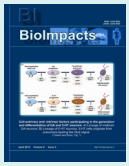
Tripathi P.P., Bozzi Y., ***BioImpacts,*** 2015, 5(2), 97-102
doi: 10.15171/bi.2015.07
http://bi.tbzmed.ac.ir/

The role of dopaminergic and serotonergic systems in neurodevelopmental disorders: a focus on epilepsy and seizure susceptibility

Prem Prakash Tripathi[1*†], Yuri Bozzi[2†]
[1] Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, USA
[2] Laboratory of Molecular Neuropathology, Center for Integrative Biology (CIBIO), University of Trento, Trento, Italy

Article Info

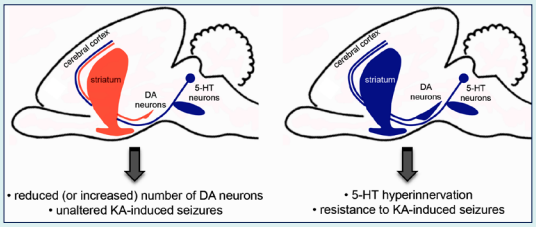


Article Type:
Mini-Review

Article History:
Received: 27 Oct. 2014
Revised: 22 Jan. 2015
Accepted: 14 Feb. 2015
ePublished: 06 Apr. 2015

Keywords:
Epilepsy
Dopamine
Serotonin
Seizure
Kainic acid
Knockout mice
Limbic system

Abstract



Introduction: The embryonic development of the vertebrate Central Nervous System (CNS) requires the induction of transcription factors regulating the expression of specific subsets of genes in restricted CNS regions. Among these transcription factors, homeobox-containing proteins play a crucial role, and altered expression of these factors can impact the embryonic as well as adult CNS functions. Importantly, the homeobox-containing genes Otx2, Engrailed-1 (En1), and Engrailed-2 (En2) have been described to crucially regulate differentiation of dopaminergic and serotonergic neurons during vertebrate CNS development. Dopaminergic and serotonergic neurons, located in midbrain and hindbrain regions respectively, diffusely innervate several forebrain areas including limbic system, contributing in regulating several physiological functions. Understanding the embryonic development of these neuronal populations is crucial to elucidate their physiological function including brain excitability in the adult brain. New evidence is emerging about the impact of an altered embryonic development of dopamine and serotonin neurons onto seizure susceptibility in the adult life.

Methods: In this mini-review, we summarized our kainic acid (KA) induced seizure susceptibility in adult mutant mouse lines with targeted manipulation of Otx2, En1, and En2 genes.

Results: Our results demonstrated that altered development of dopamine (DA) neurons does not interfere with KA seizure susceptibility, while increased serotonin (5-hydroxytryptamine, 5-HT) hyperinnervation leads to resistance to KA-induced seizure.

Conclusion: We propose that developmental alterations of serotonergic but not dopaminergic circuits play a crucial role in controlling seizure susceptibility in the adult life.

Introduction

Traditionally, the occurrence of epileptic seizures has been explained by an imbalance between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission. However, many other neurotransmitter systems are known to be involved in the epileptogenesis, including dopamine (DA)[1,2] and serotonin (5-hydroxytryptamine, 5-HT).[3] Different types of DA and 5-HT receptors are located on the neocortical and hippocampal glutamatergic or GABAergic nerve terminals, where they can cause a significant shift in the balance towards excitation in these networks.[2,3] Several lines of evidence show that *Otx1, Otx2 and En2* control patterning and regionalization of hindbrain, midbrain and forebrain areas.[4,5] Accordingly, mutations of various homeobox genes have been linked with severe postnatal neurological dysfunctions, including occurrence of epileptic seizures.[6,7]

DA and 5-HT in epileptogenesis

Classical pharmacological studies indicate that both DA and 5-HT may have an anti-epileptic action. The role of DAergic and serotonergic circuits in the genesis and control of epileptogenesis has been extensively reviewed.[1-3,7,8] Here we briefly summarize the major findings in this field.

***Corresponding author:** Prem Prakash Tripathi, Email: prem.tripathi@gmail.com
†These authors contributed equally to this work.

© 2015 The Author(s). This work is published by BioImpacts as an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by-nc/4.0/). Non-commercial uses of the work are permitted, provided the original work is properly cited.

