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Circadian/Multidien Molecular Oscillations and Rhythmicity of Epilepsy (MORE)

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Key-words: chronobiology, seizure cycles, interictal spikes, transcription, theory

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Summary

The occurrence of seizures at specific times of the day has been consistently observed for centuries in individuals with epilepsy. Electrophysiological recordings provide evidence that seizures have a higher probability of occurring at a given time during the night and day cycle in individuals with epilepsy – here referred to as the seizure rush hour. Which mechanisms underly such circadian rhythmicity of seizures? Why don't they occur every day at the same time? Which mechanisms may underly their occurrence outside the rush hour? **In this commentary, I shall present a hypothesis: MORE - Molecular Oscillations and Rhythmicity of Epilepsy, a conceptual framework to study and understand the mechanisms underlying the circadian rhythmicity of seizures and their probabilistic nature. The core of the hypothesis is the existence of circa 24h oscillations of gene and protein expression throughout the body in different cells and organs. The orchestrated molecular oscillations control the rhythmicity of numerous body events, such as feeding and sleep. The concept developed here is that molecular oscillations may favor seizure genesis at preferred times, generating the condition for a seizure rush hour. However, the condition is not sufficient, as other factors are necessary for a seizure to occur. Studying these molecular oscillations may help us understand seizure genesis mechanisms and find new therapeutic targets and predictive biomarkers. The MORE hypothesis can be generalized to comorbidities and the slower multidienn (week/month period) rhythmicity of seizures, a phenomenon addressed in another paper in this issue of Epilepsia.**

Key-words: chronobiology, seizure cycles, interictal spikes, transcription, theory

Key-points:

- Patients and experimental models display circadian rhythmicity of seizures and interictal spikes
- Body function is organized by weakly coupled molecular oscillators, which phase can be set by light
- In the brain, a large quantity of genes and proteins oscillate in a circadian manner in a brain region-dependent manner.
- Such oscillating molecular architecture may bring networks close to seizure threshold at specific times
- The MORE (Molecular Oscillations and Rhythmicity of Epilepsy) hypothesis provides a way to study the circadian rhythmicity of seizures

1. Introduction

The first detailed published description of the time of the day dependency of seizures was published nearly a century ago ¹. In this observational study, the authors reported when seizures occurred in 66 patients living in a colony (2524 seizures reported over 6 months). Figure 1 reproduces their Figure 1A. They identified three peaks of occurrence during the day (**including** one after awakening – D1) and two during the night (**including** one just after going to bed – N1 and one before awakening – N2). At the individual patient level, seizures had a higher probability of occurring at a given time; we can call a seizure rush hour. The authors also noted that some individuals could have several rush hours and seizures outside of the rush hours. Finally, the rush hour appeared to be patient-specific. The observation of non-random distribution of seizures has been repeated in numerous studies, detailed in the next section. From these observations, we can extract four key questions.

1. Which mechanisms underly the existence of a rush hour in an individual with epilepsy?
2. Why is the rush hour not always associated with a seizure?
3. Why are there seizures outside the rush hour?
4. Why do different patients have different rush hours?

These questions have been present for a long time, but the mechanisms remain poorly understood. Understanding these mechanisms may benefit clinical practice (for chronotherapy and seizure forecasting) and research (to identify new therapeutic targets). Recent advances in circadian rhythm research provide the entry points to the hypothesis I wish to introduce **here**: MORE – Molecular Oscillations and Rhythmicity of Epilepsy. The MORE hypothesis provides a framework to address these four questions, focusing primarily on how a seizure rush hour can be explained and studied.

First, to introduce the problem, the phenotype is presented: the clinical and experimental evidence of a circadian regulation of seizures. Then, will come the property that is at the core of circadian rhythms: the existence of molecular oscillations in virtually all cells and body organs. In other words, the levels of expression of hundreds of genes and proteins oscillate during the night and day cycle, changing the way organs function, enabling them to perform different actions at different times. Finally, all known pieces of information will be assembled into the MORE hypothesis, proposing that these molecular oscillations can produce periods during which seizures are more likely. Several important issues will also be discussed, including why seizures are a probabilistic event, why they can occur outside the rush hour and why they are patient-specific. Whenever possible, interesting future lines of research are highlighted.

2. Clinical and experimental evidence

Two cautionary notes:

1. The word circadian is used in its primary meaning: from latin *circa* ("about") and "*dies*" ("day"), i.e. a circadian rhythm is an oscillation with a period of around 24h. It is not necessarily linked to the night and day cycle, as it can persist in most organisms in the absence of changes in lighting conditions.
2. Most of the following observations stem from intracranial recordings obtained in patients with drug-resistant epilepsy under heavy medication. Therefore, the resulting interpretations and proposals may only be valid for such cases. For example, anti-seizure drugs may affect the circadian rhythm itself and/or sleep-wake patterns. We do not know whether the following considerations apply to absence epilepsy, which is rarely explored with long-duration recordings, but see ².

The advent of electrophysiological recordings allowed a more precise measurement of seizures (versus behavioral only studies). A circadian regulation of seizure was noted in patients with mesial temporal lobe epilepsy³. But, as in the Langdon study, it is a probabilistic event as seizures could occur at any time, the probability just being higher in the afternoon³. Numerous studies have been published trying to correlate the type of seizure (frontal versus temporal), age (children versus adults) with time preference (diurnal versus nocturnal)⁴⁻¹⁰. More recently, very long duration (months to decades) electrophysiological information was obtained in patients, providing strong statistical evidence of a circadian component to seizures (>80% of patients), which can be used to improve seizure forecasting¹¹⁻¹⁵. Although time preferences are displayed, a vast number of electrophysiological seizures in a given period can occur at any time during the night and day cycle outside of the seizure rush hour (Figure 1B graciously made by Philippa Karoly from the data published in¹⁴). Figure 1B also clearly demonstrates that the seizure rush hour is specific to each patient. For example, patient 11 has **an increased seizure probability during** the afternoon, while seizures mostly occur at the beginning of the night and at the beginning of the day in patient 10. In the latter, a fair number of seizures also occur during the afternoon. In patient 14, the number of seizures appears more evenly distributed during a 24h period. However, patient 14 had very few seizures, which raises an interesting question. Is the lack of evidenced circadian rhythmicity in patient 14 due to low statistical power (not enough seizures), or is it because the seizure rush hour only applies to a subset of patients (perhaps those with a high seizure frequency)? The visual inspection of Figure 1B also reveals the dynamic nature of epilepsy during long-term recordings. If patient 11 displays the same pattern of circadian rhythmicity throughout the 655 recorded days,

patient 10 shows a daytime preference during the first third of the recording period, before switching to a pattern that includes the night as a time of occurrence of seizures.

Long-term recordings in patients tell us something important: the circadian rhythmicity of seizures is not universal across and within patients. Of course, life experiences (e.g. highly stressful situations) may have a major impact on brain function, which may supersede the mechanisms responsible for the circadian rhythmicity of seizures. It is difficult to control for such variables in patients. In contrast, external conditions are better controlled in animal facilities. Experimental models of epilepsy also demonstrate circadian rhythmicity patterns of seizures in the kindling¹⁶, tetanus toxin^{17; 18}, kainic acid^{19; 20}, but see²¹, pilocarpine²²⁻²⁴, but see²⁵ and also WAG/Rij absence²⁶ rat models. Similar findings are reported in mice in the pilocarpine²⁷ and various genetic (ScnA1, Kcna1, Succinic Semialdehyde Dehydrogenase)²⁸⁻³⁰ models. As in humans, seizures in experimental models do not occur for each circadian cycle, and many seizures occur outside the seizure rush hour. Other authors argued that seizures emerge from theta oscillation during or at the end of REM sleep but not from the circadian cycle^{18; 31}. Discrepancies may stem from the fact that few studies check the circadian nature when light cues are not provided anymore. In constant darkness or dim light conditions, seizures and sharp wave discharges maintain circadian (close to 24h) rhythmicity^{16; 26}. Alternatively, since the circadian rhythmicity of seizures is not found in all patients, some experimental models may not display a seizure rush hour, which would be very interesting to study. Different experimental conditions can significantly affect phenotypic traits in experimental models^{32; 33}, which may disrupt circadian rhythmicity (or drive it).

In conclusion, despite a priori better-controlled conditions, seizure occurrence is also a probabilistic event in experimental animals. There is a period of increased seizure

probability in individual animals, but many seizures can also occur anytime during the night and day cycle.

The take home message #1 from both clinical and experimental data is that there exists circadian rhythmicity of seizures in most, **but not all**, cases. However, if the circadian factor is a strong contributing factor, it is not the only one. Said differently, if we can identify the mechanisms underlying the seizure rush hour, they will not be enough to explain why seizures occur.

