Errant ensembles: dysfunctional neuronal network dynamics in schizophrenia

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Abstract
Most complex psychiatric disorders cannot be explained by pathology of a single brain region, but arise as a consequence of dysfunctional interactions between brain regions. Schizophrenia, in particular, has been described as a ‘disconnection syndrome’, but similar principles are likely to apply to depression and ADHD (attention deficit hyperactivity disorder). All these diseases are associated with impaired co-ordination of neural population activity, which manifests as abnormal EEG (electroencephalogram) and LFP (local field potential) oscillations both within and across subcortical and cortical brain regions. Importantly, it is increasingly possible to link oscillations and interactions at distinct frequencies to the physiology and/or pathology of distinct classes of neurons and interneurons. Such analyses increasingly implicate abnormal levels, timing or modulation of GABA (γ-aminobutyric acid)-ergic inhibition in brain disease. The present review discusses the evidence suggesting that dysfunction of a particular class of interneurons, marked by their expression of the calcium-binding protein parvalbumin, could contribute to the broad range of neurophysiological and behavioural symptoms characteristic of schizophrenia.

Introduction
Apportioning mechanistic blame in complex neurobiological diseases presents a unique challenge: how can we design rational therapies for brain disorders that impact processes such as cognition without a detailed understanding of the molecular, synaptic, neuronal, network and systems mechanisms that enable high-order brain function under physiological conditions? Furthermore, attempting to pinpoint the underlying causes of complex pathologies can quickly generate circular arguments. For example, if some abnormal neuroanatomical hallmark (e.g. a change in protein expression levels) correlates with disease symptoms, is this a root cause or a compensatory pathophysiological response to the disease state? If a neurophysiological signature (e.g. task-related oscillatory activity in electroencephalographic recordings) proves abnormal in patients, does it reflect the cause of atypical behaviour, or does it simply reflect that behaviour being atypical? Schizophrenia is a primary example of such a complex disorder; its wide range of positive (e.g. hallucinations and psychosis), negative (e.g. blunted affect and social withdrawal) and cognitive symptoms, plus the variance of their presentation and diagnosis across the patient population, almost inevitably originates from a complex array of causal factors. Nevertheless, commonalities are increasingly becoming apparent among this noisy array of genetic and environmental parameters.

In particular, recent clinical and animal model work supports the growing consensus that dysfunctional GABA (γ-aminobutyric acid)-ergic interneurons are central contributors to a number of the symptoms of schizophrenia, and therefore present tangible targets for future therapeutic intervention. Interneuron dysfunction is probably a victim of abnormal neurodevelopment or insult rather than a truly elemental cause of disease, and the cascade of interneuronal changes and their contributions to full-blown schizophrenia are not understood. Also, interneuron populations are widespread and diverse; thus interneuronal dysfunction is likely to manifest as a disparate set of interdependent symptoms. However, behaviour-dependent neuronal network oscillations during cognition and state-dependent oscillations during sleep offer windows through which to observe the complex interplay between GABAergic interneurons and their pyramidal cell partners, and to diagnose mechanisms of dysfunction in disease models and patients.

The present review briefly summarizes current evidence implicating interneuronal dysfunction in schizophrenia. This dysfunction is in turn reflected by abnormal oscillatory activity and may underpin interdependent abnormalities in both sleep and cognitive processing. A number of these issues have been reviewed more comprehensively elsewhere [1–4].

