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Review

Sex dimorphism in seizure-controlling networks

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ABSTRACT

Males and females show a different predisposition to certain types of seizures in clinical studies. Animal studies have provided growing evidence for sexual dimorphism of certain brain regions, including those that control seizures. Seizures are modulated by networks involving subcortical structures, including thalamus, reticular formation nuclei, and structures belonging to the basal ganglia. In animal models, the substantia nigra pars reticulata (SNR) is the best studied of these areas, given its relevant role in the expression and control of seizures throughout development in the rat. Studies with bilateral infusions of the $GABA_A$ receptor agonist muscimol have identified distinct roles of the anterior or posterior rat SNR in flurothyl seizure control, that follow sex-specific maturational patterns during development. These studies indicate that (a) the regional functional compartmentalization of the SNR appears only after the third week of life, (b) only the male SNR exhibits muscimol-sensitive proconvulsant effects which, in older animals, is confined to the posterior SNR, and (c) the expression of the muscimol-sensitive anticonvulsant effects become apparent earlier in females than in males. The first three postnatal days are crucial in determining the expression of the muscimol-sensitive proconvulsant effects of the immature male SNR, depending on the gonadal hormone setting. Activation of the androgen receptors during this early period seems to be important for the formation of this proconvulsant SNR region. We describe molecular/anatomical candidates underlying these age- and sex-related differences, as derived from *in vitro* and *in vivo* experiments, as well as by [^{14}C]2-deoxyglucose autoradiography. These involve sex-specific patterns in the developmental changes in the structure or physiology or $GABA_A$ receptors or of other subcortical structures (e.g., locus coeruleus, hippocampus) that may affect the function of seizure-controlling networks.

Contents

Abbreviations: AR, androgen receptors; DES, diethylstilbestrol; 2-DG, [^{14}C]2-deoxyglucose; DHT, dihydrotestosterone; E, 17β-estradiol; ER, estrogen receptors; $GABA_AR$, $GABA_A$ receptors; GP, lateral globus pallidus; KCC, K^+/Cl^- cotransporter; IPSC, inhibitory post-synaptic current; -ir, immunoreactivity; LC, locus coeruleus; L-VSCC, L-type voltage sensitive calcium channel; NE, norepinephrine; NKCC, $Na^+/K^+/Cl^-$ cotransporter; PN, postnatal day; PPT, pedunculopontine tegmental nucleus; SC, superior colliculus; SNpc, pars compacta of the substantia nigra; SNR, pars reticulata of the substantia nigra; SNRa, anterior part of SNR; SNRp, posterior part of SNR; STN, subthalamic nucleus; T, testosterone; VM, ventro-medial thalamic nucleus.

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Introduction

Various subcortical structures play a critical role in modulating seizures. A great deal of data has been provided concerning a role for thalamus, especially in models of primary generalized epilepsy (see the review by Avanzini et al., 2000), and several structures belonging to the basal ganglia–cortical circuits have been extensively studied. Among them a pivotal role in seizure modulation seems to be played by the substantia nigra pars reticulata (SNR) as well as the superior colliculus (SC) and subthalamic nucleus (STN) through their connections with the SNR (Garant and Gale, 1987; Lado et al., 2003; Velísková et al., 1996a). There is also evidence concerning the role of monoaminergic subcortical nuclei, and in particular for the noradrenergic (NE) locus coeruleus (LC), and the serotonergic (5HT) raphe nuclei. These structures are part of the brainstem reticular formation, are formed by neurons whose axons widely innervate the cortical mantle, such as the noradrenergic axons, and exert a modulatory role on their targets.

In the last two decades, it has been clearly shown that the SNR has significant sex-related effects on different types of seizures, and that some of these vary significantly during development. Most of the present review will primarily address the sex-specific features of the SNR, including its differentiation, morphology and function in seizure control, and will discuss potential mechanisms and implications of such a dimorphism. Lastly, we will also address the existing evidence for a potential dimorphic role of other seizure-controlling structures.

The SNR: a predominantly GABAergic basal ganglia nucleus

The SNR constitutes the main part of substantia nigra (SN). The SNR is formed almost exclusively of fast-spiking GABAergic cells (Gerfen, 2004; Schultz, 1986), which are much less densely packed than their dopaminergic counterpart of the pars compacta of the SN (SNpc). A few dopaminergic cells can also be found in the posterior SNR (SNRp) (González-Hernández and Rodríguez, 2000). The SNR together with the globus pallidus internal (GPi), which in rodents is represented by the entopeduncular nucleus, is the main output structure of the basal ganglia.

SNR neurons receive afferents from the STN, lateral globus pallidus (GP) and from the striatum. They send axon terminals to structures outside of the basal ganglia, namely the thalamus, the pedunculopontine tegmental nucleus (PPT) and the SC (Gerfen, 2004); in addition, each GABAergic SNR neuron sends axon collaterals to neighboring SNR neurons (Deniau et al., 1982; Grofova et al., 1982). Specific subregions of the SNR receive projections from specific subregions of the afferent nuclei, and such a topographical segregation is maintained also in the targets of SNR efferents (reviewed in Gerfen, 2004), The thalamic targets of SNR include bilateral projections to the ventromedial (VM), parafascicular, centromedian and paracentral nuclei and unilateral projections to the centrolateral, mediodorsal and thalamic reticular nucleus (Gulcebi et al., 2012).