Although there is no clear-cut picture emerging from seizure data, important information can be gained when looking at another electrophysiological signature of epilepsy: interictal spikes. Contrary to seizures, interictal spikes are highly reliable events, occurring at a frequency several orders of magnitude greater than seizures every day. In patients and animal models, interictal spikes display a strong circadian rhythmicity^{11; 14; 24; 34}, which was maintained in five patients kept in constant dim light conditions³⁵. Going back to clinical data (Figure 1C), we can make another important observation. **The distribution of interictal spikes during the night and day cycle** is patient specific. Most of the spikes occur during the night in patient 11 (although seizures occur during the daytime) and during the day in patient 14. Interestingly, there are two peaks (one during the night, one during the night) in patient 10. However, one peak may correspond to the first third of the 322 day-long recording, and the second peak to the rest as the seizure timing pattern changes.

Since there appears to be a very reliable **circadian rhythmicity** of interictal activity every day, we can hypothesize the existence of molecular mechanisms that control interictal spikes' daily emergence. For example, there could be a daily change in the excitability of

neuronal networks among many possibilities, which would account for a parallel change in the frequency of interictal spikes. However, it is important to keep in mind that even if we manage to unravel the mechanisms underlying the rhythmicity of interictal spikes, they may not apply to the seizure rush hour since the two events do not necessarily align in time in patients (e.g. the antiphase pattern in patient 11).

The take home message #2 is that there is circadian rhythmicity of interictal spikes, which does not necessarily align with seizures' circadian rhythmicity. It will be particularly interesting to investigate whether some mechanisms are common to interictal and ictal rhythmicities.

3. A body of coupled oscillators

The circadian regulation of seizures and interictal activity is hardly surprising. After all, many (most?) body functions/states oscillate over time with a quasi 24h period, including body temperature, blood pressure, sleep, and alertness (Figure 2). We now know much about how these oscillations are generated and controlled. Before discussing the MORE hypothesis's bases, it is important to present how these body oscillations are generated and controlled. The core idea is the ubiquitous presence of circadian molecular oscillators.

3.1. Molecular oscillators

Let us consider a very simple unicellular organism: the Cyanobacterium *Synechococcus elongatus*. This cyanobacterium expresses (among others) three proteins – KaiA, KaiB, and KaiC – with interesting properties. KaiC has both autophosphorylation (the ability to add a

phosphate group on one of its amino acids) and autodephosphorylation (removal of a phosphate group) activities, KaiA can enhance KaiC autophosphorylation, and KaiB decreases the effect of KaiA. If you place these three proteins in a test tube (respecting their intracellular ratio) with energy (1 mM ATP), KaiC phosphorylation oscillates with a period of about 24 hours (Figure 3).³⁶ Thus, in the absence of any external cue (e.g. light/dark cycle), give these three proteins some energy, and they start to oscillate at a period close to the earth rotation on its axis. This property is not limited to cyanobacteria. When human red blood cells, which have no nucleus or DNA, are isolated, peroxiredoxins (PRX, antioxidant proteins) display a quasi 24h redox oscillation³⁷. The emergence of such biological oscillations is grounded in physics, particularly Ilya Prigogine's work on systems operating far from thermodynamic equilibrium, such as living systems. On this topic, I recommend reading this excellent paper³⁸. Suffice to say that the key property that allows the emergence of oscillations is the existence of feedback processes (i.e. KaiA has a positive action on KaiC and KaiB a negative action on KaiA), which can provide sufficient conditions for the emergence of molecular oscillations.

The take home message #3 is that life on Earth uses a substrate of built in molecular oscillators, which continuously (if energy and nutrients are provided) change the internal state of the system with a circa 24h period.

The Cyanobacterium provides a simple example of molecular oscillators. If we now have a peek at a map of human metabolism (still at the single cell level)

[https://en.wikipedia.org/wiki/Metabolism#/media/File:Human_Metabolism -](https://en.wikipedia.org/wiki/Metabolism#/media/File:Human_Metabolism_-_Pathways.jpg)

[Pathways.jpg](https://en.wikipedia.org/wiki/Metabolism#/media/File:Human_Metabolism_-_Pathways.jpg), we shall notice the presence of numerous feedback/regulatory processes for

most chemical reactions (feedback loops). This creates a complex system, i.e. a system whose behaviour is difficult to understand because of the (often nonlinear) interactions between its numerous parts. We can speculate that the presence of so many feedback loops may allow the system to oscillate by itself if we could isolate it in a test tube with nutrients, just like KaiC. In reality, cells and organs do not use a protein-only based mechanism to generate molecular oscillations, if only because proteins have a limited half-life spanning several orders of magnitude, from minutes to days ³⁹. In most cells, molecular oscillations stem from the oscillation of their mRNA transcripts. This general principle is so universal and simple that one can engineer it. Using three transcriptional repressor systems that are not part of any natural biological clock, one can build an oscillating molecular network in *Escherichia coli* ⁴⁰, whose period can be programmed and controlled ⁴¹. Therefore, it is no wonder that seizures and interictal spikes display some circadian rhythmicity. This property is grounded in a core aspect of living organisms on Earth: their genes and molecules oscillate daily.

Since seizures are generated by the brain it is necessary to spend some time on the Suprachiasmatic Nucleus (SCN) of the hypothalamus, which is considered as the master clock of the body and which controls sleep, alertness, temperature, hormone levels and other functions in a circadian manner ^{42; 43}. This system is more complex, but conceptually, it is as simple as the examples above, composed of feedback loops.

3.2. The Suprachiasmatic Nucleus and circadian rhythms

The core mechanism is mentioned here *en passant* for the sake of the demonstration. Excellent reviews have been published on the topic if the reader is interested in the detailed mechanisms and the explanation of the technical terms. The SCN has two molecular actors,

CLOCK and BMAL1, which cause the transcription of genes, which ultimately feedback and inhibit CLOCK and BMAL1 transcriptional activity⁴⁴. In the SCN, the transcription factors CLOCK and BMAL1 activate the expression of *Period* (*Per1–3*) and *Cryptochrome* (*Cry1/2*) genes during the day (Figure 4). PER and CRY proteins start to accumulate in the cytoplasm and move to the nucleus during the night to inhibit the transcription mediated by CLOCK/BMAL1. An epigenetic mechanism then modifies histone tails, ultimately leading to a dissociation of the CLOCK/BMAL1 transcription complex and suppressing the production of *Per* and *Cry* mRNAs. The gradual degradation of PER/CRY complexes releases the CLOCK/BMAL1 dimer from PER/CRY suppression, thus re-initiating the clock cycle by induction of *Per* and *Cry* transcription. What is essential for us is the result: the levels of mRNAs and proteins within the SCN oscillate with a quasi 24h period. Subsequently, numerous other functional outcomes controlled by the SCN oscillate daily, such as body temperature and neuronal activity (Figures 2 and 5). Within SCN cells (but also other cells), the clock machinery is closely linked with metabolism, as some molecular actors of the clock have both an epigenetic action and a metabolic action (in some of the reactions found in the metabolic map)⁴⁵. We are thus in the presence of a very complex system of coupled chemical equations.

Like in cyanobacteria, SCN circadian molecular oscillations are an intrinsic property, i.e. molecular oscillations persist when the SCN is isolated, even at the single neuron level, each with its own period⁴⁶. In humans, the intrinsic oscillator has a period >24h⁴⁷, and its period is reset daily by light⁴⁸. The effect of light involves a chain of events along the retino-thalamic pathway, ultimately leading to an increase in *per1* mRNA⁴⁹. The external cue (light) thus resets the intrinsic oscillator phase by directly acting within the translation/transcription loop via its action on *per1* (Figure 4). In this case, light is the

Zeitgeber (the time giver or synchronizer). Two pathologies are interesting to consider in this context. Mutations on the *per2*⁵⁰ and *cry1*⁵¹ clock genes produce advanced and delayed sleep phase syndromes, respectively, while sleep duration remains normal. These mutations slightly alter the circadian clock's intrinsic period, changing the way it is entrained by light.

It is important to stress that our internal oscillators are built-in with their own circa 24h (hence circa dies) period. This means that in the absence of Zeitgeber (or daily synchronization), the oscillations follow their internal “free-running” or built-in period, which can differ from one person to the other. For example, individual free-running sleep-wake cycles were reported in a group of scientists living in constant daylight in the high arctic⁵². If an event is regulated in a circadian manner, it should persist in constant light conditions, which is rarely performed in epilepsy research. The circadian regulation of seizures persists in constant darkness in experimental epilepsy¹⁶, suggesting that the phenomenon is generated internally.

The take home message #4 is that there exists a built-in molecular oscillator in the human brain, with a period of circa 24h specific to each individual person, but which can be synchronized by light.

In theory, whichever species we consider, observing just one oscillating system in the body – the SCN – whose cyclic activity is reset each day by light, we could potentially explain the cycles shown in Figure 2. We could even propose hypotheses to explain the circadian regulation of seizures and interictal spikes. Figure 5b provides the intuition. SCN cells

increase their firing during the day, perhaps a sign of hyperexcitability. We could stop here and focus research exclusively on the SCN. Unfortunately (for our pursuit of mechanisms), the reality is more complex. Our body is made of multiple oscillating systems.