θ and γ rhythms in the hippocampus and cortex
Processes such as learning, memory, attention and decision-making are emergent properties of co-ordinated, extended networks spanning cortical and subcortical structures. How can co-ordination both within and between brain...
regions be measured, and can the roles of individual elements be deciphered? Co-ordination of neuronal populations across different timescales is reflected by oscillatory EEG (electroencephalogram) and LFP (local field potential) signals at a range of frequencies, spanning cycle lengths of seconds [e.g. 0.1 Hz cortical slow waves during non-REM (rapid eye movement) sleep], hundreds of milliseconds (e.g. 5–10 Hz θ rhythms during spatial exploration) and tens of milliseconds (e.g. 40–80 Hz γ rhythms during visual attention and 150–250 Hz ripples during quiet wakefulness and slow-wave sleep). These oscillatory signals can only arise by virtue of co-ordinated rhythmic activity. Conversely, if the timing of rhythmic activity across neurons and networks were mutually independent, population signals would average out to a flat line. Thus EEG and LFP recordings during cognition in humans and animals provide critical clues as to the nature of neuronal co-ordination underpinning brain function. In particular, these signals are dominated by synaptic events (i.e. summed excitation and/or inhibition postsynaptic potentials) rather than fast action potential firing, and therefore represent sensitive measures from which to infer synaptic function and/or dysfunction. Combining mechanistic, LTP (long-term potentiation)-based studies of synaptic plasticity with more holistic, physiological measures of intrinsic network function, gauged by LFP and/or EEG recordings during cognition, therefore embodies a powerful approach.

A representative, allocortical model of embedded oscillatory units and networks resides in the mammalian hippocampus, where classical electrophysiological recordings combined with recent molecular genetic tools have given rise to a schematic map of the connectivity and contributions of principal pyramidal cells and their surrounding interneuronal networks. Given the well-defined roles of the hippocampus in learning and memory, this model also increasingly allows for links between oscillations and function. For example, 5–10 Hz θ rhythms co-ordinate networks of pyramidal neurons encoding information about an animal’s position (‘place cells’) during navigation, and fast 100–200 Hz ripples synchronize the firing of recently co-activated place cells during sleep, potentially leading to plasticity at synapses between them. Thus CA1 of the hippocampus exemplifies a region whose physiology relies on the complex interplay between pyramidal cells and at least 21 (at last count) GABAergic interneuronal types, each with distinct patterns of protein expression, neurotransmitter release, connectivity and rhythmic activity relative to neighbouring cells [5].

Intracellular recordings from individual hippocampal pyramidal neurons reveal membrane potential oscillations at frequencies that reflect the predominant θ and γ frequencies recorded in LFP. Synaptic inputs from surrounding interneurons shape these intrinsic biophysical properties to generate the θ rhythm, a 4–12 Hz oscillation in the hippocampal LFP generated by the synchronized activities of inhibitory and excitatory populations [6]. The θ rhythm and underlying hippocampal firing rates are coupled by a characteristic temporal relationship between pyramidal cell and interneuronal spike-timing and the phase of the concurrent θ cycle: spikes are ‘phase-locked’ to θ. Thus the spikes of a given neuron are not distributed randomly across the θ cycle, but rather consistently tend to occur during a restricted phase window of the oscillation, a window imposed by oscillating levels of inhibition. Hippocampal pyramidal cell phase-locking therefore constitutes an important demonstration of the power of interneuronal networks in shaping principal cell activity.

The amplitude of γ rhythms is also phase-locked to the hippocampal θ rhythm: the power of γ is modulated, cycle-by-cycle, by the phase of local θ oscillations. This is a primary example of cross-frequency coupling, with higher-frequency rhythms embedded in, and amplitude modulated by, concurrent lower-frequency cycles. Like θ rhythms, γ frequency oscillations are not restricted to the hippocampus, and are also associated with a range of cognitive processes, and with dynamically modulated phase-locking of spike times during distinct phases of cognitive behavioural tasks [7]. It has been proposed that nested θ and γ oscillations organize (‘bind’) associated features of episodic memories encoded in the hippocampus, and that these processes may be impaired in schizophrenia, contributing to cognitive symptoms of the disease [8]. More generally, description of cross-frequency coupling has extended beyond the hippocampus and been linked to co-ordination of cross-structural interactions [9]. In the case of θ–γ coupling, examples include within human neocortex during auditory and linguistic tasks [10] and between rat striatum and hippocampus during decision-making [11].

