The multifaceted role of inhibition in epilepsy: seizure-genesis through excessive GABAergic inhibition in autosomal dominant nocturnal frontal lobe epilepsy

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Purpose of review

While epilepsy describes a heterogeneous array of syndromes, the conventional view is that there is a common underlying failure in the ability of GABAergic inhibition to overcome excessive synaptic excitation. This review explores the possibility that enhanced GABAergic inhibition in the neocortex could also be proepileptogenic.

Recent findings

Recently, two mouse strains carrying mutant alleles of the α4 subunit of the nicotinic acetylcholine receptor that are associated with autosomal dominant nocturnal frontal lobe epilepsy have been found to show spontaneous seizures. Recordings from neocortical pyramidal neurons *in vitro* show that the autosomal dominant nocturnal frontal lobe epilepsy mutations are associated with large selective increases in nicotine-evoked GABAergic inhibition, which may be key factor in epileptogenesis, as the seizures *in vivo* are blocked by subconvulsive doses of the $GABA_A$ receptor antagonist, picrotoxin.

Summary

The precise links between the observed gain of neocortical inhibition and development of seizures in autosomal dominant nocturnal frontal lobe epilepsy mice remain unknown. Recent insights into the functional properties of cortical GABAergic circuits, however, suggest several possible pathways to be explored, whose elucidation could enable selective therapeutic interventions.

Keywords

autosomal dominant nocturnal frontal lobe epilepsy, GABA, inhibition, interneurons, nicotinic acetylcholine receptors, synchronization

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Introduction

The principal excitatory neurons in the healthy neocortex display sparse activity, with low mean firing rates, and spike patterns that are often reminiscent of a Poisson process. Recordings of the bulk neuronal activity, using an electroencephalogram (EEG) or electrocorticogram (ECoG), reveal that the ensemble spike activity is synchronized during a variety of network oscillations, whose frequency and spatial scale vary with behavior. At one extreme are the ultraslow oscillations (<1 Hz) observed during slow wave sleep, that propagate across the entire neocortical mantle, and also recruit neurons in the thalamus and striatum [1–4]. In contrast, the activity within local cortical circuits can display rhythmic synchronization over much shorter time scales, reaching the high γ-frequency range (80–120 Hz) [5] and possibly ultra-fast frequencies (>200 Hz) [6,7]. Thus, the spatio-temporal correlations in physiological neuronal activity can range from long-range synchronization of slow oscillations, to the millisecond precision of local spike activity.

While epileptic seizures are behaviourally defined phenomena, accurate diagnosis often depends on the identification of paroxysmal activity in the EEG. Seizures in some epileptic syndromes are associated with stereotypical EEG patterns, such as the spike-wave discharges of around 3 Hz in absence epilepsy. Electrographic seizures, however, do not conform to a universal pattern, and are instead detected as atypical large-amplitude EEG oscillations, which are generally referred to as 'hypersynchronous'. The term hypersynchrony is vague, as it does not distinguish between changes in correlated activity over spatial versus temporal scales, or how neuronal synchrony differs from that observed over the full gamut of physiological brain oscillations. Hypersynchrony, however, does capture the fact that the degree of coherence during ictal

oscillations is inappropriate for the normal behavioral state of the system, and reflects the breakdown of sparse neocortical activity into population bursts.

Excitatory neurons in the neocortex are not only reciprocally connected, but also synapse onto a smaller population of GABAergic inhibitory interneurons, which act to dampen recurrent excitation in individual neurons, and to control the propagation of neuronal activity. Many in-vitro and in-vivo models have demonstrated that degrading GABAergic inhibition can lead to run-away excitation and epileptiform bursts. Thus, treatments for epileptic syndromes are generally designed to suppress the overall levels of neuronal excitability. GABAergic interneurons, however, also play an intimate role in synchronizing neuronal spiking [8,9•], and thus it is possible that a gain of inhibition could also lead to some forms of pathological synchronization. What is not immediately apparent is whether such increased inhibition would alone be able to collapse the sparse nature of neocortical activity, recruiting more neurons to each oscillatory cycle, and eventually leading to seizure activity.