3.3. Our body is a collection of different molecular oscillators

Many organs also display the ability to produce molecular oscillations autonomously, i.e. in the absence of external cue. Although the SCN is presented as the master clock in the body, synchronizing other clocks in the brain and peripheral organs, the SCN is not necessary. Perhaps every cell in the body displays circadian rhythmicity, and molecular clocks can be found in many organs (Figure 6) ⁵³.

The liver molecular clock can be reprogrammed by nutritional challenge ⁵⁴, with feeding cycles entraining the liver independently of the SCN ⁵⁵. Note that food cues are also important to entrain clocks, although they are considered as secondary Zeitgeber, in comparison to light ⁴⁹. When *Bmal1* (which is key for SCN feedback oscillation) is knocked out in the body except in the liver, only 10% of the liver transcripts continue to oscillate to regulate metabolic processes ⁵⁶. This example shows that even if the liver can oscillate without the SCN, the latter exerts a strong influence on its genes and proteins' circadian regulation. These factors add another layer to an already complex system, and it doesn't stop at body organs. Our symbiotic microbiota has endogenous oscillations with a 25h period, which can be entrained by melatonin and, of course, feeding ⁵⁷. Although microbiota oscillations are entrained by the host circadian clocks, the relationship is bi-directional, and perturbing this relationship can be associated with pathologies ⁵⁷. In general, mutations affecting the circadian clocks (regardless of their location in the body) may lead to physiological disorders (Figure 6), such as sleep disorders ⁵⁸ and cancer ⁵⁹.

The take home message #5 is that there are circadian molecular oscillators everywhere in our body. A defect in one oscillator may have local, far away, or widespread consequences.

3.4. A body of coupled oscillators

If most body oscillators possess an intrinsic period, which may differ from one cell/organ to another, how is our body synchronized? An interesting property characterizes oscillators: if you couple them weakly, they will synchronize; uncouple them, and they will oscillate independently. The famous experiment of metronomes provides the best example of this concept (<https://www.youtube.com/watch?v=Aaxw4zbULMs>). In the metronome example, five metronomes are individually set to 176 beats per minute and placed on a foam board. They are out of rhythm with each other. When empty cans are placed underneath the board, the board can move from side to side (allowing for physical coupling), and the metronomes influence one another's synchronization and become in sync. All organs in our body exchange information in the form of electrical or molecular signals (most of which remain unknown), effectively providing a multi-directional weak coupling⁶⁰. The metronomes provide a simplistic view, as the molecular oscillations in our body are not in phase across regions and organs.

Let us pause briefly to focus on two concepts that are very important for our attempt to address the rhythmicity of seizures. We can speculate that if we were to constantly feed a body with fuel (nutrients, O₂), in the absence of Zeitgeber, free-running molecular oscillations and a dynamic regime would appear in the body (the metronomes remain synchronized). This is the first concept: the internal organization relies on weakly coupled built-in oscillators. The second concept is that many external (e.g. light, food, and stress) or

internal (e.g. the release of hormones) events can provide a signal strong enough to alter/reset local or global molecular oscillations. Since our environment is highly dynamic, some of our internal molecular oscillators can react to such changes, with transient or sustained changes. Imagine that I block with one finger one metronome during a short time and then release it. It will start to oscillate again but not in phase with the others until the weak coupling between them drives it back to synchrony. But if we change the coupling mechanism, making it different between each metronome pair (imagine different boards connecting different metronomes), we will start to see major changes in the system dynamics. Since our external environment and internal milieu are constantly changing, there exist multiple possibilities to alter our oscillators; hence the latter are unlikely to show the same pattern every day. Such alterations can occur across multiple time scales to alter circadian rhythms, such as transient stressful experiences^{61; 62} and aging – a strong modulator of the internal state – that affects circadian patterns⁶³. Our way of living and daily experiences may alter molecular oscillations somewhere in the body and change its operating mode. This provides us with an entry point to study why seizures do not occur every day at the same time: the body is not in the same state from one day to the next. Obviously, the organism can cope with most changes in the environment, and molecular oscillations may not deviate much from a global equilibrium or homeostatic state. Departures far from an equilibrium state may result in a pathological state, e.g. favoring seizures. A very strong event may even change the seizure rush hour (this is perhaps what happened to patient 10).

3.5. A multi-directional system to build a hypothesis

Although brain oscillators can influence/drive oscillations in other organs, it is essential to keep in mind that the reverse is also true. If the brain sends signals to the periphery, the periphery also sends strong signals to the brain, directly modulating its activity and cognitive performance⁶⁴. As mentioned above, everything may be formalized with nonlinear coupled differential equations, generating a complex system, which requires a holistic (global) approach to understand it. In such a coupled system of oscillators, the seizure may originate from any oscillator (or a combination of them) in the body, which renders the identification of the mechanisms very complex.

Yet a simple conceptual framework can be proposed. Intuitively, given the presence of oscillating transcripts and proteins throughout the body, we can propose that seizures' circadian rhythmicity is grounded in molecular oscillations. For example, the seizure threshold, defined as the minimum change required to have a seizure, is ultimately controlled by molecular-biophysical processes, which could go up and down daily.

Alternatively, or simultaneously, a set of oscillating proteins may increase the network's excitability, as shown in Fig. 5B for the firing of SCN neurons. Either condition would increase the probability of seizure occurrence. For the sake of the demonstration, let us go back to the cyanobacteria example and imagine that a mutation in KaiC leads to an increase of its autophosphorylation. If the seizure threshold is reached when enough KaiC proteins are phosphorylated, we have a potential mechanism to explain the seizure rush hour as we will get close to the seizure threshold every day at the same time. Then, all we have to do is to design a drug that decreases KaiC phosphorylation and deliver it during the rising phase of the oscillation. In this scheme, we don't need to change the molecular oscillation frequency or its phase, just its amplitude. Of course, this is an invented scheme, but it provides a generic principle.

Since we are made of coupled oscillators throughout the body, it is important to stress again that processes controlling the seizure may not be located in the brain. For example, let us assume that one microbiota species becomes dysfunctional in a patient and starts to release a large quantity of molecule X in the afternoon. It turns out that X can rapidly cross the blood brain barrier to find its main target in the temporal lobe. There, it starts to depolarize excitatory cells driving the network to seizure threshold. In this invented example, a seizure rush hour will be evidenced in the afternoon, and the cause will be molecule X released by a microbiota species.

However, the variability in the human data presented in Figure 1 already alerts us that the answer will not be that simple. Several conditions should be present **for a seizure to occur**. Perhaps one needs the microbiota molecule X + lack of sleep + a late lunch. This exemplifies the difficulty of unraveling the mechanisms. If we were to study sleep in this patient, we would end up finding a correlation between lack of sleep and seizures. But there will be instances when lack of sleep is not associated with seizures because, in this example, lack of sleep is not sufficient, as late lunch is also necessary. We dig further, and we finally find that it is the combination of lack of sleep and late lunch that correlates best with seizures. All we have to do is to tell the patient to work on their sleep and feeding time. Note that in that scheme, we have not identified the true cause: the molecule X produced by the microbiota, but we have found a solution for the patient, which is what matters. There lies the main difficulty in research. It is always difficult to interpret correlations. If we were to launch a research program on sleep and feeding in experimental models of epilepsy because we found a correlation between sleep/feeding and seizures, the cause of seizures (the X factor) will always remain unknown. This is what Figure 1 is also telling us. There exists a correlation between seizure occurrence and time of the day in individual patients, but not every day

and not always at the same time, which means that there must be other actors. This leads us to phrase the key question. What is the role of circadian oscillations in epilepsy? Do they contain the cause (i.e. molecule X from the microbiota in the example) or are they one of the conditions for seizures to occur (i.e. late lunch or lack of sleep). My intuition is that sometimes they are a cause and sometimes they are a co-factor, because so many different mechanisms can produce a seizure in an individual.

The take home message #6 is that circadian molecular oscillations provide a necessary condition for circadian rhythmicity of seizures; whether they are causally related to seizure genesis remains to be determined.

3.6. An ever-changing molecular landscape

Figure 5C shows that the levels of mRNAs of the clock machinery are oscillating during the night and day cycle. However, these oscillations are not limited to clock genes. The CLOCK-BMAL1 complex can control a very large number of genes, which will oscillate accordingly. The quantification of 12 mouse organs' transcriptomes revealed that 43% of all protein coding genes show circadian rhythmicity⁶⁵. In the brain, the SCN is considered the master clock, which can entrain ancillary clocks in numerous other brain areas⁶⁶. As a result, many other genes oscillate, and numerous brain regions (potentially all of them) are characterized by a complex circadian remapping of their molecular architecture, including in humans⁶⁷⁻⁷⁰. The total set of genes (and proteins) that oscillate in a circadian manner is brain-region specific⁶⁹. For example, 10% of the hippocampus' genes, 6% in the forebrain, and 19% in the SCN^{69; 71} oscillate. These percentages are underestimates as these studies mixed different cell types (different classes of neurons and glial cells, endothelial cells, pericytes,

etc.). Future research is needed to obtain cell type- and cell compartment-specific information about the circadian molecular remapping. A majority of genes and proteins may display circadian rhythmicity. In keeping with this proposal, two studies report that at least 70% of the synaptic transcriptome, proteome, and phosphorylome of forebrain synapses oscillate ^{71; 72}. Again, this may be underestimated as all forebrain synapses were mixed (circadian rhythmicity of synaptic mRNA/proteins may be brain region dependent). Possibly all cells, regions and organs are characterized by a circadian oscillation of their molecular landscape, allowing our organism to adapt to the natural alternation of day and night, sleep pressure, food availability etc.. In the brain, such molecular oscillations may change the way brain cells operate and communicate with each other across spatial scales (from synapses to microcircuits, to networks, to the brain, to the entire body). Indeed, circadian rhythmicity exists for synaptic responses ⁷³, long term synaptic plasticity ^{74; 75}, and the memory-associated MAPK pathway ⁷⁶ in the hippocampus; and for functional connectivity related to memory in humans ⁷⁷ as well as learning and memory processes ⁷⁸⁻⁸⁰.