**Parvalbumin-expressing interneurons and oscillations**

The activity of GABAergic interneurons, particularly those expressing the calcium-binding protein parvalbumin [PV+ interneurons (parvalbumin-positive interneurons)], is central to γ rhythm generation, and recent studies have shown that selective optogenetic activation of PV+ interneurons in the neocortex is sufficient to trigger emergent γ-frequency activity [12]. This exemplifies why GABA-releasing interneurons should not habitually be described as ‘inhibitory’: depending on their network context, they may act in feedback loops with pyramidal cells to have a net excitatory effect on local neuronal populations [13]. Importantly, PV+ interneurons are also mediators of θ–γ coupling: knockout of functional GABA A receptors selectively from PV+ interneurons effectively decoupled γ amplitude from θ phase in CA1 of mouse hippocampus [14]. These functions are consistent with PV+ interneuronal connectivity: many PV+ terminals release GABA on to the axon initial segment of pyramidal cells, and are therefore well placed to exert control over timing of pyramidal cell firing. Thus the study of γ rhythmicity in general, and θ–γ cross-frequency coupling in particular, constitutes a useful metric through which to infer PV+ interneuronal function and/or dysfunction from LFP and EEG recordings.
Although the mechanisms of co-ordinated oscillations and cross-frequency coupling beyond the hippocampus remain to be demonstrated, it seems likely that PV+ interneurons play central roles. For example, a number of studies have now shown that hippocampal and prefrontal cortical $\theta$ rhythms are dynamically modulated in line with cognitive behavioural demands in rodents [15,16], and frontal $\theta$ rhythms are consistently associated with cognitive processing in primates [17] and humans [18,19]. The hippocampal formation sends monosynaptic, glutamatergic projections directly to deep layers of the medial frontal cortex, which synapse on to both cortical pyramidal cells and interneurons [20]. Thus these projections are well placed to mediate and/or modulate the extent and co-ordination of oscillatory activity across limbic–cortical networks. Indeed, recent work has begun to dissect the nature and timing of activity in different classes of prefrontal cortical interneurons with respect to hippocampal oscillations, showing that firing of PV+ cortical interneurons is phase-locked to the CA1 $\theta$ rhythm in rats [21]. Dysfunction of these interneurons will therefore impair co-ordinated interactions, not only among populations within brain structures such as the hippocampus, but also between functionally connected regions such as the hippocampus and frontal cortex.

Oscillatory activity in schizophrenia
Most psychiatric and neurological disorders are associated with abnormal oscillatory activity recorded using clinical EEG or MEG (magnetoencephalography) techniques. Schizophrenia is no exception (reviewed in [2]), although there is some disagreement as to the most consistent and relevant abnormalities (see [22]). Of course, comparison across studies is complicated by variations in patients, medication, recording sites, analytical methods, behavioural state and – perhaps most critically – the inherently dynamic nature of the signals themselves. Task-dependent changes in oscillatory power at different frequencies can generally only be quantified during carefully controlled behavioural testing that allows trial-averaging of signals, but trial length may limit the possibilities for examining different frequencies (e.g. tests of working memory with short delays preclude quantification of task-dependent slow oscillations), and analyses that rely on windowing of signals may smooth out highly dynamic oscillations at higher frequencies.

Working memory exemplifies a cognitive process during which activity in multiple brain regions must be co-ordinated, relies on prefrontal cortical function, is associated with memory-related oscillatory activity at $\theta$ and $\gamma$ frequencies [19,23] and is consistently impaired in schizophrenia [24]. Working memory tests have therefore been the focus of a number of EEG studies in patients with schizophrenia, revealing deficits in memory-related oscillations at $\gamma$ [25] and other frequencies [26] during distinct task phases. $\gamma$ frequency deficits are largely corroborated by studies of stimulus-evoked $\gamma$, suggesting that $\gamma$ abnormalities might be apparent in a range of cortical areas and behavioural contexts in schizophrenia.

Some studies of cognitive processing in schizophrenia have revealed impaired covariance of oscillations across pairs of brain regions, presumably reflecting dysfunctional connectivity [27]. However, the extent to which impaired EEG coherence reflects (rather than mediates) impaired behaviour, and the extent to which different EEG features relate to positive, negative and cognitive disease symptoms remains to be established.