DA and epileptogenesis

The use of dopaminergic ligands specific for different subclasses of DA receptors allowed to demonstrate that DA has an anti-epileptic action in a wide variety of animal models.[1,9] There are subtypes of dopamine receptors that are proconvulsant as well as anticonvulsant.[10,11] Studies performed on mice lacking specific DA receptor subtypes revealed the important opposite role of D1-like receptor (D1R) and D2-like receptors (D2R) signaling in regulating seizure activity. Activation of D1R usually exerts pro-epileptogenic, whereas D2R stimulation can block seizure. Importantly, physiological balance of DAergic activity at D1R and D2R would be crucial for determining the complex neuromodulatory response to seizure-promoting stimuli.[2,8,11,12]

The mesolimbic pathway that links ventral tegmental area to other limbic areas is involved in the DAergic control of seizures. Indeed, DAergic neurons innervate limbic areas and express different types of DA receptors.[13] Dopamine D2 receptor knockout ($D2R^{-/-}$) mice show an increased susceptibility to KA-induced seizures and CA3 hippocampal cell death,[8,11] as indicated by activation of pro-apoptotic markers such as Bax[11] and caspase-3.[14] Indeed, loss of D2R signaling in $D2R^{-/-}$ mice results in the reduction of Akt (Ser473) phosphorylation and increase of GSK-3β phosphorylation,[2,14,15] rendering CA3 hippocampal neurons more susceptible to KA-induced apoptosis.

5-HT and epileptogenesis

The link between 5-HT and seizure inhibition was originally suggested more than five decades ago.[16] Drugs such as selective serotonin reuptake inhibitors (SSRI) employ an anti-epileptic action by increasing the extracellular 5-HT levels against both limbic and generalized seizures.[17,18] In contrast, depletion of brain 5-HT can lower seizure threshold to promote seizures. Indeed, limbic system and ventral midbrain are involved in 5-HTergic control of seizure.[1]

Expression of various types of 5-HT receptors is evident in most of the networks associated with seizure onset. Based on their structure and function, 5-HT receptors have been classified into seven receptors and total fourteen subtypes.[19,20] Mutant mice lacking 5-HT_{1A} show increased lethality after KA-induced seizures, while administration of 5-HT_{1A} agonists reduces seizures in rats. Similarly, mice lacking 5-HT_{2C} receptors also show increased seizure latency and reduced seizure threshold.[21]

Taken altogether, all these pharmacological and genetic manipulation studies clearly demonstrate that some subtypes of DA and 5-HT receptors have a proconvulsant effect while others have an anticonvulsant action.[22] Thus, seizure origin and spread will vary when different subtypes of DA and 5-HT receptors are stimulated. However, little is known about the impact of an altered embryonic development of DA and 5-HT neurons onto seizure susceptibility in the adult life. In the following paragraph, we briefly review the genetic networks involved in the generation and differentiation of DA and 5-HT neurons during embryonic brain development.

Generation and differentiation of dopaminergic and serotonergic neurons

In the mammalian nervous system, individual population of neurons develop in a stereotypic position identified by their coordinates along the antero-posterior (A-P) and dorso-ventral (D-V) axes.[23] The formation of A-P and D-V axes is controlled by three organizing centers: floor plate (FP), mid-hindbrain boundary (MHB) and anterior neural ridge (ANR).

The MHB is only defined with the use of expression patterns of specific genes (e.g. En1, En2, Pax5, Pax8, Fgf8, Fgf17, and Fgf18) and cover a broad domain that terminates at the midbrain-hindbrain boundary (e.g. Otx2 and Gbx2). The second organizing center of the midbrain/hindbrain region is the FP. Sonic hedgehog (Shh) is the key-signaling molecule of the FP. During neurogenesis, dopaminergic and serotonergic neuron progenitors within the neuroepithelium are committed by the combined action of Fgf8 and Shh, originating from the MHB and the FP, respectively.

By embryonic stage 7.5 (E7.5) in mouse, the transcription factors Otx2 and Gbx2 are expressed in an interdependent fashion in the embryo and antagonize each other in brain regionalization.[24] At early stages, Fgf8, Wnt1, and Otx2 are expressed in the caudal midbrain regions that generate midbrain DA neurons. In contrast, Fgf8 and Gbx2, but not Wnt1, are expressed in the region that generate rostral 5-HT progenitors (Fig. 1). The transcription factors En1 and En2 are instead expressed in both anterior hindbrain and caudal midbrain.

