2002; 136: 215–38.
38. Wonders CP, Anderson SA. The origin and specification of cortical interneurons. Nat Rev Neurosci 2006; 7: 687–96.
39. Hensch TK. Critical period plasticity in local cortical circuits. Nat Rev Neurosci 2005; 6: 877–88.
40. Parnavelas JG. The origin and migration of cortical neurones: new vistas. Trends Neurosci 2000; 23: 126–31.
41. Cheng L, Arata A, Mizuguchi R, Qian Y, Karunaratne A, Gray PA, Arata S, Shirasawa S, Bouchard M, Luo P, Chen CL, Busslinger M, Goulding M, Onimaru H, Ma Q. Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. Nat Neurosci 2004; 7: 510–7.
42. Cheng L, Samad OA, Xu Y, Mizuguchi R, Luo P, Shirasawa S, Goulding M, Ma Q. Lbx1 and Tlx3 are opposing switches in determining GABAergic versus glutamatergic transmitter phenotypes. Nat Neurosci 2005; 8: 1510–5.
43. Glasgow SM, Henke RM, Macdonald RJ, Wright CV, Johnson JE. Ptf1a determines GABAergic over glutamatergic neuronal cell fate in the spinal cord dorsal horn. Development 2005; 132: 5461–9.
44. Gross MK, Dottori M, Goulding M. Lbx1 specifies somatosensory association interneurons in the dorsal spinal cord. Neuron 2002; 34: 535–49.
45. Mizuguchi R, Kriks S, Cordes R, Gossler A, Ma Q, Goulding M. Ascl1 and Gsh1/2 control inhibitory and excitatory cell fate in spinal sensory interneurons. Nat Neurosci 2006; 9: 770–8.
46. Muller T, Brohmann H, Pierani A, Heppenstall PA, Lewin GR, Jessell TM, Birchmeier C. The homeodomain factor lbx1 distinguishes two major programs of neuronal differentiation in the dorsal spinal cord. Neuron 2002; 34: 551–62.
47. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. Trends Neurosci 2007; 30: 194–202.
48. Lennington JB, Pope S, Goodheart AE, Drozdowicz L, Daniels SB, Salamone JD, Conover JC. Midbrain dopamine neurons associated with reward processing innervate the neurogenic subventricular zone. J Neurosci 2011; 31: 13078–87.
49. Smidt MP, Burbach JP. How to make a mesodiencephalic dopaminergic neuron. Nat Rev Neurosci 2007; 8: 21–32.
50. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain 1999; 122 (Pt 8): 1437–48.
51. Ding Y, Won L, Britt JP, Lim SA, McGehee DS, Kang UJ. Enhanced striatal cholinergic neuronal activity mediates L-DOPA-induced dyskinesia in parkinsonian mice. Proc Natl Acad Sci USA 2011; 108: 840–5.
52. Schultz W. Reward signaling by dopamine neurons. Neuroscientist 2001; 7: 293–302.
53. Seeman P, Guan HC, Van Tol HH. Dopamine D4 receptors elevated in schizophrenia. Nature 1993; 365: 441–5.
54. Ungerstedt U, Ljungberg T, Steg G. Behavioral, physiological, and neurochemical changes after 6-hydroxydopamine-induced degeneration of the nigro-striatal dopamine neurons. Adv Neurol 1974; 5: 421–6.
55. Vasudevan A, Won C, Li S, Erdelyi F, Szabo G, Kim KS. Dopaminergic neurons modulate GABA neuron migration in the embryonic midbrain. Development 2012; 139: 3136–41.
56. Dessaud E, McMahon AP, Briscoe J. Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. Development 2008; 135: 2489–503.
57. Puelles E. Genetic control of basal midbrain development. J Neurosci Res 2007; 85: 3530–4.
58. Zervas M, Blaess S, Joyner AL. Classical embryological studies and modern genetic analysis of midbrain and cerebellum development. Curr Top Dev Biol 2005; 69: 101–38.