Recently, two mouse strains that carry mutant alleles associated with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) have been found to show spontaneous seizures that appear to result from a gain of inhibition [10••]. The precise mechanisms underlying seizure generation in these mice remain poorly understood, and are the focus of current research. Here, we briefly review the known links between ADNFLE mutations and neocortical function, and integrate these with recent findings on GABAergic transmission, in order to speculate as to how such increases in inhibition could be proconvulsive.

Autosomal dominant nocturnal frontal lobe epilepsy and nicotinic acetylcholine receptor mutations

ADNFLE is characterized by clusters of frontal lobe motor seizures that arise most frequently during slow wave sleep. In many cases, seizures are not apparent in the EEG, which may be due to muscle artifacts or an obscured epileptic focus, but analysis of video-polysomnography and family history can enable diagnosis [11]. Three loci associated with ADNFLE have been mapped to mutant genes coding for the α4 (CHRNA4), α2 (CHRNA2) and β2 (CHRNB2) subunits of the nicotinic acetylcholine receptor (nAChR), while candidate genes for alternate loci remain to be identified [11,12]. Mammalian nAChRs are cation-selective ionotropic receptors, which are formed by pentameric combinations of 11 nAChR subunits (α2–7, α9, α10, β2–4). Permutations in subunit composition endow distinct nAChR subtypes with different properties, including agonist sensitivity, activation/desensitization kinetics and Ca^{2+} permeability. While the precise subunit composition of native nAChR is generally not known, however, the majority of functional nAChRs expressed throughout the brain appear to be either heteromeric α4β2-containing nAChRs or homomeric α7 nAChRs [13,14]. It remains a mystery as to why mutations in the α4, α2 or β2 nAChR subunits should lead to focal frontal lobe epilepsy, but such localized pathophysiology is suggestive of a cortical rather than subcortical origin of seizure generation. The immediate question is what common effect do the various ADNFLE mutations in the nAChR subunits have on cholinergic transmission and network activity in the frontal cortex?

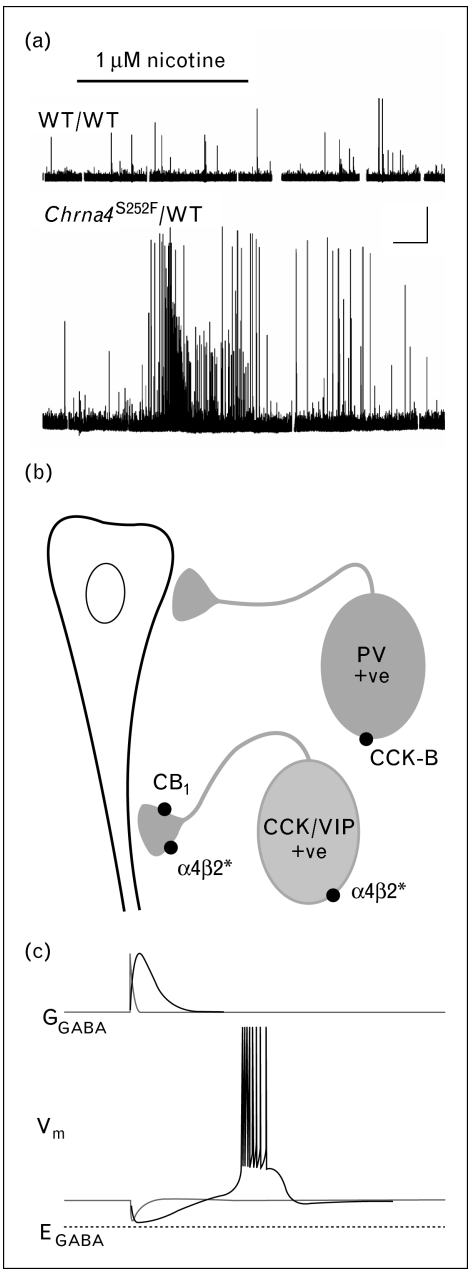
Insights from transgenic mouse models of autosomal dominant nocturnal frontal lobe epilepsy