Communication via $GABA_A$ receptors ($GABA_AR$) helps orchestrate the net activity of SNR neurons, although other neurotransmitter systems such as glutamatergic receptors play a role too (Zhou and Lee, 2011). $GABA_ARs$ are pentameric ligand-activated ionic channels permeable to chloride ions and, to a lesser extent, to bicarbonate (Farrant and Kaila, 2007; Galanopoulou, 2008b). The precise composition of $GABA_ARs$ in terms of subunits types determines their kinetics, affinity to drugs, subcellular localization (extra- or post-synaptic), or region-specific expression in a complex manner (Galanopoulou, 2008b; Mohler, 2006). Usually, functional channels comprise two α and two β subunits. When the fifth subunit is a δ subunit, $GABA_AR$ are extrasynaptic and responsible for tonic current generation. In contrast, $GABA_AR$ with a γ subunit are most commonly post-synaptic, generating phasic inhibitory post-synaptic currents (IPSCs), but can also be found at extrasynaptic sites (reviewed in Galanopoulou, 2008b).

$GABA_AR$ signaling classically induces neuronal hyperpolarization, due to an influx of Cl^-, which follows the electrochemical Cl^- gradient between the extracellular and the intracellular compartments. The intracellular Cl^- concentration ($[Cl^-]_i$) is regulated by cation Cl^- cotransporters. These include Cl^- exporters, like K^+/Cl^- cotransporters (KCCs), and Cl^- importers like the $Na^+/K^+/Cl^-$ cotransporters (NKCCs). During development, there is a gradual shift in the expression and activity of two main representatives, mainly NKCC1 which declines (Plotkin et al., 1997) and KCC2 that increases with age (Rivera et al., 1999). As a result, the $[Cl^-]_i$ is considerably higher in most studied immature neurons compared to the mature ones. Thus, activation of $GABA_ARs$ in immature neurons with high $[Cl^-]_i$ triggers depolarizing potentials due to the efflux of Cl^- and hyperpolarizing potentials in mature neurons with low $[Cl^-]_i$, due to Cl^- influx. The early depolarizing $GABA_AR$ signaling is essential for normal brain development, as it results in depolarization-induced activation of L-type voltage-sensitive calcium channels (L-VSCCs) and NMDA receptors (reviewed in (Ben-Ari, 2002; Farrant and Kaila, 2007; Galanopoulou, 2008b). In fact, precocious termination of the depolarizing GABA effects may have serious adverse effects in the way normal neurons develop and arborize to form synaptic connections (Cancedda et al., 2007; Wang and Kriegstein, 2008, 2011). It is worth noting that in the normal brain the $GABA_AR$-mediated depolarizations are not necessarily excitatory, as they can still induce a weaker form of inhibition, shunting inhibition, when neuronal depolarization exceeds the reversal potential of $GABA_ARs$ (Staley and Mody, 1992).

The role of SNR in seizure control in males

In the early 1980s, it was shown that in male adult rats, microinfusions of muscimol (a $GABA_AR$ agonist) in the SNR exert anticonvulsant effects toward different types of experimental seizures such as tonic hindlimb extension in the maximal electroshock test and tonic and clonic seizures produced by pentylenetetrazole and bicuculline (Iadarola and Gale, 1982), as well as toward flurothyl-induced clonic seizures (Okada et al., 1986), a seizure model which allows a precise quantification of the seizure threshold. [^{14}C]2-deoxyglucose (2-DG) autoradiographic studies showed that the SNR was differently involved in kainic acid-induced seizures in adult male rats versus male rat pups (Albala et al., 1984) and, surprisingly, that bilateral muscimol microinfusions into the SNR of 15-17 postnatal days (PN) old male rat pups were proconvulsant in flurothyl-induced clonic seizures (Garant et al., 1995; Moshé and Albala, 1984; Okada et al., 1986; Sperber et al., 1987). The age-related differences in the modulation of the nigral output systems following various pharmacological manipulations were documented in several studies summarized in Table 1 and point out that there is an age-related role of the SNR in the control of seizures but also in motor control. Indeed, given the known higher propensity of the immature CNS to seizures and status epilepticus (Moshé and Albala, 1983; Moshé et al., 1983), the SNR has been considered as an important candidate to justify such an age-related shift in seizure susceptibility and expression that may be involved in human seizures too.

In parallel with the growing amount of data on the role of SNR on seizures, another important observation was made in the mid-90s. Detailed analysis on the effects of the spatial features relating the microinfusions of pharmacologic agents into specific regions of SNR along its anterior–posterior axis led to the discovery that actually the SNR of adult male rats can be divided into two functionally separate regions, an anterior (SNRa) and a posterior one (SNRp), bearing different effects in the flurothyl-induced clonic seizures (Moshé et al., 1994; Sperber et al., 1999; Velísková et al., 1996b; Velísková and Moshé, 2001). In particular, in adult male rats, bilateral muscimol infusions are anticonvulsant if infused in the SNRa and proconvulsant if infused in the SNRp (Sperber et al., 1999). Region-specific effects were also noted for the $GABA_AR$ agonist (*Z*)-3-[(aminoiminomethyl)thio] 2-propenoic acid (ZAPA) and γ-vinyl-GABA which increases local GABA levels (Velísková et al., 1996b). In contrast, bicuculline (a $GABA_AR$ antagonist) infusions were proconvulsant into the SNRa and without any effect in the SNRp (Velísková et al., 1996b) (see also Table 1).