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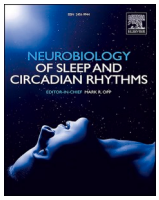

Question what is "known"

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When Mark Opp, editor, invited Marcos Frank and me to write OPEDs on the state of the field of sleep research, Marcos suggested (facetiously?) that he would title his OPED "everything you know about sleep is wrong". I countered by saying that I would title my OPED "everything you know about sleep is right." My reason for that quick repartee was focused on the word "know". What can you truly know? You can know good data. Thanks to the universally accepted ethic in modern science that no ends justify the means, we trust each others' data. Regardless of field, nationality, or any other characteristic, data from well done scientific experiments are accepted as facts. Of course, methodologies must be critically evaluated, but if the methods are solid, the data are facts that we can "know". However, we cannot and should not believe the speculations that arise in the interpretation of data. Such speculations are at best hypotheses, they generate new questions and they stimulate future experiments. That is the way science progresses. But if speculations become entrenched as common knowledge that is assumed to be true, they can skew the direction of future research to the detriment of the field.

Thinking about the problem of speculative interpretations of data rising to the status of accepted fact, I realized that questioning assumptions on which experiments are based is an important quality of mind for scientists, and it should be instilled into young investigators entering our field – or any field. I therefore prepared a talk on the subject for Trainee Day at SLEEP2020. This article is a narrative version of that talk.

An assumption at the very foundation of sleep research is: the need for sleep builds up during wake and is discharged through sleep. That statement seems reasonable given our daily experiences, even if we factor in the circadian influence on sleep-wake propensities. However, it ignores an important fact – sleep consists of two entirely different states, NREM and REM. Lumping NREM and REM as sleep results in the expansion of the initial assumption to: the need for both NREM and REM sleep builds up during wake. That expanded assumption has skewed research into the functions of sleep as I describe below. But first, I list several facts that must be accommodated by any hypothesis about sleep functions:

- NREM always come first except in some pathological conditions.
- NREM and REM cycle with a periodicity characteristic of the species.
- NREM sleep is deeper (greater delta power) early in the sleep phase and less deep in the late sleep phase.
- REM bouts are shorter early in the sleep phase and longer in late sleep phase.
- NREM sleep takes precedence in recovery from sleep deprivation.

I will use research on the NREM/REM cycle, which I call the sleep cycle, as an example of how assumptions based on interpretation of even excellent data can bypass alternative possibilities and skew the direction of future research. The sleep cycle has a periodicity of about 90 min in humans, 10 min in rats, and 23 min in cats. Across many species of mammals these periodicities are a function of brain size – one of the few quantitative characteristics of sleep across mammalian phylogeny. Attention to the sleep cycle was stimulated by the elegant electrophysiology done by Hobson. McCarley, and Wzynski that offered a mechanistic explanation of the cycling. In their classic paper (Hobson et al., 1975), they described two populations of brainstem neurons that fired in antiphase with each other. One population was REM-off and the other population was REM-on. The REM off cells inhibited the REM-on cells, but also had recurrent inhibition on themselves. The REM-on cells were excitatory to the REM-off cells and had their own recurrent excitation. Their paper concluded with the statement: "…we see these results as relevant to the hypothesis that reciprocal interaction between

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functionally interconnected cell populations may determine the cyclical alternation of behavioral states." The electrophysiological details have increased over the years (Pace-Schott and Hobson 2002; Fuller et al. 2007) and have been modeled to describe a control system for NREM/REM cycling.

A distinction frequently ignored is that control is not the same as regulation. A controlling system for the NREM/REM cycle could be a simple fixed period oscillator. A regulatory system, in contrast, has an optimum value or set point and must use feedback information to keep its regulated variable close to its set point. This is a homeostatic relationship. Identification of the feedback parameter can offer clues as to the function of the system. Since the prevailing assumption was that sleep (both NREM and REM) is in a homeostatic relationship with wake, it was easy to assume that the NREM/REM cycle was controlled by a fixed period oscillator and did not involve a homeostatic mechanism of regulation.