The take home message #7, and perhaps the most important one, is that neuronal networks are in different structural (i.e. molecular) and functional states from one moment to the next (Figure 7A). The circadian component must be considered when studying the functions of all body organs, including the brain.

Experimental epilepsy data directly support the proposal that the circadian cycling of genes and proteins is also important to consider in epilepsy research. Seizure induction in control animals is regulated in a circadian manner ^{69; 81; 82}. These results may be due to the circadian rhythmicity of the molecular targets of the method used to trigger seizures (e.g. the

molecules on which pentylenetetrazole binds). Since the molecular landscape (and perhaps the excitability) of neuronal networks oscillate in a circadian manner, it is not surprising that the threshold for seizure induction changes accordingly. In keeping with this proposal, synaptic excitability changes in a circadian manner in control conditions⁷³, and excitability is enhanced in the afternoon (i.e. during the high seizure phase) in the kindling model⁸³. We now have our entry point to the MORE hypothesis: as the molecular architecture of neuronal networks oscillates so does the seizure probability.

4. The MORE hypothesis

We now have all the puzzle pieces to start to study the mechanisms of the seizure rush hour. We do not know how seizures start (the causal event), but as an axiom, we can propose that a threshold must be reached for a transition between normal and epileptic activity to occur (Figure 7B). Many theoretical and experimental studies support the existence of such a threshold, independently from the assumed mathematical mechanism underlying seizure genesis⁸⁴⁻⁸⁹. Let us consider this image. You live along the Atlantic coast, and your house is just on the beach but sitting on an elevated walled platform that prevents water from submerging your garden at high tide. Most of the time, your garden is not flooded – this is the normal state. When it is flooded, let us call that a seizure. The seizure threshold is when the water reaches the edge of the wall. Every day, there is an oscillatory pattern of water rising and falling, which corresponds to molecular oscillations that periodically change the network (the network gets periodically close to the threshold). But every day, if the water does not rise high enough, the network remains at a "safe" distance from the threshold. However, during the equinox's high tides, the water will rise a lot at high tide, getting close to the top of the wall (increased probability of flooding). But this may

not be sufficient because the wall is high enough. A conjunction of events (an additional external or internal event or cue), such as strong wind from the sea direction, a storm at sea, or an increased gravitational pull from the moon, is needed. This combination of factors will end up with your garden being flooded – a seizure. When observed over the years, you will find a high probability of getting flooded during equinox tides, but not for all of them. Importantly, since several high tides can occur during the equinox, you can get flooded several times, provided that a storm is raging long enough. Seizures would thus occur in clusters, which is observed in 50% of patients ^{11; 15; 90}.

This simple example illustrates the CORE hypothesis. Molecular oscillations change the architecture of neuronal networks in a circadian manner (Figure 7A). As a result, networks in the epileptogenic zone would get close periodically to the seizure threshold (Figure 7B). Of course, rather than the network getting close to the threshold, it could be the threshold itself that oscillates in a circadian manner, or a combination of the two phenomena. But these oscillations are not sufficient; additional factors are needed, as seizures do not occur every day at the same time (Figure 7B). The identification of the additional factors may constitute the most formidable challenge for future clinical and experimental research. They may come from other organs, the microbiome, and the environment. Also, they may change in time. For example, as the body ages, molecular oscillations may change, altering the rush hour's mechanisms. Clinical data shows that the time of high seizure probability can switch by 12h in some patients (e.g. patient 10, Baud Epilepsia 2020).

The take home message #8 is that body-wide Molecular Oscillations provide appropriate conditions for the Rhythmicity of Epilepsy. However, before using the MORE hypothesis to

interpret existing data, propose and test hypotheses, it is important to consider the hypothesis in chronic epilepsy.

5. Circadian molecular remapping in epilepsy

As we discover the rules governing the dynamical molecular architecture of brain regions and their functional consequences in the healthy brain, it is important to stress that these rules may not translate as such to epilepsy. The circadian rhythmicity of genes and proteins can be strongly modified in pathological conditions, as demonstrated in the liver following nutritional challenge⁵⁴ or in the human prefrontal cortex in schizophrenia⁹¹. In experimental epilepsy, most of the genes/proteins oscillating in the ventral hippocampus of control animals are not oscillating anymore in experimental epilepsy; a new set of genes/proteins is now oscillating⁶⁹. Interestingly, some core clock genes gain in oscillatory amplitude, as well as their downstream targets. As a result, the hippocampus's oscillatory molecular landscape in experimental epilepsy is very different from that found in control animals. Perhaps, animals with spontaneous seizures should be studied for themselves and not compared to controls, which may operate with a different functioning mode. This proposal is supported by the fact that the time of the day when animals are more susceptible to seizure genesis with PTZ is different in controls and animals with chronic epilepsy⁶⁹. Alternatively, the hippocampus may still operate in the same way, but using a different set of oscillating genes – a form of homeostasis⁹². A modified oscillatory molecular landscape may also affect the molecular oscillations in other brain regions and organs, changing their coupling. In the latter case, seizures may not be due to a down/up-regulation of a given protein; they may be due to a global reorganization of the molecular landscape dynamics.

The transcriptome and proteome's circadian regulation was analyzed in one brain region and one model of epilepsy⁶⁹. Future work is needed to determine whether molecules' circadian rhythmicity is also modified in other brain regions and organs in the same experimental model. Other experimental models, in different strains and species, in both sexes need to be investigated (in particular genetic ones). They may provide cues to explain clinical data, particularly the fact that seizure rush hours can be very different from one patient to the next. In particular, it would be interesting to study a rodent model with a daytime seizure rush hour with another model with a nighttime seizure rush hour.

The take home message #9 is that circadian molecular oscillations are different in experimental epilepsy than controls. These alterations may be at the core of seizure rhythmicity and/or seizure genesis.

6. Interpreting clinical data within the MORE hypothesis

Figure 1 clearly illustrates that seizures have an increased probability of occurring at certain phases of the circadian cycle, that is to say, seizures do not occur for all cycles. Many seizures also occur outside the period of high seizure probability. As mentioned previously, the cycling of the molecular landscape provides favoring conditions for seizures to occur, but since they do not occur for all cycles, additional factors are necessary. How can we explain the occurrence of seizures outside the rush hours? How can we explain that the rush hour is patient-specific?

Clinical data demonstrate that individual patients express different types of seizures in terms of dynamics⁸⁹ and spatial patterns of propagation⁹³. Interestingly, seizures with similar propagation patterns tend to be clustered in time⁹³. Different types of seizures may

involve different underlying molecular mechanisms. Conversely, experimental data demonstrate that many different mechanisms can produce the same seizure in the same brain area ⁸⁶. **If the different molecular mechanisms have distinct circadian rhythmicity, there will be different periods of increased seizure probability.**

Although equinox high tides increase the probability for your garden to get flooded (a periodic event), other events can flood your garden at any time during the day: a hurricane, a tsunami, or the rupture of a water pipe in your house, independently of the tide, and thus time. **Powerful external events can strongly alter neuronal networks, driving them directly over seizure threshold, such as a highly stressful event.**

The fact that molecular oscillations are brain region-specific ⁶⁹ may explain why the time of high seizure probability can vary in patients with different epileptogenic zones. Different brain regions may display different sets of oscillating genes, hence different periods of enhanced seizure probability. Such a simple scheme may account for the existence of two circadian peaks in some patients, as already noted a century ago ¹, and a shift in the period of increased probability (e.g. patient 10) as the epileptogenic zone changes over time in individuals.

7. Using the MORE hypothesis to explore mechanisms

Although we do know that brain processes (e.g. memory and learning) and neuronal properties (e.g. synaptic plasticity) display circadian rhythmicity, the underlying mechanisms remain unknown. This constitutes uncharted territory, in both health and disease, and offers fantastic future research opportunities. Where to start in epilepsy?