Importantly, there is evidence that pharmacological modulation of GABA receptor function may alleviate some $\gamma$ rhythm deficits [28]. This is consistent with rodent models of schizophrenia, some of which are characterized by damage to PV+ interneurons [29,30] that may underlie deficits in sensory-evoked $\gamma$ [31]. Do dysfunctional PV+ interneurons underlie the abnormal oscillatory activity seen in patients?

PV+ interneurons: a broad range of symptoms from a broad range of roles?
Converging evidence presents PV+ interneurons as central victims of schizophrenia (reviewed in [1]). Parvalbumin itself is a slow-acting, cytosolic calcium buffer, and decreased expression levels in tissue from patients may reflect abnormal calcium signalling in PV+ interneurons. More striking is the loss of GABA-synthesizing enzymes from PV+ interneurons in post-mortem cortical and hippocampal tissue, although importantly different brain regions have not been surveyed comprehensively in post-mortem studies. Most of the non-parvalbumin-expressing interneurons appear intact [32]; hence, factors such as relatively late postnatal maturation, high NMDA ($N$-methyl-$D$-aspartic acid) receptor expression or high firing rates may render PV+ interneurons selectively vulnerable.

Given that PV+ cells are found throughout cortical and some subcortical areas, inhibitory deficits across large swathes of limbic and cortical networks would sit comfortably with the diverse nature of schizophrenia’s symptoms. Since PV+ interneurons are key contributors to $\gamma$ rhythms in general, and to cross-frequency co-ordination of $\theta$ and $\gamma$ rhythms in particular, PV+ interneuronal dysfunction in schizophrenia presumably contributes to abnormal cortical $\gamma$ rhythms, and potentially to impaired co-ordination of oscillations across cortical regions at lower frequencies [3]; studies of cross-frequency coupling in schizophrenia have not yet been reported. However, a cursory glance at parvalbumin expression patterns in the rodent brain (e.g. [14]) in fact reveals their prominence elsewhere, most overtly in the cerebellum and the reticular nucleus of the thalamus. Is there evidence indicative of PV+ interneuronal dysfunction in these brain regions in schizophrenia and related disorders?

Anatomical studies have consistently revealed cerebellar structural abnormalities in schizophrenia (e.g. [33]). As well as providing circumstantial evidence for cerebellar roles extending beyond motor control, these abnormalities may also reflect early steps in the development of the disease [34]. The prevalence of PV+ interneurons in the cerebellum may relate to structural changes in schizophrenia, and there
is some evidence for altered GABAergic function in this region in both patients [35] and animal models [36]. Whether altered cerebellar interneuronal function contributes to cognitive symptoms by interfering with cerebellar–cerebral communication remains to be established, although once again failures in co-ordinated oscillatory activity may prove a useful metric to test this (see [37,38]).

Of all brain regions, the thalamus could be taken as best placed to act as a central co-ordinator. Thus if thalamic nuclei are rendered vulnerable by virtue of their high PV+ interneuron content, might thalamic abnormalities in schizophrenia contribute to the disease's broad range of symptoms [39]? This is certainly the case in the context of thalamo-cortical loops and oscillatory activity therein. Pathological activity in these loops has been linked with psychosis [40] and impaired sensorimotor gating [41], but systems-level study of thalamo-cortical interactions has commonly been related to sleep, a brain state increasingly recognized as holding significant sway over cognitive processes in health and disease.

**Overlapping networks during sleep and cognition**

The study of sleep in the context of complex psychiatric disorders has at least two powerful motivations: (i) the sleep state and its associated stages and neurophysiological signatures offer useful opportunities to diagnose neuronal network dysfunction; and (ii) whether or not sleep abnormalities reflect elemental disease causes, it is clear that they contribute to cognitive symptoms.

The different stages of the mammalian sleep cycle are associated with distinct oscillatory modes in different brain regions. Broadly speaking, REM sleep bears more in common with wakefulness than non-REM/slow-wave sleep, particularly in the hippocampus, where the 5–10 Hz theta rhythm predominates during both conditions. In contrast, non-REM sleep is marked in the hippocampus by large irregular activity and sharp wave–ripple events, short ~100 ms bursts of 150–250 Hz high-frequency activity generated in CA3 and propagated to CA1. In the neocortex, non-REM sleep is dominated by cortical slow waves, spindles and K-complexes. These latter events are driven by thalamo-cortical interactions and thus constitute a useful metric of thalamo-cortical connectivity and function [42].