A different approach was taken in the early 1990's by Joel Benington. He asked what would be the characteristics of the sleep cycle if it were not controlled by a fixed period oscillator but instead was due to a homeostatic relationship between NREM and REM sleep. He hypothesized that NREM sleep need accumulated during wake and REM sleep need accumulated during the expression of NREM sleep. Thus, he postulated a homeostatic relationship between wake and NREM sleep and a homeostatic relationship between NREM and REM. If this hypothesis were true, the sleep cycle would not be due to a fixed period oscillator but by a homeostatic regulatory mechanism. Such a regulatory relationship would answer all of the bulleted questions posed above. But, how could you tell the difference between a wake related model with a fixed period oscillator and a sleep related model with a homeostatic switch? The answer was simple. If the sleep cycle is controlled by a fixed period oscillator and consists of two phases, NREM and REM, variations in the durations of the two phases should be inversely related to maintain a constant period length. If however, the sleep cycle is driven by a regulatory mechanism, when the independent phase changed in duration, the dependent phase should change in the same direction – a proportional relationship. Recordings of almost 4500 sleep cycles in 12 undisturbed rats, showed a clear proportional relationship, long REM bouts were followed by longer NREM bouts (Benington and Heller 1994, 1995). The interpretation of these results was that maximum discharge of accumulated REM need permitted longer NREM episodes, but residual REM need resulted in a more rapid interruption of NREM to discharge that REM pressure. Additional experiments showed that interference of REM bouts by the experimenter resulted in more frequent attempts to enter REM causing fragmentation of NREM bouts (Benington et al., 1994). These results clearly supported the possibility that NREM serves a need created during wake and REM serves a need created during NREM. Similar results were reported for human sleep (Barbatto and Wehr, 1998). In the ensuing 20+ years there has been no research, to my knowledge, on the possible functional relationships between NREM and REM sleep – an opportunity for new investigators.

A homeostatic explanation of the sleep cycle has serious implications for a wide range of past and present sleep research probing relationships between sleep states and waking functions. Although it was widely recognized that you could not eliminate NREM without preventing expression of REM (which you would predict from the NREM/REM homeostatic model), some thought that you should be able to do selective REM deprivation. Many studies have employed that approach using methods ranging from the classic upside down flower pots to rotating disks over water. What those experiments failed to recognize is that selective REM deprivation rapidly impairs the quality and continuity of NREM sleep. As explained above, if a REM bout is curtailed, the next attempt to enter REM comes sooner, and this effect is cumulative resulting in shorter and shorter NREM bouts. Since each attempt to enter REM and return to NREM takes about a minute, only 2 h of selective REM deprivation in the rat resulted in about 45–50 attempts to enter REM per hour, and that left not much quality NREM sleep (Benington et al., 1994). So, how can the results from "selective REM deprivation" experiments be interpreted in terms of REM sleep functions exclusively?

Selective REM deprivation fragments sleep, and that has been shown to have a powerful effect on one recognized function of sleep – memory consolidation. Rolls et al. (2013) used optogenetic stimulation of hypocretinergic cells to fragment sleep. The behavioral test was Novel Object Recognition (NOR). Mice were trained just before the rest phase and then during the first 4 h of the rest phase they received trains of brief stimulations at various frequencies. The mice were tested for NOR 24 h after training. Stimulation at 30 or 60 s intervals eliminated NOR performance. Stimulation at longer intervals did not. Looking specifically at the data from the 60 s interval stimulations as compared to controls (no stimulation), there was an increased number of transitions to wake, but no differences in total NREM or REM sleep, and no differences in power spectral profiles of NREM or REM. These results make perfect sense in the context of the elegant studies of Matthew Wilson, Gyorgy Buszaki, and colleagues supporting the idea that memory traces in the form of temporally sequenced firing patterns of place cells are transferred from hippocampus to cortex in the process of memory consolidation during NREM sleep (eg. Ji and Wilson 2007; Lee and Wilson 2002; Buszaki 1998, 2015; and as recently reviewed by Findlay et al., 2021). The juxtaposition of these different studies suggests that fragmentation of sleep without decreasing NREM or REM quantity can disrupt and therefore destroy a recognized function of sleep – memory consolidation. Is it not, therefore, possible that the fragmentation of NREM sleep by selective REM deprivation could account for any effects ascribed to REM by any method of so-called selective REM deprivation? I have to conclude that any studies that interpret results from selective REM deprivation experiments as revealing a function of REM cannot be accepted at face-value.

What does my discussion of these old studies have to do with my assignment of commenting on the state of our field? The state of our field is excellent as evidenced by the growth of publications, the advent of excellent new journals such as this one and also the new *SLEEP Advances* journal, the new *Frontiers in Neurosciencs: Sleep and Circadian Rhythms*, the organization of new exciting meetings such as the Sleep Gordon Conferences and the Sleep Research Society Advances in Sleep and Circadian Sciences meetings, growth of funding for our science, and the increased translational activity taking our basic research on sleep and circadian systems into the clinic. We have benefited greatly from the development of new technologies such as optogenetics, chemogenetics, genetic engineering, new methods of brain imaging including those enabling recording of neural activity in real time, and new applications of AI and machine learning to extract more information from our traditional electrophysiological recordings. My discussion of old data is meant to alert new investigators in our field (and also old investigators looking for new challenges) that there are many important and exciting questions hiding behind widely held assumptions. Important qualities of mind are to be questioning, challenging, looking for alternative explanations, and designing experiments that can disprove existing explanations and assumptions. We can look forward to many exciting years of sleep research ahead. Maybe we will finally understand why we sleep.

Author contribution

The author is solely responsible for the content of this article.

Declaration of competing interest

I have no conflicts of interest related to my State of the Art oped.

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