7.1. Interictal activity

Interictal activity may constitute a good entry point, because, in contrast to seizures, it shows strong circadian rhythmicity every day^{11; 14; 24; 34}. Since the genesis of interictal bursts also seems to require threshold crossing⁹⁴, we can propose the hypothesis that circadian molecular oscillations in neuronal networks are accompanied by changes of excitability, which would favor the genesis of more and more interictal spikes as excitability increases. In keeping with this idea, excitability is enhanced in the afternoon in the kindling model⁸³. Perhaps, the first step towards understanding the mechanism underlying the seizure rush hour is to understand the principles regulating the circadian rhythmicity of interictal spikes. Simple hypotheses may be tested. We know that GABAergic function is regulated in a circadian manner in the SCN in control conditions, including the sensitivity to GABA action and the GABAergic drive received by neurons⁹⁵. **Decreased GABAergic neurotransmission at a given time during the day in an epileptogenic network may increase interictal activity (but also increase seizure probability)**. Following the same idea, a microdialysis study reveals a circadian rhythmicity of extracellular glutamate levels in experimental epilepsy⁹⁶. In the latter study, variations of glutamate in control animals were blunted, highlighting that the circadian "rules" are altered in epilepsy. More generally, the levels of many amino acids display circadian rhythmicity in control conditions⁹⁷. **Circadian regulation of metabolites (including those involved in metabolism) may be causally related to the rhythmicity of interictal spikes and/or seizures.**

Adding a level complexity, it has been argued that different types of interictal spikes may reflect pro-epileptic or safe states for the network^{88; 98; 99}. It will be interesting to determine whether different types of spikes display different circadian patterns. Finally, it is important to remember that interictal spike and seizure rhythmicity can be dissociated (Figure 1B).

Understanding what happens at the level of interictal spikes may not improve our understanding of seizure rhythmicity. But it is a good starting point.

7.2. The potential role of clock genes

Given the circadian rhythmicity of seizures, it is tempting to link seizures to the genes and molecules that drive molecular oscillations^{100; 101}. Although there is no apparent cell loss in the SCN in the pilocarpine¹⁰² and kindling¹⁰³ seizure models; light-induced c-Fos immunoreactivity is disrupted in the pilocarpine model¹⁰⁴, perhaps signaling a defect in the daily phase resetting of the SCN clock. In keeping with the latter idea, a different daily profile of melatonin receptors has been reported in the hippocampus in the pilocarpine model as compared to controls¹⁰⁵. Several studies report alterations in the molecular clock in epilepsy. For example, CLOCK, CRY1, PER1, and DBP protein levels are decreased in epileptic tissue obtained from surgical resection¹⁰⁶. In experimental epilepsy, there is altered rhythmicity of clock genes¹⁰⁷ and key players of the molecular clock, including RORa receptor¹⁰⁸, acidic amino acid-rich basic leucine zipper (PAR bZIP) transcription factors (TFs)¹⁰⁹, and sirtuin 1 in the Kcna1 SUDEP model¹¹⁰. Whether these alterations explain the circadian rhythmicity of seizures or seizures themselves is not known. One way to potentially test this hypothesis is to check whether altering the molecular clock leads to epilepsy.

Several studies support the latter proposal. In BMAL1 knock out mice, the seizure threshold in the electroshock model is decreased⁸¹. Disruption of CLOCK in excitatory cells leads to epileptiform discharges¹⁰⁶, and loss of PAR bZIP genes leads to seizures¹¹¹. However, these studies are difficult to interpret as the genetic manipulation will be present in the first dividing cells, perhaps affecting developmental processes. For example, the cell cycle and

the circadian clock are bidirectionally coupled oscillatory networks. In particular, BMAL1 (among others), is involved in both the circadian clock and the control of the cell cycle ¹¹². Disrupting BMAL1, or other molecular clock players may disrupt cell cycle or developmental processes, which will be the real cause of seizures, not removing the gene. To better illustrate this important concept, let us consider the following study. Disrupting KCNQ2 during a critical period of development leads to epilepsy, but seizures do not stop when the functional KCNQ2 protein is reintroduced after the critical period ¹¹³. Altering KCNQ2 may have triggered a misconstruction of the brain during development, which provided the necessary conditions for seizure occurrence later in life. In other words, if altering CLOCK and PAR bZip leads to epilepsy, they may not be causally related to seizures themselves. A conditional model, in which genes can be turned on and off at will, is necessary to explore causality ¹¹³.

It remains that dysregulation of core clock genes may change the way transcripts and proteins oscillate, as shown in the hippocampus ⁶⁹. In this case, if the SCN is still oscillating as in control, there will be a different type of coupling between the SCN and the hippocampus in terms of phase and of the nature of transcripts/proteins, as if the oscillators would become uncoupled ¹⁰⁰. Two oscillators, like the SCN and the hippocampus, can become directly uncoupled, which means that the activity of one never affects the activity of the other. Given the large number of oscillators in the body, an indirect coupling may still be maintained via other brain regions/organs. However, the phase relationships between the different oscillators may be shifted, and one can propose that a “wrong” or “mistimed” signal may reach the hippocampus in the low seizure threshold condition ¹⁰⁰, a condition that would not have occurred in the healthy brain, in keeping with the MORE hypothesis. My intuition is that we see a large modification of the coupling of the different body

oscillators, resulting in altered phase and amplitude relationships. The number of potential targets is enormous (causal factors may be located outside the brain). Except if one has great intuition, it may be interesting to start with main drivers (like clock genes just discussed) and macroscopic markers, like sleep.

7.3. Sleep

Some authors argue that seizure occurrence correlates better with sleep patterns than with circadian rhythms. Many studies have been published on sleep and circadian rhythm, sleep disturbances causing or caused by pathologies, and on sleep and epilepsy^{114; 115}. But sleep is just a state of the organism. As vital as it is, it remains a direct readout of what happens at the molecular level (i.e. it is not a cause but a consequence). We can quantify and characterize it, just like seizures, but it remains grounded in molecular events. For example, sleep deprivation will have direct molecular consequences, acting as a strong external cue, with possible consequences on the circadian machinery, perhaps lowering the seizure threshold. Except if external factors affect sleep, it is difficult to claim that sleep is causally related to seizures, because sleep and seizures are the consequences of molecular events (with possible shared pathways). The causes will always be found at the molecular level (or below it). If seizures emerge from theta oscillations during REM sleep, neither theta oscillations nor REM sleep cause seizures. The molecular mechanisms underlying REM sleep or theta oscillations are the cause. Melatonin and its receptors could be one such mechanism¹⁰⁰. Here also, the field is wide open. However, when using experimental rodent models, it is important to keep in mind that their sleep architecture is very different from humans.

7.4. Key players

Another interesting avenue of research is to focus on key players. Clock genes belong to this category, as they can control many downstream targets. Many other proteins/pathways can be investigated, particularly those that control gene expression and which expression is changed in epilepsy, including NRSF ¹¹⁶, NRF2 ¹¹⁷, CREB ¹¹⁸ and mTOR ¹¹⁹. mTOR constitutes an interesting lead to follow because of its circadian regulation and its involvement in epilepsy ^{100; 119}. The information regarding the circadian expression of hippocampal transcripts in control and experimental epilepsy ⁶⁹ can be obtained in the open database <http://circadiomics.igb.uci.edu/> (mouse ventral hippocampus at the bottom of the list). For example, Creb1, tends to oscillate in antiphase in the ventral hippocampus between control and epileptic mice. In contrast, NRSF shows an upregulation for all time points. Such a database can be used to assess the fate of downstream targets. Major differences in energy metabolism were thus predicted, and the predictions were verified experimentally ⁶⁹. If metabolism operates differently at different times of the day in control and epilepsy, what could be the functional consequences? This is an important topic to pursue, since, as mentioned previously, metabolism and the clock machinery are strongly linked to each other (they cannot be dissociated). The same approach can assess other major biological pathways, e.g. inflammation, glial cell function, cell cycle etc. Given the large number of transcription factors and downstream genes involved in seizure rhythmicity, where do we start? The differences between rodents and humans may help us.

7.5. Diurnal versus nocturnal species

Surprisingly, the timing of the oscillation of clock genes and the action of light on the SCN are similar in diurnal and nocturnal species ¹²⁰. Although Per1 peaks at midday in both

types, the general behavior is very different. This suggests that the same core oscillator can be used to do different things. As a result, some processes are in phase, while others are in antiphase in both species. For example, body temperature and hypothalamic serotonin oscillate in antiphase, while melatonin release remains in phase in diurnal and nocturnal animals. Understanding how the same core oscillator in the SCN can drive opposite behaviors at the whole-body level may provide a breakthrough for our understanding of the circadian regulation of seizures.

Interestingly, patients with temporal lobe epilepsy and an experimental rat model display similar seizure rhythmicity³. A possibility is that the molecular determinants of the seizure rush hour should be in phase (e.g. like melatonin, whose concentration rises and falls at the same time in rats and humans), and not in antiphase (e.g. like serotonin, which is at its maximum concentration in humans when it is at its lowest in rat) in both species. Of course, this reasoning applies if the mechanisms underlying seizure rhythmicity are similar in humans and rodents. Experimental and theoretical data demonstrate many biophysical ways to generate the same activity in a neuronal network⁹², including seizures⁸⁶. Hence, the mechanisms may be different between and within species.