Studies exploring the effects of ADNFLE nAChR mutations in heterologous in-vitro expression systems have produced inconsistent results, suggesting a variety of changes in receptor properties that lead to both increases and decreases in nAChR function [11,12]. These differences may reflect the details of expression and the particular experimental paradigms used. Currently, the most consistent finding is that heterozygous mutant receptors show an increase in acetylcholine sensitivity. A more accurate and cohesive understanding of the links between genotype and phenotype would be enabled by the development of mouse models expressing nAChR mutations found in ADNFLE patients.

The initial attempt to address this issue involved the development of knock-in mice expressing mutations at the Leu9′ position in the M2 region of the α4 nAChR subunit, which, while not a known ADNFLE mutation, leads to hypersensitive α4 nAChR subunit-containing receptors [15]. More recently, knock-in mice carrying the ADNFLE *Chrna4*^{S248F} mutation have been generated [16•]. Unfortunately, none of these mouse strains display spontaneous behavioral or electrographic seizures. In both studies, though, the expression of at least one mutant allele was found sufficient to confer paroxysmal motor behaviors in response to subconvulsive doses of nicotine (e.g. 2 mg/kg systemic injections of nicotine) [15,16•]. These behavioral symptoms, however, could not be associated with any ictal events in the EEG or ECoG. Further study may reveal epileptic foci in these mouse models, and they will no doubt provide valuable insights into the semiology of ADNFLE, but without an electrophysiological correlate, it will be difficult to explore the mechanisms by which nAChR mutations lead to paroxysmal activity.

More immediate insights may be gleaned from an alternative set of mouse strains carrying either the ADNFLE *Chrna4*^{S252F} or the *Chrna4*$^{+L264}$ mutant alleles [10••],

Figure 1 Enhanced inhibition following nicotinic acetylcholine receptor activation in a mouse model of autosomal dominant nocturnal frontal lobe epilepsy and the possible mechanisms underlying the ensuing pathological synchrony



(a) Spontaneous and nicotine-evoked inhibitory postsynaptic currents recorded in layer V pyramidal neurons of the motor cortex in slices from wild type (WT, upper) and heterozygous *Chrna4*^{S252F}/WT (lower) mice. Pyramidal neurons were voltage clamped at 0 mV, with 75% series resistance compensation. Gaps in the current traces indicate periods when seal tests were performed. Nicotine (1 μM) was applied in the perfusate. Vertical scale bar 200 pA; horizontal scale bar 60 s. (b) Putative circuit mediating nicotine-evoked increases in inhibitory currents. Pyramidal neuron is shown in black, with two subtypes of GABAergic neuron shown in grey: parvalbumin-positive (PV +ve) interneurons that target perisomatic sites and cholecystokinin (CCK) and vasoactive intestinal peptide (VIP)-positive interneurons. Cholecystokinin/VIP-positive interneurons have been shown to express α4β2-containing nicotinic acetylcholine receptors (α4β2*) and cannabinoid receptors (CB1) [20,21].

for which both the homozygous and heterozygous mice display abnormal ECoG and spontaneous recurrent seizures. Recordings from pyramidal neurons in the motor cortex of these ADNFLE mutant mice *in vitro* demonstrate a large increase in nicotine-evoked inhibition (Fig. 1a). Furthermore, the abnormal ECoG and spontaneous seizures were found to be transiently inhibited by subconvulsive doses of picrotoxin, a use-dependent antagonist of fast GABAergic transmission. This suggests the counterintuitive conclusion that the paroxysmal activity produced by both ADNFLE mutations results from a gain of inhibition.