59. Le Dreau G, Marti E. Dorsal-ventral patterning of the neural tube: a tale of three signals. Dev Neurobiol 2012; 72: 1471–81.
60. Kala K, Haugas M, Lillevali K, Guimera J, Wurst W, Salminen M, Partanen J. Gata2 is a tissue-specific post-mitotic selector gene for midbrain GABAergic neurons. Development 2009; 136: 253–62.
61. Nakatani T, Minaki Y, Kumai M, Ono Y. Helt determines GABAergic over glutamatergic neuronal fate by repressing Ngn genes in the developing mesencephalon. Development 2007; 134: 2783–93.
62. Lahti L, Achim K, Partanen J. Molecular regulation of GABAergic neuron differentiation and diversity in the developing midbrain. Acta Physiol (Oxf) 2013; 207: 616–27.
63. Achim K, Salminen M, Partanen J. Mechanisms regulating GABAergic neuron development. Cell Mol Life Sci 2014; 71: 1395–415.
64. Katarova Z, Sekerkova G, Prodan S, Mugnaini E, Szabo G. Domain-restricted expression of two glutamic acid decarboxylase genes in midgestation mouse embryos. J Comp Neurol 2000; 424: 607–27.
65. Guimera J, Vogt Weisenhorn D, Echevarria D, Martinez S, Wurst W. Molecular characterization, structure and developmental expression of Megane bHLH factor. Gene 2006; 377: 65–76.
66. Tsunekawa N, Yanagawa Y, Obata K. Development of GABAergic neurons from the ventricular zone in the superior colliculus of the mouse. Neurosci Res 2005; 51: 243–51.
67. Edwards MA, Caviness VS Jr., Schneider GE. Development of cell and fiber lamination in the mouse superior colliculus. J Comp Neurol 1986; 248: 395–409.
68. Achim K, Peltopuro P, Lahti L, Li J, Salminen M, Partanen J. Distinct developmental origins and regulatory mechanisms for GABAergic neurons associated with dopaminergic nuclei in the ventral mesodiencephalic region. Development 2012; 139: 2360–70.
69. Marchand R, Poirier LJ. Isthmic origin of neurons of the rat substantia nigra. Neuroscience 1983; 9: 373–81.

70. Miyoshi G, Bessho Y, Yamada S, Kageyama R. Identification of a novel basic helix-loop-helix gene, Heslike, and its role in GABAergic neurogenesis. J Neurosci 2004; 24: 3672–82.
71. Guimera J, Weisenhorn DV, Wurst W. Megane/Heslike is required for normal GABAergic differentiation in the mouse superior colliculus. Development 2006; 133: 3847–57.
72. Peltopuro P, Kala K, Partanen J. Distinct requirements for Ascl1 in subpopulations of midbrain GABAergic neurons. Dev Biol 2010; 343: 63–70.
73. Jo AY, Park CH, Aizawa S, Lee SH. Contrasting and brain region-specific roles of neurogenin2 and mash1 in GABAergic neuron differentiation *in vitro*. Exp Cell Res 2007; 313: 4066–81.
74. Achim K, Peltopuro P, Lahti L, Tsai HH, Zachariah A, Astrand M, Salminen M, Rowitch D, Partanen J. The role of Tal2 and Tal1 in the differentiation of midbrain GABAergic neuron precursors. Biol Open 2013; 2: 990–7.
75. Waite MR, Skidmore JM, Billi AC, Martin JF, Martin DM. GABAergic and glutamatergic identities of developing midbrain Pitx2 neurons. Dev Dyn 2011; 240: 333–46.
76. Martin DM, Skidmore JM, Philips ST, Vieira C, Gage PJ, Condie BG, Raphael Y, Martinez S, Camper SA. PITX2 is required for normal development of neurons in the mouse subthalamic nucleus and midbrain. Dev Biol 2004; 267: 93–108.
77. Hatten ME. New directions in neuronal migration. Science 2002; 297: 1660–3.
78. Corbin JG, Nery S, Fishell G. Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. Nat Neurosci 2001; 4 Suppl: 1177–82.