Finally, when recorded over extended periods, individuals with epilepsy and experimental models display different types of seizures^{89; 121}. It will be interesting to test whether specific types of seizures occur at specific circadian times and whether their temporal distribution is similar in humans and rodents.

The take home message #10 is a simple hypothesis: biological factors oscillating in phase (or with a delay) with seizure rhythmicity are promising candidates, with the proviso that some

of these factors may come from body organs other than the brain and that they may be regulated on a slower time scale (multidien cycles).

8. Multidien rhythms

Clinical and experimental data based on long-lasting recordings show that even if there is a time-of-the-day preference for seizure occurrence^{11-15; 24}, seizures do not occur on every circadian cycle. Slower rhythms (from 7 days to 45 days) organize the distribution of seizures on a slow time scale^{11; 15; 24}. This is not limited to epilepsy. Cardiovascular diseases show circaseptan (around 7 days) periodicity, which can be explained by a weekend effect¹²². However, the weekend effect cannot simply explain the circaseptan and hemiseptan periodicity of COVID-19 data¹²³. The biological origins of such slow rhythms are unknown. Still, they are conceptually similar to catamenial epilepsy, which is characterized by increased seizure probability during a specific part of the cycle in female patients¹²⁴. As for circadian molecular oscillations, we are starting to (re)discover the existence of slower than circadian rhythms. According to the MORE hypothesis, there should be slow molecular oscillations to explain such slow rhythms. Experimental data is lacking, although melatonin and progesterone appear to follow circaseptan rhythmicity in healthy humans (some relevant works from Franz Halberg and Peter Celer are only available on ResearchGate); but these studies need to be replicated. These slow rhythms may control the daily molecular remapping on a slow time scale, i.e. the molecular map at any given time is not the same from one day to the next, but it could be the same every 7-45 days, according to the slow period. The MORE hypothesis can also account for seizures' multidien rhythmicity because it is grounded in molecular oscillations. The fact that multidien cycles have a circaseptan period in rodent models makes it reasonably tractable experimentally²⁴.

What could cause multidien cycles? A simple hypothesis would be that some circadian molecular oscillators are not reset by cues such as light or food, but would follow their own slower frequency, thus creating a multidien cycle. This may contribute to the fact that seizures do not occur every day during the period of increased probability. Rather, they could occur at a specific phase of the slower oscillation in conjunction with the daily rush hour.

9. The impact of interictal and ictal activities on circadian rhythms

It is possible that interictal activity and spontaneous seizures, especially when they occur in clusters, act as strong feedback cues and alter circadian molecular oscillations in the networks involved in seizure genesis and propagation of their downstream targets, which can be body-wide. This key issue remains to be investigated, but we already know that seizures can change biological rhythms (as assessed by temperature) in a phase-dependent manner ¹²⁵. The fact that intense network activation can up-regulate Per1 ¹²⁶, one key player in the circadian clock, suggests that interictal/ictal activity may alter the clock machinery, and thus the circadian regulation of the molecular architecture. A very simple hypothesis can be proposed: as the peak of interictal activity increases during its multidien cycle ^{11; 24}, Per1 expression (or any other gene regulated by neuronal activity) may increase accordingly, hence its target genes, moving the architecture of neuronal networks closer to the seizure threshold over days. Said differently, seizures may produce a phase shift of the molecular oscillations changing the timing but not the basic mechanisms. It is important to remember that there may not be direct causality in a complex system with mutually coupled oscillators, i.e. the multidien cycle of Per1 (or any other), interictal and ictal activities could just be emergent properties of the network.

Investigating the role of interictal and ictal activity on the molecular architecture and its circadian rhythmicity now becomes possible, e.g. with fluorescent reporters that will enable us to follow genes and proteins' expression over days in freely moving animals in correlation with electrophysiological activity¹²⁷.

Conclusion

Several key concepts have been presented in the MORE hypothesis. Most body organs and their inner parts, up to individual cells, are characterized by a molecular architecture that oscillates in a circadian manner. The primary driver resides in the suprachiasmatic nucleus. All these oscillators are weakly coupled to each other, and the periodic organization of a living organism results from these oscillations. These oscillations depend upon and can be modified by internal and external cues. It is possible that an oscillating molecular architecture brings some neuronal networks close to the seizure threshold at specific times during the day. Providing drugs to increase the threshold only when this is needed or when the molecular target is most present may improve treatments and perhaps decrease the drugs' side effects by decreasing the time of exposure. **This is at the core of chronotherapy.** However, since seizures do not occur every day at the same time, other co-factors are necessary, which adds a level of difficulty to control them. When looking for mechanisms, it is important to keep in mind that circadian molecular oscillations are different in control and epilepsy; i.e. it may be difficult to translate the healthy brain's results to epilepsy. Finally, **the fact that the body is constantly changing during one's life adds another layer of complexity.** Since the rules driving molecular oscillations change over time, so may seizure mechanisms at the individual level.

The dynamic (time-dependent) nature of the molecular architecture of brain networks is essential to consider to understand not only seizure mechanisms but also comorbidities. Epilepsies are associated with numerous comorbidities, including depression and cognitive deficits. Since depression and learning/memory processes are regulated in a circadian manner ^{74; 75; 128}, it will be important to establish the molecular landscapes that favor their expression.

The MORE hypothesis is proposed as a framework to ask questions. Although the observation of the circadian rhythmicity of seizures is century-long, it is only in the last years that we are starting to glimpse its possible molecular underpinnings and the complexity of the task in front of us. Arguably, knowing how to ask the most relevant questions may be the surest path to a much-needed understanding of seizure mechanisms and treatments. I hope that the MORE hypothesis will be a first step in this direction.

I confirm that I have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

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Figure Legends

Figure 1. Seizure rush hour in patients with epilepsy. A. Figure 1 reproduced with its legend with permission from ref ¹. B. Distribution of seizures during 24 h (horizontal axis) across all recorded days (vertical axis – the last recording day is indicated at the top of each axis) in six patients S10-S15. Black dots are seizures with identical electroencephalographical signature, some of which can be recognized clinically; grey dots are electroencephalographically distinct subclinical events. C. Distribution of seizures and interictal spikes during the night (gray rectangle) and day cycle.

Figure 2. Processes displaying circadian rhythmicity in humans. From Wikipedia.

Figure 3. The relative density of the phosphorylated (P) and non-phosphorylated (NP) forms of KaiC oscillate with a quasi 24h period, while the relative total density of KaiC remains constant. Reproduced with permission from ref ³⁶.

Figure 4. Core clock feedback loops (top) and genes controlled in a circadian manner (bottom). Arrows indicate positive control, hyphen negative control. The meaning of all abbreviations can be found in the original paper. The figure is reproduced here to show the complexity of the regulatory loops, with CLOCK/BMAL1 at its core to regulate transcription. The bottom part shows the complexity of the mechanisms regulating the clock-controlled genes. Reproduced with permission from ref ⁵³.

Figure 5. A. Circadian regulation of activity and body temperature in a nocturnal mouse. Dark bars indicate the dark phase. Reproduced with permission from ref ¹²⁹. B. Circadian

regulation of the spontaneous firing activity of SCN neurons maintained *in vitro*. The bars indicate the subjective night. Reproduced with permission from ref ¹³⁰. C. Expression levels of six circadian genes. Note the differences in phase. Reproduced with permission from ref ⁶⁸.

Figure 6. Examples of different clocks in the body and the functions they control. Clock alterations in these organs can result in pathologies. Reproduced with permission from ref ⁵³. The oscillators in the different organs are coupled to one another directly or indirectly (via other organs). Molecular oscillations in one organ can thus influence the activity/function and the molecular oscillations in another organ. The state of the body at any given time is the result of these coupled oscillations in interaction with the environment.

Figure 7. Schematic illustration of the MORE hypothesis. A. Invented example of the state of a given neuronal network at 9:00 (left panel) and 15:00 (right panel). Circadian oscillators induce time-dependent changes in the expression of genes, proteins and metabolites. At 9:00, there is strong transcription of the NF- κ B in the pyramidal cell, some pyramidal cell synapses contain many receptors, and the mitochondria produce a small amount of ATP in astrocytes. The molecular architecture is such that network activity is low. In the afternoon, transcription of NF- κ B and the number of receptors are decreased, while mitochondria produce a lot of ATP in astrocytes. Some peptides produced by the gut microbiome enter the brain via blood vessels. Network activity is high. It is important to note the determinants of seizure rhythmicity may stem from molecular oscillations in any cell type in any organ. B. Molecular circadian oscillations bring the neuronal networks of the epileptogenic zone near

seizure threshold at specific times. A seizure occurs when other internal (e.g. neuromodulators) or external (e.g. stress) factors drive the network above the threshold.