The concomitant action of MHB and FP activates a series of transcription factors including Otx2, Lmx1a, Lmx1b, En1, En2, Msx1, Msx2, Ngn2 and Mash1 in the midbrain. Otx2, Lmx1b, and En1/2 genes start expressing by E9;[25] Lmx1a, Msx1, and Msx2 expression starts around E9.5, while expression of Ngn2 and Mash1 starts just before E11.[26] Midbrain DA neurons would be therefore specified by D-V as FP cells and A-P by Otx2 signals while hindbrain neurons, such as 5-HT cells, would originate from precursors lacking the Otx2 signal.[27] DA and 5-HT neurons are localized in caudal midbrain and rostral hindbrain, respectively.[28]

Generation and differentiation of dopaminergic neurons

In mouse, first DA neurons are born at around E9.5.[29] Initially, Otx2 and Shh induce ectopic TH-positive cells and further Lmx1a induces Msx1 which in turn activates Ngn2. Activated Ngn2 regulates the progression of differentiated Sox2-positive late progenitors into Nurr1-positive post-mitotic mesencephalic dopaminergic (mesDA) neurons. The correct specification and maintenance of postmitotic immature DA precursor requires activity of the Aldh1, En1/2, Pitx3, and Lmx1b transcriptional regulators. Postmitotic DA precursors induce expression of Nurr1[30] and Lmx1b (Fig. 1), which allow differentiation of

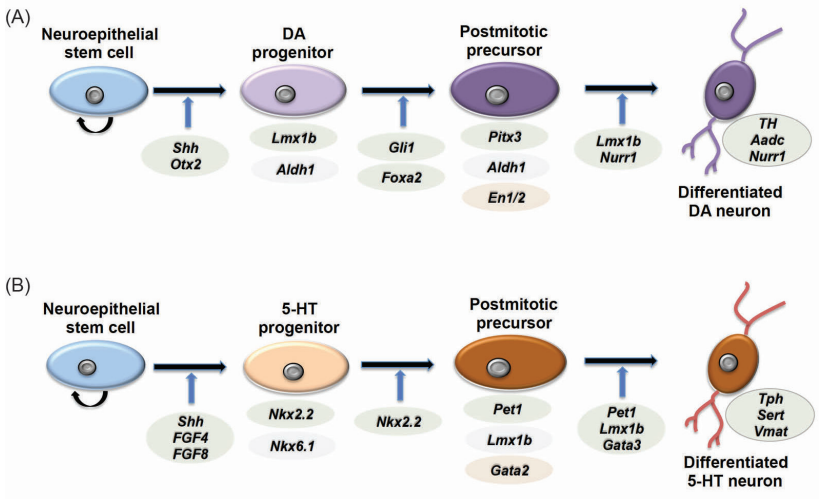


Fig. 1. Cell-extrinsic and -intrinsic factors participating in the generation and differentiation of DA and 5-HT neurons. (A) Lineage of midbrain DA neurons. Initial induction of TH- positive cells is provided by Otx2 and sonic hedgehog (Shh). During the next stage, DA progenitors exit the cell cycle and transform into postmitotic DA precursors. Indeed, Shh induces Gli1 activation and Foxa2 induction. Once positioning and identity of the DA neurons are determined, specific differentiation programs are activated by Lmx1b and Nurr1. (B) Lineage of 5-HT neurons. 5-HT cells originate from precursors lacking the Otx2 signal. Initial specification and patterning of 5-HT progenitors requires the combined activation of Shh, FGF4 and FGF8. Shh signaling induces the expression of Nkx2.2 and Nkx6.1 for specification of its ventral progenitor identity and activates expression of Gata2 and Gata3. Gata2 is necessary and sufficient to activate Lmx1b and Pet1to specify 5-HT neurons. Pet1 differentiates rostral and caudal groups of 5-HT cells while Gata3 differentiates only caudal 5-HT cells.

immature DA neurons that induce En1/2 expression.[31] Otx2 is further expressed in postmitotic mesDA neurons during later part of embryogenesis and in the adult brain.[32] During maturation of DA neurons, other genes necessary for the synthesis and maintenance of DA are expressed including TH (tyrosine hydroxylase) and aromatic amino acid decarboxylase (Aadc), vescicular monoamine transporter 2 (Vmat2), and dopamine transporter (Dat).

Generation and differentiation of serotonergic neurons

Expression of immature 5-HT neurons starts around E10.75 in the mouse from rhombomeres r1–r7 neural progenitors. Rostral hindbrain 5-HT neurons have been shown to depend on the activity of the Shh signaling and FGF signaling especially Fgf8 and Fgf4.[33] Expression of Nkx2.2 is then essential for the specification of 5-HT neurons.[34] Once the position of the precursors is defined, three transcriptional regulators (Nkx2.2, Pet1 and Gata3a) are required to establish the serotonergic phenotype. Nkx2.2 cooperates with other factors to direct conversion of 5-HT precursors to 5-HT postmitotic neurons. Lmx1b, Gata3, and Pet1 are strictly limited to the raphe nuclei and required for transition and correct specification of postmitotic precursor to differentiated 5-HT neuron. The full maturation of the axon terminals is achieved by the activation of specific genes that describe the serotonergic phenotype: tryptophan hydroxylase (Tph), 5-HT transporter (Sert) and the vesicular monoamine transporter (Vmat).[34]

Taken altogether, the MHB organizer determines the competence of the territory to develop dopaminergic and serotonergic neurons along the A-P and D-V axes during development. Alteration of MHB territory by Otx2 and Gbx2 antagonism can expand or reduce the DA or 5-HT neuron population.[27,35,36] Indeed, manipulations of the Otx2 expression result in the A-P ($En1^{Cre/+}$; $Otx2^{flox/flox}$ mice)[27] or D-V ($Otx1^{Cre/+}$; $Otx2^{flox/flox}$ mice)[36] transformation of cell fate with consequent alteration of positioning and extension of DA and 5-HT neuronal population.