79. Anderson SA, Marin O, Horn C, Jennings K, Rubenstein JL. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 2001; 128: 353–63.
80. Won C, Lin Z, Kumar TP, Li S, Ding L, Elkhal A, Szabo G, Vasudevan A. Autonomous vascular networks synchronize GABA neuron migration in the embryonic forebrain. Nat Commun 2013; 4: 2149–63.
81. Tan SS, Valcanis H, Kalloniatis M, Harvey A. Cellular dispersion patterns and phenotypes in the developing mouse superior colliculus. Dev Biol 2002; 241: 117–31.
82. Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, Hannon G, Abeliovich A. A MicroRNA feedback circuit in midbrain dopamine neurons. Science 2007; 317: 1220–4.
83. Nunes I, Tovmasian LT, Silva RM, Burke RE, Goff SP. Pitx3 is required for development of substantia nigra dopaminergic neurons. Proc Natl Acad Sci USA 2003; 100: 4245–50.
84. van den Munckhof P, Luk KC, Ste-Marie L, Montgomery J, Blanchet PJ, Sadikot AF, Drouin J. Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. Development 2003; 130: 2535–42.
85. Smidt MP, Smits SM, Bouwmeester H, Hamers FP, van der Linden AJ, Hellemons AJ, Graw J, Burbach JP. Early developmental failure of substantia nigra dopamine neurons in mice lacking the homeodomain gene Pitx3. Development 2004; 131: 1145–55.
86. Chung S, Hedlund E, Hwang M, Kim DW, Shin BS, Hwang DY, Kang UJ, Isacson O, Kim KS. The homeodomain transcription factor Pitx3 facilitates differentiation of mouse embryonic stem cells into AHD2-expressing dopaminergic neurons. Mol Cell Neurosci 2005; 28: 241–52.
87. Langston JW, Langston EB, Irwin I. MPTP-induced parkinsonism in human and non-human primates – clinical and experimental aspects. Acta neurologica Scandinavica Supplementum 1984; 100: 49–54.
88. Flores C, Manitt C, Rodaros D, Thompson KM, Rajabi H, Luk KC, Tritsch NX, Sadikot AF, Stewart J, Kennedy TE. Netrin receptor deficient mice exhibit functional reorganization of dopaminergic systems and do not sensitize to amphetamine. Mol Psychiatry 2005; 10: 606–12.
89. Fazeli A, Dickinson SL, Hermiston ML, Tighe RV, Steen RG, Small CG, Stoeckli ET, Keino-Masu K, Masu M, Rayburn H, Simons J, Bronson RT, Gordon JI, Tessier-Lavigne M, Weinberg RA. Phenotype of mice lacking functional deleted in colorectal cancer (Dcc) gene. Nature 1997; 386: 796–804.
90. Nishikawa S, Goto S, Yamada K, Hamasaki T, Ushio Y. Lack of Reelin causes malpositioning of nigral dopaminergic neurons: evidence from comparison of normal and Reln(rl) mutant mice. J Comp Neurol 2003; 461: 166–73.
91. Ohyama K, Kawano H, Asou H, Fukuda T, Oohira A, Uyemura K, Kawamura K. Coordinate expression of L1 and 6B4 proteoglycan/phosphacan is correlated with the migration of mesencephalic dopaminergic neurons in mice. Brain Res Dev Brain Res 1998; 107: 219–26.
92. Demyanenko GP, Shibata Y, Maness PF. Altered distribution of dopaminergic neurons in the brain of L1 null mice. Brain Res Dev Brain Res 2001; 126: 21–30.
93. Shults CW, Kimber TA. Mesencephalic dopaminergic cells exhibit increased density of neural cell adhesion molecule and polysialic acid during development. Brain Res Dev Brain Res 1992; 65: 161–72.
94. Kania A, Johnson RL, Jessell TM. Coordinate roles for LIM homeobox genes in directing the dorsoventral trajectory of motor axons in the vertebrate limb. Cell 2000; 102: 161–73.