A possible role for cholecystokinin-positive interneurons and inhibitory avalanches

A first step to understanding the links between increased GABAergic inhibition and the generation of seizures will be to elucidate the classes of interneurons expressing the mutant ADNFLE nAChR. Cortical GABAergic interneurons can be classified into different subtypes based on their firing patterns, axonal targets, and expression of neuropeptides and Ca^{2+}-binding proteins [17]. The majority of layer I neocortical interneurons have been shown to express both α4β2 and α7 nAChR [18], but these cells appear to target other interneurons rather than pyramidal neurons, and are unlikely to explain the observed effects of nicotine in the ADNFLE mutant mice *in vitro*. Nicotine has also been shown to excite interneurons in the deep layers of the visual cortex, which have been characterized electrophysiologically by the presence of low-threshold Ca^{2+} spikes [19]. Separate studies identifying interneurons on the basis of mRNA expression suggest that α4β2 nAChR activation predominantly excites interneurons in the sensorimotor cortex that coexpress vasoactive intestinal peptide (VIP) and cholecystokinin [20,21] (see Fig. 1b). A large proportion of cholecystokinin-positive interneurons are large basket cells that target the perisomatic regions of pyramidal neurons [22••]. While no set of features uniquely identifies neocortical interneurons expressing postsynaptic α4β2 nAChR, one apparent consensus is that they are distinct from the cholecystokinin-positive basket cells, rather

Figure 1 (*Continued*)

The release of cholecystokinin could augment the increase in inhibitory currents impinging on pyramidal neurons, by depolarizing parvalbumin-positive interneurons through activation of cholecystokinin-B receptors [23•]. (c) Inhibitory currents can drive action potential firing through rebound excitation. In this example, GABAergic conductances (G_{GABA}) with different kinetics are injected into a single-compartment neuron expressing a strong T-type calcium current. While both inhibitory conductances drive the membrane potential (V_m) towards the reversal potential of GABA (E_{GABA}), only the prolonged hyperpolarization is sufficient to de-inactivate the T-type current, and enable rebound bursting. This example is only meant to demonstrate the principle of rebound excitation.

targeting the dendrites of pyramidal neurons or other interneurons [18–21].

The picture is far from complete, and with no evidence for interneuronal expression of α2 nAChR subunit [18,20], there is little indication as to how mutations in the α2, α4 and β2 nAChR may all yield similar functional affects. The link between α4β2-containing nAChR and cholecystokinin-positive interneurons, however, is tantalizing. It has recently been shown [23•] that cholecystokinin can depolarize perisomatic-targeting parvalbumin-positive interneurons through postsynaptic cholecystokinin-B receptors, and thereby produce a barrage of inhibition onto downstream pyramidal neurons (see Fig. 1b). While the mechanisms of cholecystokinin release from interneurons remain unknown, activation of hypersensitive Ca^{2+}-permeable postsynaptic α4β2 nAChR is likely to provide a powerful stimulus for peptide secretion *in vivo*. Therefore, it is possible that activation of ADNFLE nAChR could lead to an avalanche of inhibition in the neocortex.

How could enhanced inhibition lead to seizures?

As nAChR activation has been shown to increase inhibition onto interneurons, which can result in disinhibition of pyramidal neurons [18], one could posit a simple model whereby ADNFLE nAChR mutations lead to excessive disinhibition, and thus seizures. Recordings from ADNFLE-mutant mice, however, demonstrate that any increases in interneuron–interneuron inhibition are overridden by the excitatory pre and postsynaptic effects of ADNFLE-mutant nAChR activation on interneurons, yielding a net massive increase in nicotine-evoked inhibition onto pyramidal cells [10••].