Altered development of DA and 5-HT neurons regulate seizure susceptibility: indications from classical and conditional mutant mice

We investigated seizure susceptibility in mutant mice with conditional inactivation of the Otx2 gene in DA precursor cells. In these mice, Otx2 was conditionally inactivated in mesDA progenitors by a Cre recombinase expressed under the control of the En1 gene ($En1^{Cre/+}$; $Otx2^{flox/flox}$). Otx2 conditional inactivation resulted in great reduction of midbrain DA neurons and significantly increased the number of 5-HT neurons in the ventral midbrain, CA3 subfield of hippocampus and cerebral cortex by neurotransmitter fate switch in the ventral midbrain and this alteration is maintained throughout life.[27,37] Due to this increased 5-HT hyper-innervation, $En1^{Cre/+}$; $Otx2^{flox/flox}$ mice were resistant to kainic-acid (KA) induced seizures. Indeed, depletion of brain 5-HT in these mice restored 5-HT content, fully re-establishing KA-seizure susceptibility.[37]

In parallel experiments, we evaluated KA induced seizure susceptibility in mice with conditional overexpression of the Otx2 gene in DA precursor cells. In these mice, Otx2 was conditionally overexpressed by a Cre recombinase under the transcriptional control of the En1 gene ($En1^{Cre/+}$; $tOtx2^{ov/+}$). Otx2 overexpression resulted in a 35% increase of mesDA progenitors neurons in the VTA of the anterior as well as posterior mesencephalon, without any alteration in 5-HT neurons during embryonic and postnatal

development.[32,38] En1$^{Cre/+}$; tOtx2$^{ov/+}$ mice did not show significantly altered KA induced seizure susceptibility when compared to control animals.[39] Importantly, an increased inhibitory tone in limbic areas by higher number of parvalbumin cells observed in these mice might therefore contribute to justify the effects of KA-induced seizure susceptibility in these mutants.[40]

We also evaluated KA induced seizure susceptibility in En1$^{+/-}$; En2$^{-/-}$ mutant mice (En^{HT} mice) that display gradual loss of DAergic neurons of the substantia nigra.[41] It is important to point out that the postnatal DA cell loss in HT mice is not accompanied by increased number of 5-HT cells. En^{HT} mice did not show significantly altered seizure susceptibility when compared to control animals.[7] We also investigated KA induced seizure susceptibility in

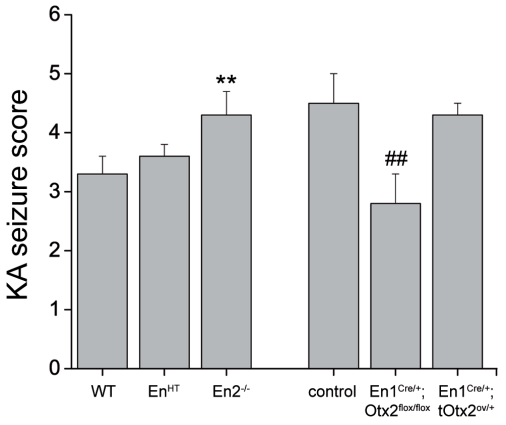


Fig. 2. Fig. 2 shows KA seizure susceptibility in different En1/2 and Otx2 mutant mouse strains. En2$^{-/-}$ mice showed a significantly increased susceptibility to KA-induced seizures as compared to WT and En1$^{+/-}$; En2$^{-/-}$ (En^{HT}) mice. Conversely, En1$^{Cre/+}$; Otx2$^{flox/flox}$ mice show a marked resistance to KA seizures, as compared to their controls and En1$^{Cre/+}$; *t*Otx2$^{ov/+}$ mice. WT, En2$^{-/-}$ and En^{HT} mice were from a C57Bl/6x129Sv mixed genetic background,[41,42] whereas control, En1$^{Cre/+}$; Otx2$^{flox/flox}$and En1$^{Cre/+}$; *t*Otx2$^{ov/+}$ were generated in a KA-sensitive DBA2 background.[37] For these reasons, the two sets of experiments were analyzed separately. Bars represent the maximum seizure rating scale value scored by each genotype (n = 8-10 animals per group) over a period of two hours after intraperitoneal (i.p.) administration of KA (20 mg/kg). Data are expressed as mean ± s.d. ** *P*<0.001, one-way ANOVA followed by post-hoc Tukey test (*En2*$^{-/-}$ vs. WT and *En*^{HT}). ## *P*<0.001, one-way ANOVA followed by post-hoc Tukey test (En1$^{Cre/+}$; Otx2$^{flox/flox}$vs. control and En1$^{Cre/+}$; *t*Otx2$^{ov/+}$). Seizures were scored as described:[37] stage 0: normal behavior; stage 1: immobility; stage 2: forelimb and/or tail extension, rigid posture; stage 3: repetitive movements, head bobbing; stage 4: forelimb Clonus with rearing and falling (limbic motor seizure); stage 5: continuous rearing and falling; stage 6: severe whole body convulsions (tonic-clonic seizures); stage 7: death. Data are re-adapted from our previous studies.[7,37,39,42]