95. Sharma K, Sheng HZ, Lettieri K, Li H, Karavanov A, Potter S, Westphal H, Pfaff SL. LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. Cell 1998; 95: 817–28.
96. Sharma K, Leonard AE, Lettieri K, Pfaff SL. Genetic and epigenetic mechanisms contribute to motor neuron pathfinding. Nature 2000; 406: 515–9.
97. De Marco Garcia NV, Jessell TM. Early motor neuron pool identity and muscle nerve trajectory defined by postmitotic restrictions in Nkx6.1 activity. Neuron 2008; 57: 217–31.
98. Muller M, Jabs N, Lorke DE, Fritzsch B, Sander M. Nkx6.1 controls migration and axon pathfinding of cranial branchiomotoneurons. Development 2003; 130: 5815–26.
99. Shirasaki R, Pfaff SL. Transcriptional codes and the control of neuronal identity. Annu Rev Neurosci 2002; 25: 251–81.
100. Nobrega-Pereira S, Kessaris N, Du T, Kimura S, Anderson SA, Marin O. Postmitotic Nkx2–1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. Neuron 2008; 59: 733–45.
101. Hussman JP. Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. J Autism Dev Disord 2001; 31: 247–8.
102. Levitt P, Eagleson KL, Powell EM. Regulation of neocortical interneuron development and the implications for neurodevelopmental disorders. Trends Neurosci 2004; 27: 400–6.
103. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2003; 2: 255–67.

104. Lewis DA. GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. Brain Res Brain Res Rev 2000; 31: 270–6.
105. Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology 2001; 25: 1–27.
106. Jetty PV, Charney DS, Goddard AW. Neurobiology of generalized anxiety disorder. Psychiatr Clin North Am 2001; 24: 75–97.
107. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 2002; 71: 877–92.
108. Kim TG, Yao R, Monnell T, Cho JH, Vasudevan A, Koh A, Peeyush KT, Moon M, Datta D, Bolshakov VY, Kim KS, Chung S. Efficient specification of interneurons from human pluripotent stem cells by dorsoventral and rostrocaudal modulation. Stem Cells 2014; 32: 1789–804.
109. Maroof AM, Keros S, Tyson JA, Ying SW, Ganat YM, Merkle FT, Liu B, Goulburn A, Stanley EG, Elefanty AG, Widmer HR, Eggan K, Goldstein PA, Anderson SA, Studer L. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell 2013; 12: 559–72.
110. Maroof AM, Brown K, Shi SH, Studer L, Anderson SA. Prospective isolation of cortical interneuron precursors from mouse embryonic stem cells. J Neurosci 2010; 30: 4667–75.
111. Maisano X, Litvina E, Tagliatela S, Aaron GB, Grabel LB, Naegele JR. Differentiation and functional incorporation of embryonic stem cell-derived GABAergic interneurons in the dentate gyrus of mice with temporal lobe epilepsy. J Neurosci 2012; 32: 46–61.
112. Liu Y, Liu H, Sauvey C, Yao L, Zarnowska ED, Zhang SC. Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. Nat Protoc 2013; 8: 1670–9.
113. Kim JH, Auerbach JM, Rodriguez-Gomez JA, Velasco I, Gavin D, Lumelsky N, Lee SH, Nguyen J, Sanchez-Pernaute R, Bankiewicz K, McKay R. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature 2002; 418: 50–6.
114. Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y. Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron 2000; 28: 31–40.
115. Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, Isacson O. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. Proc Natl Acad Sci USA 2002; 99: 2344–9.
116. Yang D, Zhang ZJ, Oldenburg M, Ayala M, Zhang SC. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in parkinsonian rats. Stem Cells 2008; 26: 55–63.

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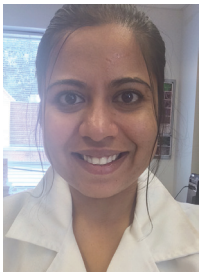

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