If the primary pathological effect of ADNFLE nAChR mutations is an increase in GABA release onto pyramidal neurons in response to acute cholinergic activation, it is more difficult to explain how this could lead to seizures. One possibility is that the chloride influx during these barrages could overwhelm the mechanisms of chloride extrusion, and eventually lead to epileptogenic depolarizing GABAergic events [24]. It will certainly be important to check the $GABA_A$ receptor reversal potentials in ADNFLE mutant mice, and monitor chloride homeostasis during cholinergically induced GABA release. GABA, however, does not necessarily have to be depolarizing to produce both synchronization and excitability. Inhibitory events can interact with intrinsic voltage-dependent conductances, and produce rebound excitation (Fig. 1c). This effect of membrane hyperpolarization may be mediated by activating inward (depolarizing) currents, such as I_h, by deactivating outward (hyperpolarizing) currents, such as I_M, or by deinactivating inward currents, such as voltage-gated Na^+ and Ca^{2+} currents. Indeed, the systemic administration of $GABA_A$ or $GABA_B$ receptor agonists can lead to spike-wave seizures in animal models [25,26], whose generation appears to depend on rebound bursting mediated by low-threshold T-type Ca^{2+} channels [26]. It has been proposed that such a mechanism of postinhibitory rebound bursting contributes to seizure generation in thalamocortical circuits in human absence epilepsy [27], which is consistent with the fact that clinically effective treatments for absence seizures, including ethosuximide, can act to reduce T-type Ca^{2+} currents [28].

An association between seizure activity and increased inhibition has also been observed in several models of temporal lobe epilepsy [29–31]. These changes are likely to represent homeostatic responses to dampen network activity, but could also be potentially proconvulsant by increasing network synchronization [30] or producing hyperexcitability through increased I_h-mediated rebound excitation [32]. Massive GABA release in the neocortex in response to cholinergic activation, leading to activation of $GABA_A$ and possibly also $GABA_B$ receptors, could invoke analogous mechanisms to produce seizures in ADNFLE.

Potential for therapeutic effects of cannabinoids

ADNFLE seizures arise predominantly out of slow wave sleep, when cortical acetylcholine levels are at their lowest. It is possible that ictogenesis is actually triggered by transitions to rapid eye-movement sleep or the awake state, when cholinergic tone increases. Understanding why these states are subsequently protective against seizures could offer routes to selective treatment. Hypersensitive ADNFLE nAChR could remain desensitized during sustained acetylcholine release, which would favor a treatment strategy aimed at replicating this receptor state during slow wave sleep with low doses of nicotine (see [16•]). The additional actions of acetylcholine at muscarinic acetylcholine receptors (mAChR), however, may also act to counteract the effects of ADNFLE nAChR activation. Activation of presynaptic mAChR can directly suppress GABAergic transmission [33], while activation of postsynaptic mAChR can also induce endocannabinoid release from pyramidal cells [34], which acts retrogradely to suppress inhibition from interneurons [22••,34,35].

There is anecdotal evidence for the beneficial effects of marijuana use in managing some forms of epilepsy. Cannabinoid receptor activation suppresses both excitatory and inhibitory synaptic transmission, and it is the effect on excitation which has been shown to provide protection against kainic acid-induced seizures in the hippocampus [36]. Cannabinoid receptor agonists,

however, may also have therapeutic potential when there is a gain of inhibition rather than excitation. The majority of studies exploring the role of cannabinoid receptors in suppressing GABAergic inhibition have focused on cholecystokinin-positive basket cells in the hippocampus [22••,34,35]. It has recently been shown, however, that VIP-positive interneurons in the neocortex, which are sensitive to nAChR agonists, also express cannabinoid receptors [21]. Therefore, cannabinoid receptor agonists provide a potential tool for the study and treatment of seizures in ADNFLE.

Conclusion

Treatments designed to suppress the overall levels of neuronal excitability have proved successful in treating some epileptic syndromes, but such gross changes in the balance between excitation and inhibition have severe side effects. Moreover, a large proportion of epilepsy cases remain drug resistant. Clues to tackling these problems may come from a more detailed understanding of how the dynamics of inhibition are altered in epilepsy, and how this contributes to seizure generation. The recent characterization of two mouse models harboring mutant ADNFLE nAChR suggests that seizures can result from synchronized increases in neocortical inhibition, although other developmental changes and subcortical abnormalities cannot yet be ruled out. Further studies in these mice promise an attractive avenue for linking genotype to seizure type, and could yield a deeper understanding of how 'hypersynchrony' is generated and can be more selectively curtailed.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 213).

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