Since its discovery in rats, subsequently replicated in humans, much has been made of the phenomenon that the timing of hippocampal ripples is correlated with the timing of neocortical spindles, prompting the hypothesis that ripple–spindle correlation during non-REM sleep enables consolidation of memories encoded in the hippocampus across cortical networks [43,44]. Arguments for a role of ripple–spindle co-ordination in memory consolidation are also bolstered by the discovery that the structured ‘replay’ of neuronal activity during sleep often coincides with ripples, and a recent demonstration that normal hippocampal ripple activity is required for spatial memory consolidation in rats [45]. An intriguing facet of ripple–spindle co-ordination lies in the respective triggers of the two events: ripples are essentially hippocampal, and spindles are thalamo-cortical. By implication, thalamic activity must somehow be controlling the timing of ripple onset, aligning it (within tens of milliseconds) with spindle onset. Is this finely tuned system of thalamic–limbic–cortical interactions during sleep affected in schizophrenia? If so, what might be the functional consequences for cognition?

Sleep disruption has been recognized as a feature of schizophrenia since the original description of the disease, although, as for aberrant EEG oscillations, details vary from study to study, reflecting the diverse nature of symptoms and the varied effects of medications. A bad night’s sleep impacts a range of mnemonic, attentional and emotional processes [46]. Nevertheless, growing consensus recognizes the importance of impaired slow-wave sleep in schizophrenia, its potential links to altered GABAergic function [47] and its potential impact on cognition. In particular, impaired slow-wave sleep is likely to have an impact on memory consolidation processes [4], potentially via its effects on limbic–cortical (ripple–spindle) interactions. Since there is also some evidence for impaired cortical spindling in patients [48], it is tempting to speculate that dysfunctional PV+ interneurons result in altered thalamo-cortical activity that manifests as impaired slow-wave sleep and attenuated declarative memory consolidation. Related slow-wave sleep impairments may pertain to impaired cerebellar interactions disrupting procedural learning in schizophrenia [49]. Note, however, that some intended therapies may have a negative impact on these systems: recent evidence suggests that some antipsychotics may reduce sleep spindling [50].

Although deciphering the mechanistic detail linking interneurons, oscillations, thalamo-cortical/limbic–cortical interactions and cognitive processes relies on further work at the cellular and network level in animal models, it is clear that sleep has become a therapeutic target in schizophrenia. Particularly interesting in this regard is the possibility that cortical oscillatory activity during sleep can be targeted directly by transcranial stimulation [51,52]. However, while treating sleep disruption is very likely to benefit patients, the full extent to which it may alleviate cognitive, or other, schizophrenia symptoms remains unknown.

**Conclusions**

Post-mortem evidence strongly suggests that GABAergic signalling through PV+ interneurons goes awry in schizophrenia. Unfortunately, we are not yet in a position to detail the sequence of events that lead to PV+ interneuronal dysfunction or to understand when and how this particular post-mortem feature arises relative to other established pathologies of pyramidal neurons, white matter or glutamatergic and dopaminergic neurotransmitter systems. It may be that PV+ interneuron function undergoes some plastic, compensatory change in a bid to counter upstream causes of the disease. However, given the roles of this
class of interneurons in shaping the timing of oscillatory activity throughout the brain, their dysfunction intuitively manifests as aberrant oscillations at a range of frequencies, in a range of structures and under a range of behavioural conditions. Study of co-ordinated neuronal network activity in both patients and animal models is therefore central to our understanding of complex diseases that impact higher-order information processing in the brain. Therapies that aim to normalize aberrant oscillations in both wakefulness and sleep, through pharmacology or direct electrophysiological stimulation, therefore, hold promise, particularly for the treatment of cognitive symptoms in schizophrenia and related disorders.

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References


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