En2$^{-/-}$ mice, which showed no alteration in the number of DA and 5-HT neurons at all ages. Surprisingly, En2$^{-/-}$ mice showed increased KA-induced seizures susceptibility. We further investigated the possible reason and consequence for this increased susceptibility, discovering a reduced number of inhibitory interneurons in the hippocampus and cerebral cortex of En2$^{-/-}$ mice.[42,43] Fig. 2 summarizes KA seizure susceptibility in the different En1/2 and Otx2 mutant mouse strains analyzed in our experiments.

According to this view, it was expected that reduction of DA cells in both En1$^{Cre/+}$; Otx2$^{flox/flox}$ and En^{HT} mice would contribute to increase the seizure susceptibility in these animals, while increase in DA cells in En1$^{Cre/+}$; tOtx2$^{ov/+}$ mice would contribute to lower seizure susceptibility severity. On the contrary, En1$^{Cre/+}$; Otx2$^{flox/flox}$ mice were markedly resistant to KA seizures due to 5-HT hyper-innervation, whereas En1$^{Cre/+}$; tOtx2$^{ov/+}$ mice and En^{HT} mice (in which 5-HT levels were unchanged) showed a normal susceptibility to KA induced seizures (Table 1). This is in line with earlier observation that 5-HT levels were inversely proportional to the seizure susceptibility. More importantly, altered level of DA in En1$^{Cre/+}$; Otx2$^{flox/flox}$, En1$^{Cre/+}$; tOtx2$^{ov/+}$ and En^{HT} mice had less impact in altering seizure susceptibility. Indeed En1$^{Cre/+}$; Otx2$^{flox/flox}$ mice (which have reduced level of DA) did not show increased seizure susceptibility, while En1$^{Cre/+}$; tOtx2$^{ov/+}$ and En^{HT} mice (which have higher and lower level of DA, respectively, with no alterations in 5-HT), showed unaltered seizure threshold. Thus, the altered embryonic development of 5-HT neurons seems to have a more prominent effect on the seizure control than the altered development of DA neurons (Fig. 3 and Table 1). Importantly, it is widely known that different genetic background meaning different inbred mouse strains impacts KA-induced seizure susceptibility in the mouse[44] but this issue is not discussed here due to space limitations.

Concluding remarks

Altered expression and function of homeobox genes during CNS development may lead to abnormal neuronal differentiation and circuit formation, ultimately leading to an imbalance between excitation vs. inhibition that might account for seizure susceptibility. In this review, we summarized our studies carried out in mutant mouse lines with targeted manipulation of Otx2, En1 and En2 genes. Our results suggest that altered specification of DA and 5-HT cell fate results in altered seizure susceptibility in the adult age. Classical pharmacological studies clearly showed that both DA and 5-HT may have potent anti-

Table 1. The effect of 5-HT hyper-innervation onto seizure control is more prominent than that of DA reduction in these animal models

Mouse strain	DA and 5-HT alterations	KA seizure susceptibility	References
En1$^{Cre/+}$; Otx2$^{flox/flox}$	Less DA, more 5-HT	Resistant	37
En1$^{Cre/+}$; tOtx2^{ov}	More DA, unaltered 5-HT	Not altered	39
En1$^{+/-}$; En2$^{-/-}$ (En^{HT})	Less DA, unaltered 5-HT	Not altered	7
En2$^{-/-}$	DA and 5-HT unaltered	Increased	42