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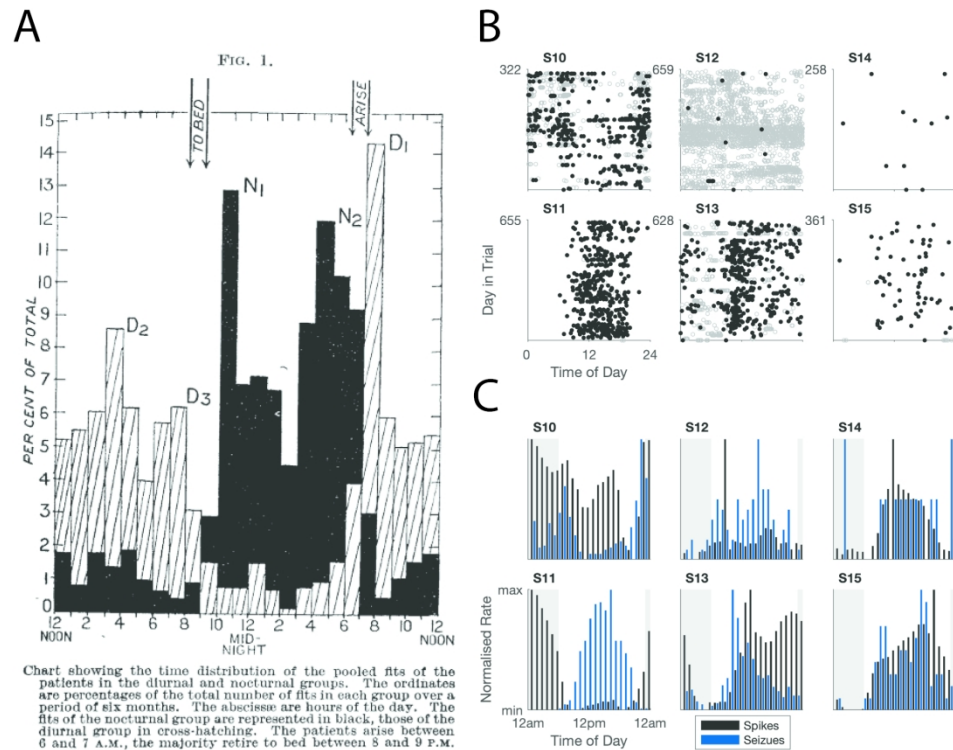
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32 Figure 1. Seizure rush hour in patients with epilepsy. A. Figure 1 reproduced with its legend with permission
33 from ref 1. B. Distribution of seizures during 24 h (horizontal axis) across all recorded days (vertical axis –
34 the last recording day is indicated at the top of each axis) in six patients S10-S15. Black dots are seizures
35 with identical electroencephalographical signature, some of which can be recognized clinically; grey dots are
36 electroencephalographically distinct subclinical events. C. Distribution of seizures and interictal spikes during
37 the night (gray rectangle) and day cycle.

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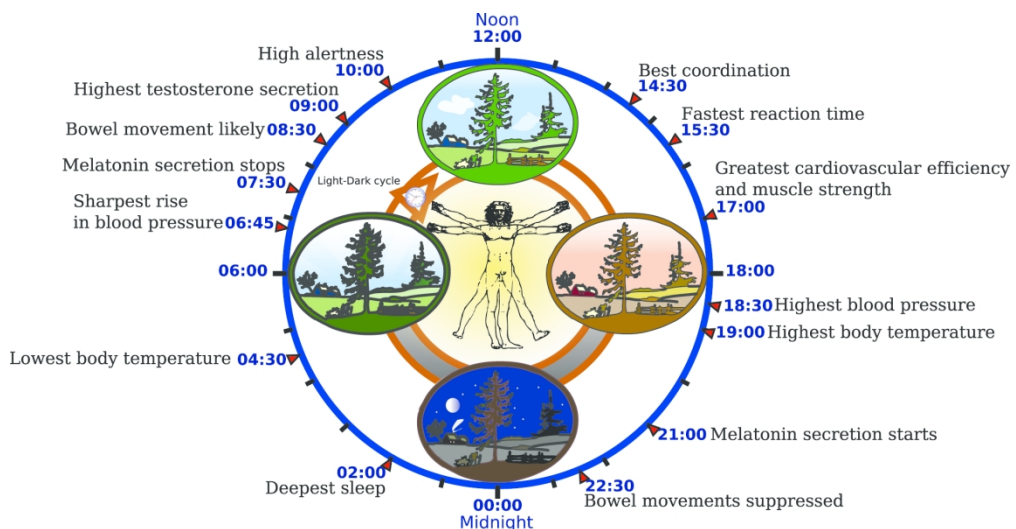


Figure 2. Processes displaying circadian rhythmicity in humans. From Wikipedia.

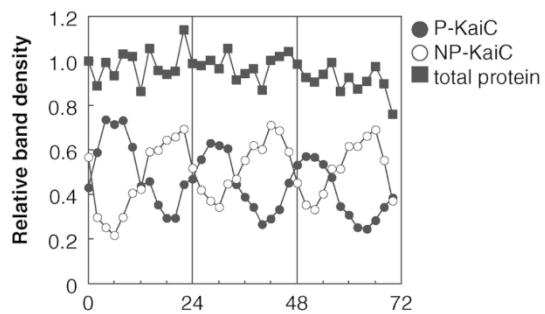
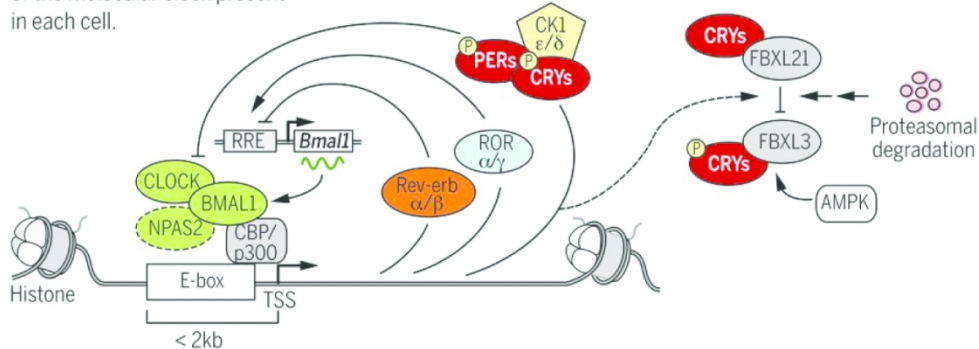


Figure 3. The relative density of the phosphorylated (P) and non-phosphorylated (NP) forms of KaiC oscillate with a quasi 24h period, while the relative total density of KaiC remains constant. Reproduced with permission from ref 36.

Core clock feedback loop

These factors comprise the gears of the molecular clock present in each cell.



Clock-controlled genes

Core clock genes engage many tissue- and signal-dependent epigenetic regulators that in turn induce rhythmic transcription genome-wide.

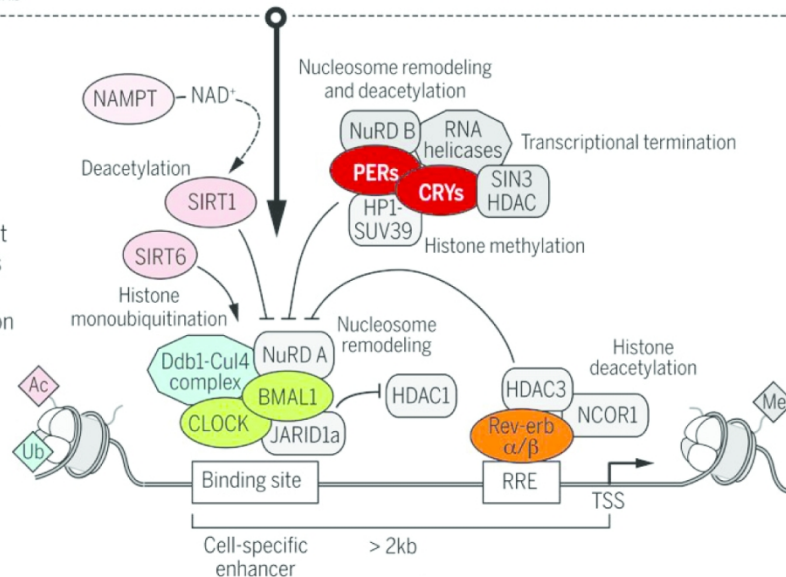


Figure 4. Core clock feedback loops (top) and genes that are controlled in a circadian manner (bottom). Arrows indicate positive control, hyphen negative control. The meaning of all abbreviations can be found in the original paper. The figure is reproduced here to show the complexity of the regulatory loops, with CLOCK/BMAL1 at its core to regulate transcription. The bottom part shows the complexity of the mechanisms regulating the clock-controlled genes. Reproduced with permission from ref 54.

