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

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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 serotoninergic neurons during vertebrate CNS development. Dopaminergic and serotoninergic 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.

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

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)\textsuperscript{1,2} and serotonin (5-hydroxytryptamine, 5-HT)\textsuperscript{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.\textsuperscript{2,3} Several lines of evidence show that Otx1, Otx2 and En2 control patterning and regionalization of hindbrain, midbrain and forebrain areas.\textsuperscript{4,5} Accordingly, mutations of various homeobox genes have been linked with severe postnatal neurological dysfunctions, including occurrence of epileptic seizures.\textsuperscript{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 serotoninergic circuits in the genesis and control of epileptogenesis has been extensively reviewed.\textsuperscript{1,3,4} Here we briefly summarize the major findings in this field.

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**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.\(^{10}\) 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.\(^{23}\) Dopamine D2 receptor knockout (D2R\(^{-/-}\)) mice show an increased susceptibility to KA-induced seizures and CA3 hippocampal cell death,\(^{4,13}\) 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 extracellular 5-HT levels against both limbic and generalized seizures.\(^{17,18}\) In contrast, depletion of brain 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.\(^{2,8}\) 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.\(^{21}\) 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, Msh1, and Msh2 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 Msh1 which in turn activates Ngn2. Activated Ngn2 regulates the progression of differentiated Sox2-positive late progenitors into Nurr1-positive postmitotic 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
immature DA neurons that induce En1/2 expression.\textsuperscript{31} Otx2 is further expressed in postmitotic mesDA neurons during later part of embryogenesis and in the adult brain.\textsuperscript{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), vesicular 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.\textsuperscript{33} Expression of Nkx2.2 is then essential for the specification of 5-HT neurons.\textsuperscript{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).\textsuperscript{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.\textsuperscript{27,35,36} Indeed, manipulations of the Otx2 expression result in the A-P (En1\textsuperscript{Cre/+}; Otx2\textsuperscript{flox/flox} mice)\textsuperscript{27} or D-V (Otx1\textsuperscript{Cre/+}; Otx2\textsuperscript{flox/flox} mice)\textsuperscript{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\textsuperscript{Cre/+}; Otx2\textsuperscript{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.\textsuperscript{27,37} Due to this increased 5-HT hyper-innervation, En1\textsuperscript{Cre/–}; Otx2\textsuperscript{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.\textsuperscript{27}

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\textsuperscript{Cre/+}; tOtx2\textsuperscript{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 En1Cre+; tOtx2flox/flox 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 (En1HT 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. En1HT mice did not show significantly altered seizure susceptibility when compared to control animals.7 We also investigated KA induced seizure susceptibility in

![Graph](image)

**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−/− (En1HT) mice. Conversely, En1Cre+; Otx2flox/flox mice show a marked resistance to KA seizures, as compared to their controls and En1Cre+; Otx2flox/flox mice. WT, En2−/− and En1HT mice were from a C57Bl/6x129Sv mixed genetic background,41,42 whereas control, En1Cre+; Otx2flox/flox and En1Cre−/−; Otx2flox mice were generated in a KA-sensitive DBA2 background.39 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 En1HT). ## P < 0.001, one-way ANOVA followed by post-hoc Tukey test (En1+/−; Otx2flox/flox vs. control and En1Cre+; Otx2flox/+). Seizures were scored as described:39 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.7,37,39-41

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 En1Cre+; Otx2flox/flox and En1HT mice would contribute to increase the seizure susceptibility in these animals, while increase in DA cells in En1Cre−/−; Otx2flox/flox mice would contribute to lower seizure susceptibility severity. On the contrary, En1Cre+; Otx2flox/flox mice were markedly resistant to KA seizures due to 5-HT hyperinnervation, whereas En1Cre−/−; Otx2flox/flox and En1HT 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 might account for seizure susceptibility. More importantly, altered level of DA in En1Cre−/−; Otx2flox/flox, En1Cre+; Otx2flox/flox and En1HT mice had less impact in altering seizure susceptibility. Indeed En1Cre−/−; Otx2flox/flox mice (which have reduced level of DA) did not show increased seizure susceptibility, while En1Cre+; Otx2flox/flox and En1HT 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 mouse44 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 on 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 |
|----------------|-------------------------|---------------------------|------------|
| En1Cre+; Otx2flox/flox | Less DA, more 5-HT | Resistant | 37 |
| En1Cre−/−; tOtx2flox | More DA, unaltered 5-HT | Not altered | 39 |
| En1+/−; En2−/− (En1HT) | Less DA, unaltered 5-HT | Not altered | 7 |
| En2−/− | DA and 5-HT unaltered | Increased | 42 |

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**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 (En1;2 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.

Convolvulant effects, acting through specific receptor pathways. It might be therefore questioned that reduction of DA cells in both En1;Otx2;flox/flox and En1;2 mice could contribute to lower seizure susceptibility in these animals. According to this interpretation, reduction of DA in the En1;Otx2;flox/flox mice would aggravate seizure severity. On the contrary, En1;Otx2;flox/flox mice were markedly resistant to KA seizures due to 5-HT hyper-innervation, whereas En1;2 and En1;Otx2;flox/flox 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.

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**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.

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**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-HTeric 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.