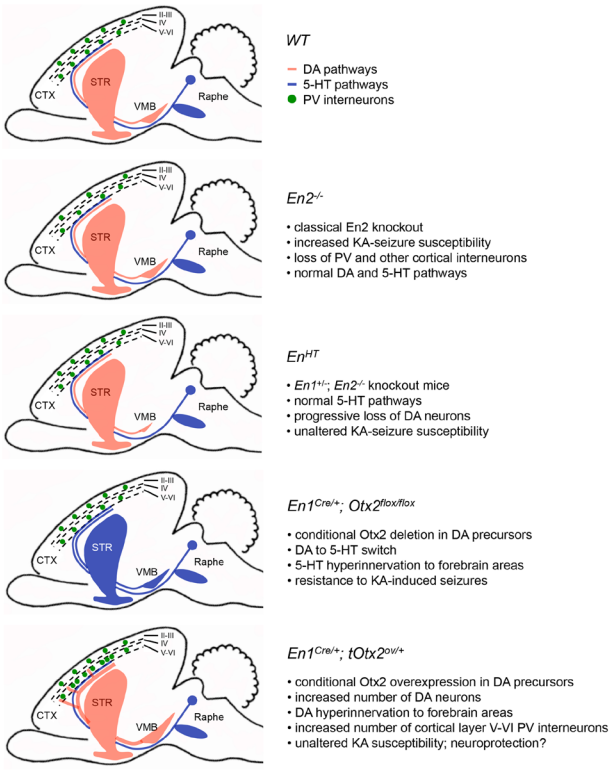


Fig. 3. Schematic representation of brain anatomical abnormalities relevant to seizure phenotypes in adult En and Otx mutant mice. Distribution of cortical PV-positive interneurons and major DA/5-HT pathways are illustrated for wild-type (WT) mice. Deletion of En2 results in reduced number of PV interneurons and increased susceptibility to KA seizures. Mice lacking En1 and En2 (En^{HT} mice) show progressive loss of DAergic neurons of the substantia nigra but unaltered KA susceptibility. Conditional deletion of Otx2 in mesDA precursors results in 5-HT hyperinnervation and resistance to KA seizures. Conditional overexpression of Otx2 in mesDA precursors results in DA hyperinnervation and increased number of PV interneurons. Symbols: DA and 5-HT pathways are indicated in red and blue, respectively; green circles indicate PV interneurons; dashed lines and Roman numbers indicate cortical layers. Abbreviations: CTX, cerebral cortex; Raphe, raphe nuclei; STR, striatum; VMB, ventral midbrain; other abbreviations are as in the text. See text and Table 1 for details and references.

convulsant effects, acting through specific receptor pathways. It might be therefore questioned that reduction of DA cells in both $En1^{Cre/+}$;$Otx2^{flox/flox}$ and En^{HT} mice could contribute to lower seizure susceptibility in these animals. According to this interpretation, reduction of DA in the $En1^{Cre/+}$;$Otx2^{flox/flox}$ mice would aggravate seizure severity. On the contrary, $En1^{Cre/+}$;$Otx2^{flox/flox}$ mice were markedly resistant to KA seizures due to 5-HT hyper-innervation, whereas En^{HT} and $En1^{Cre/+}$; $tOtx2^{ov/+}$ mice, in which 5-HT levels were unchanged, showed a normal KA seizure susceptibility. We propose that the protective role of 5-HT hyper-innervation is more conspicuous than that of DA alterations onto KA-induced seizure susceptibility. Further studies should be performed to understand whether similar mechanisms can be detected in the epileptic human brain.

Review Highlights

What is current knowledge?

√ Altered expression of homeobox-containing transcription factors markedly impact the structure and function of embryonic and adult CNS.

√ Inactivation of homeobox-containing genes can have a marked impact on specific stage of brain development, leading altered neuronal identity, neuronal circuit formation, seizure susceptibility and epilepsy in adult life.

√ Both DA and 5-HT markedly regulate seizure susceptibility through specific receptor subtype pathways.

What is new here?

√ Genetic manipulation of *En* and Otx genes leads to altered midbrain-to-forebrain DAergic and 5-HTergic pathways, resulting in altered seizure susceptibility in adult life, in some cases.

√ Genetically-induced alteration of 5-HT levels results in marked protection against KA-induced seizures while progressive loss or hyperinnervation of DA neurons results in unaltered susceptibility to KA-induced seizures.

Acknowledgments

We apologize to those authors whose studies have not been cited in this review. Authors gratefully acknowledge Dr. Antonio Simeone for providing Otx2 mutant mice and Dr. Paola Sgadò and Prof. G.U. Corsini for providing En^{HT} mice.

Ethical issues

Experiments described in this review were performed in conformity with current European Directive on the use of laboratory animals.

Competing interests

The authors declare no conflict of interests.