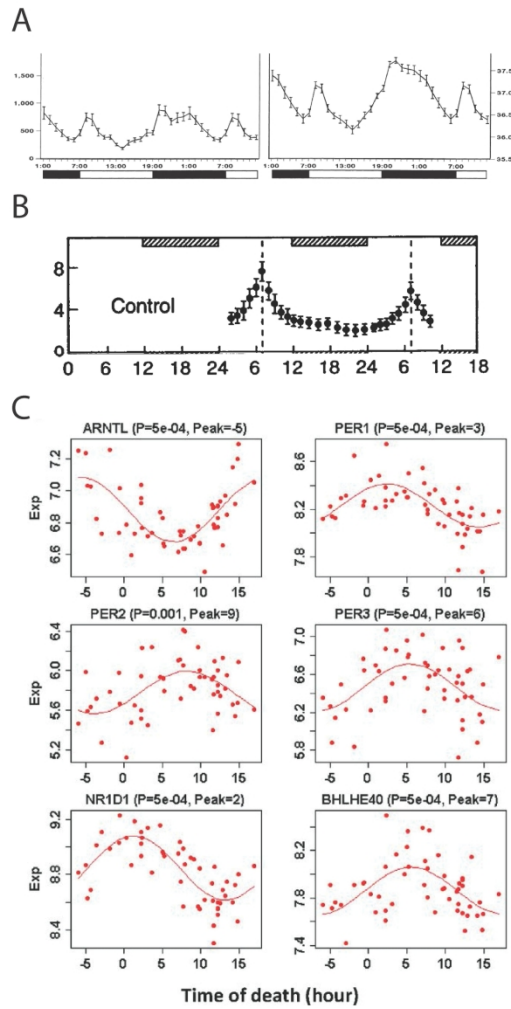


Figure 5. A. Circadian regulation of activity and body temperature in a nocturnal mouse. Dark bars indicate the dark phase. Reproduced with permission from ref 117. B. Circadian regulation of the spontaneous firing activity of SCN neurons maintained in vitro. The bars indicate the subjective night. Reproduced with permission from ref 118. C. Expression levels of six circadian genes. Note the differences in phase. Reproduced with permission from ref 82.

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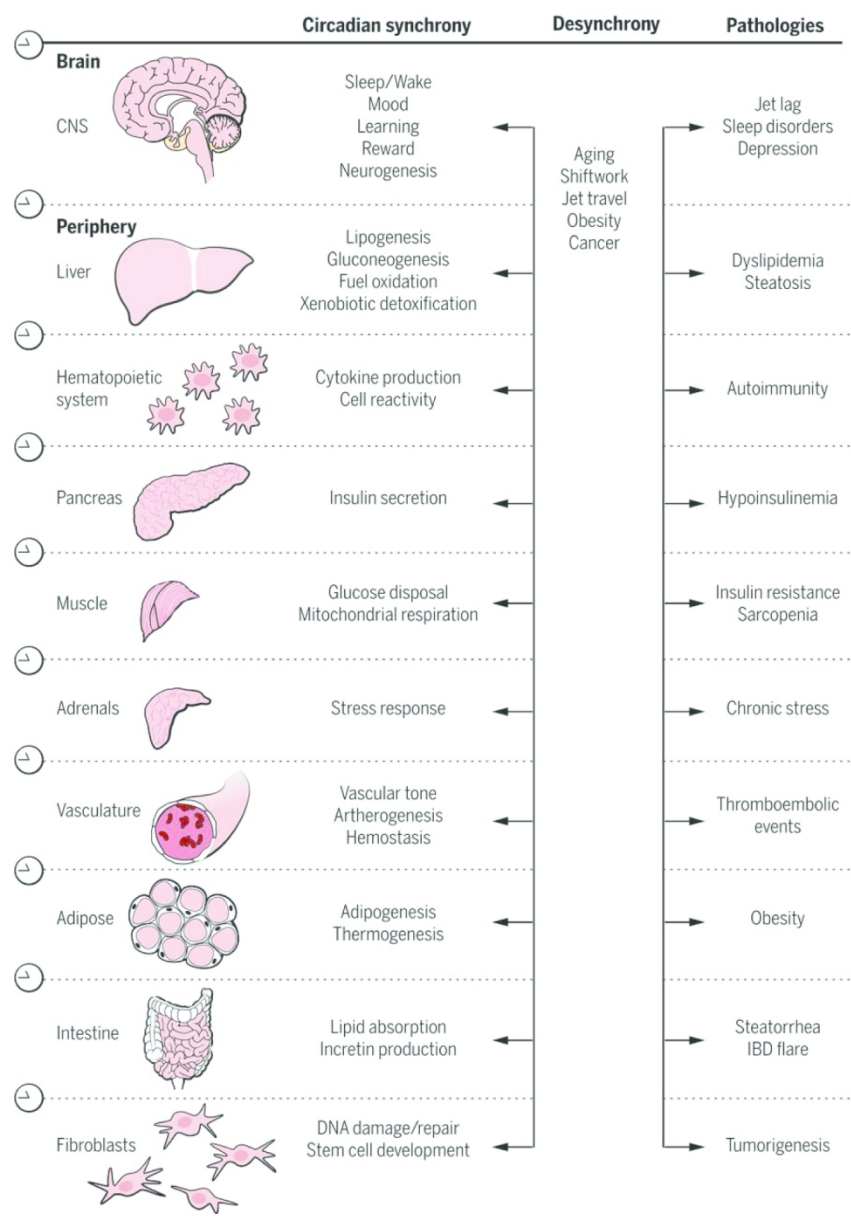
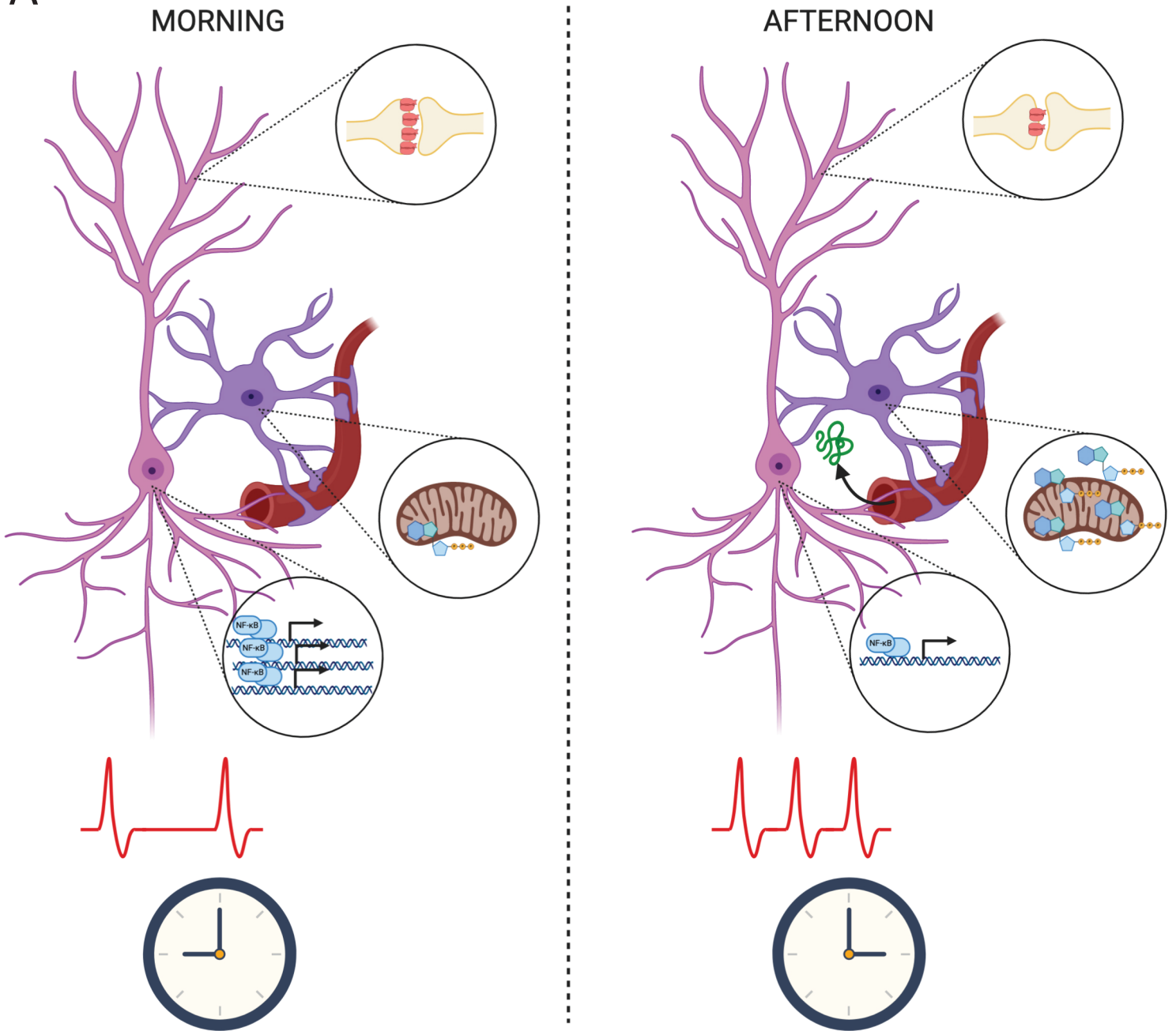


Figure 6. Examples of different clocks in the body and the functions they control. Clock alterations in these organs can result in pathologies. Reproduced with permission from ref 54.

The MORE hypothesis

A



B