References

1. Starr MS. The role of dopamine in epilepsy.*Synapse* **1996**; 22:159-94.
2. Bozzi Y, Borrelli E. The role of dopamine signaling in epileptogenesis. *Front Cell Neurosci* **2013**; 7:157. doi: 10.3389/fncel.2013.00157
3. Bagdy G, Kecskemeti V, Riba P, Jakus R. Serotonin and epilepsy. *J Neurochem* **2007**; 100:857-73. doi: 10.1111/j.1471-4159.2006.04277.x
4. Acampora D, Simeone A. Understanding the roles of Otx1 and Otx2 in the control of brain morphogenesis. *Trends Neurosci* **1999**; 22:116-22. doi: 10.1016/S0166-2236(98)01387-3
5. Simeone A, Puelles E, Omodei D, Acampora D, Di Giovannantonio LG, Di Salvio M, *et al.* Otx genes in neurogenesis of mesencephalic dopaminergic neurons. *Dev Neurobiol* **2011**; 71:665-79. doi: 10.1002/dneu.20877
6. Bozzi Y, Tripathi PP, Simeone A. Developmental basis of epilepsy and seizure susceptibility: role of Otx genes. *In Mariani Foundation Paediatric Neurology Series – XX, Genetics of epilepsy and genetic epilepsies* **2009**; 167-74.
7. Tripathi PP, Sgado P, Corsini GU, Simeone A, Bozzi Y. Developmental basis of seizure susceptibility: a focus on dopaminergic and serotonergic systems. *Current Trends in Neurology* **2009**; 3:93-101

8. Bozzi Y, Borrelli E. Dopamine in neurotoxicity and neuroprotection: what do D2 receptors have to do with it? *Trends Neurosci* **2006**; 29:167-74. doi: 10.3389/fncel.2013.00157
9. Starr MS. Regulation of seizure threshold by D1 versus D2 receptors, J. L. *Waddington (Ed.).* Academic Press **1993**; p. 235.
10. Gangarossa G, Ceolin L, Paucard A, Lerner-Natoli M, Perroy J, Fagni L, *et al.* Repeated stimulation of dopamine D1-like receptor and hyperactivation of mTOR signaling lead to generalized seizures, altered dentate gyrus plasticity, and memory deficits. *Hippocampus* **2014**; 24:1466-81. doi: 10.1002/hipo.22327
11. Bozzi Y, Vallone D, Borrelli E. Neuroprotective role of dopamine against hippocampal cell death. *J Neurosci* **2000**; 20:8643-49.
12. O'Sullivan GJ, Dunleavy M, Hakansson K, Clementi M, Kinsella A, Croke DT, *et al.* Dopamine D1 vs D5 receptor-dependent induction of seizures in relation to DARPP-32, ERK1/2 and GluR1-AMPA signalling. *Neuropharmacology* **2008**; 54:1051-61. doi: 10.1016/j.neuropharm.2008.02.011
13. Jackson DM, Westlind-Danielsson A. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol Ther* **1994**; 64:291–370.
14. Tripathi PP, Santorufo G, Brilli E, Borrelli E, Bozzi Y. Kainic acid-induced seizures activate GSK-3β in the hippocampus of D2R-/- mice. *Neuroreport* **2010**; 21:846-50. doi: 10.1097/WNR.0b013e32833d5891
15. Dunleavy M, Provenzano G, Henshall DC, Bozzi Y. Kainic acid-induced seizures modulate Akt (SER473) phosphorylation in the hippocampus of dopamine D2 receptor knockout mice. *J Mol Neurosci* **2013**; 49:202-10. doi: 10.1007/s12031-012-9927-x
16. Bonnycastle DD, Giarman NJ, Paasonen MK. Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. *Br J Pharmacol Chemother* **1957**; 12:228-31
17. Löscher W. Genetic animal models of epilepsy as a unique resource for the evaluation of anticonvulsant drugs.A review. *Meth Find Exp Clin Pharmacol* **1984**; 6:531-47
18. Yan QS, Jobe PC, Cheong JH, Ko KH, Dailey JW. Role of serotonin in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. *Naunyn Schmiedebergs Arch Pharmacol* **1994**; 350:149-52.
19. Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PP, Middlemiss DN, *et al.* Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology* **1986**; 25:563-76.
20. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, *et al.* International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* **1994**; 46:157-203.
21. Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, *et al.* Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. *Nature* **1995**; 374:542-46.
22. Wada K, Kiryu K, Kawata Y, Chiba T, Mizuno K, Okada M, *et al.* Prognosis and clinical features of intractable epilepsy: a prospective study. *Psychiatry Clin Neurosci* **1997**; 51:233-35.
23. Hynes M, Rosenthal A. Specification of dopaminergic and serotonergic neurons in the vertebrate CNS. *Curr Opin Neurobiol* **1999**; 9:26-36.
24. Martinez-Barbera JP, Signore M, Boyl PP, Puelles E, Acampora D, Gogoi R, *et al.* Regionalisation of anterior neuroectoderm and its competence in responding to forebrain and midbrain inducing activities depend on mutual antagonism between OTX2 and GBX2. *Development* **2001**; 128:4789-800.
25. Simeone A, Acampora D, Gulisano M, Stornaiuolo A, Boncinelli E. Nested expression domains of four homeobox genes in developing rostral brain. *Nature* **1992**; 358: 687-690.
26. Andersson E, Tryggvason U, Deng Q, Friling S, Alekseenko Z, Robert B, *et al.* Identification of intrinsic determinants of midbrain dopamine neurons. *Cell* **2006**; 124:393-405.
27. Puelles E, Annino A, Tuorto F, Usiello A, Acampora D, Czerny T, *et al.* Otx2 regulates the extent, identity and fate of neuronal progenitor domains in the ventral midbrain. *Development* **2004**; 131, 2037-48. doi: 10.1242/dev.01107
28. Hynes M, Rosenthal A. Specification of dopaminergic and serotonergic neurons in the vertebrate CNS.*Curr Opin Neurobiol* **1999**; 9:26-36.
29. Bayer SA, Wills KV, Triarhou LC, Ghetti B. Time of neuron origin and gradients of neurogenesis in midbrain dopaminergic neurons in the mouse. *Exp Brain Res* **1995**; 105:191 -99.
30. Zetterström RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. *Science* **1997**; 276:248-50.
31. Simeone A, Acampora D. The role of Otx2 in organizing the anterior patterning in mouse. *Int J Dev Biol* **2001**; 45:337-45
32. Di Salvio M, Di Giovannantonio LG, 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* **2010**; 54:939-45. doi: 10.1387/ijdb.092974ms
33. Ye W, Shimamura K, Rubenstein JL, Hynes MA, Rosenthal A. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **1998**; 93:755-66.
34. Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev Neurosci* **2003**; 4:1002-1012.
35. Broccoli V, Boncinelli E, Wurst W. The caudal limit of Otx2 expression positions the isthmic organizer. *Nature* **1999**; 401:164-68.
36. Puelles E, Acampora D, Lacroix E, Signore M, Annino A, Tuorto F, *et al.* Otx dose-dependent integrated control of antero-posterior and dorso-ventral patterning of midbrain. *Nat Neurosci* **2003**; 6:453-60.
37. Tripathi PP, Di Giovannantonio LG, Viegi A, Wurst W, Simeone A, Bozzi Y. Serotonin hyperinnervation abolishes seizure susceptibility in Otx2 conditional mutant mice. *J Neurosci* **2008**; 28:9271-76. doi: 10.1523/JNEUROSCI.2208-08.2008
38. Omodei D, Acampora D, Mancuso P, Prakash N, Di Giovannantonio LG, Wurst W, *et al.* Anterior-posterior graded response to Otx2 controls proliferation and differentiation of dopaminergic progenitors in the ventral mesencephalon. *Development* **2008**; 135:3459-70. doi: 10.1242/dev.027003
39. Tripathi PP, Di Giovannantonio LG, Sanguinetti E, Acampora D, Allegra M, Caleo M, *et al.* Increased dopaminergic innervation in the brain of conditional mutant mice overexpressing Otx2: effects on locomotor behavior and seizure susceptibility. *Neuroscience* **2014**; 261:173-83. doi: 10.1016/j.neuroscience.2013.12.045
40. Bozzi Y, Simeone A. Otx genes and seizure susceptibility. *Molecular & Cellular Epilepsy* **2014**; 1:e74. doi: 10.14800/mce.74
41. Sgadò P, Albéri L, Gherbassi D, Galasso SL, Ramakers GM, Alavian KN, *et al.* Slow progressive degeneration of nigral dopaminergic neurons in postnatal Engrailed mutant mice. *Proc Natl Acad Sci USA* **2006**; 103:15242-47.
42. Tripathi PP, Sgadò P, Scali M, Viaggi C, Casarosa S, Simon HH, *et al.* Increased susceptibility to kainic acid-induced seizures in Engrailed-2 knockout mice. *Neuroscience* **2009**; 159:842-49. doi: 10.1016/j.neuroscience.2009.01.007
43. Sgadò P, Genovesi S, Kalinovsky A, Zunino G, Macchi F, Allegra M, *et al.* Loss of GABAergic neurons in the hippocampus and cerebral cortex of Engrailed-2 null mutant mice: implications for autism spectrum disorders. *Exp Neurol* **2013**; 247:496-505. doi: 10.1016/j.expneurol.2013.01.021
44. Schauwecker PE, Steward O. Genetic determinants of susceptibility to excitotoxic cell death: implications for gene targeting approaches. *Proc Natl Acad Sci USA* **1997**; 94:4103-08.