

CHANGES IN GENE EXPRESSION DURING THE SLEEP-WAKING CYCLE: A NEW VIEW OF ACTIVATING SYSTEMS

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INTRODUCTION

The alternation between waking and sleep is a striking, global change of state that occurs every day in all vertebrates. Although there is hardly any behavioral or physiological parameter that is unaffected by this transition, the search for the functions as well as for the mechanisms of sleep has converged onto the brain and onto specific brain structures (see 43). Based on a broad review of the available evidence, Moruzzi hypothesized in 1972 (73) that “[...] sleep concerns primarily not the whole cerebrum, nor even the entire neocortex, but only those neurons or synapses, and possibly glia cells, which during wakefulness are responsible for, or related to, the brain functions concerned with conscious behavior; [...] sleep recovery would be responsible for the stability of the physicochemical properties of those brain structures that are affected by, or contribute to, the plastic changes occurring during the waking state”.

Moruzzi's own work on the ascending activating system (reviewed in 73) and that of many other researchers in the following years (reviewed in 107) have characterized neural structures and mechanisms that are responsible for modifying the “tonus” of neurophysiological activity in widespread brain regions. The firing of such “ascending activating systems” has thus been implicated in determining both tonic and phasic changes in neuronal activation across the sleep-waking cycle. Such changes have clear implications for the ability of the organism to orient and respond to stimuli during different states of wakefulness and sleep.

To this date, on the other hand, far less evidence has accumulated to support Moruzzi's hypothesis that sleep might be important for the recovery of physicochemical properties of brain structures that are implicated in plastic changes. The present paper explores the possibility that a better understanding of the functional consequences of sleep may be obtained by extending the notion of electrophysiologic activation to the realm of gene expression. It is suggested that, in the transition between waking and sleep there is, in conjunction with a widespread change in neuronal firing patterns, a widespread change in patterns of gene expression in the brain. It is then hypothesized that ascending activating systems subserve the diffuse, tonic and phasic activation of both neuronal responses and of gene expression. Finally, it is suggested that changes in gene expression may represent a key functional

consequence of sleep, especially with respect to brain mechanisms of synaptic plasticity. Some of these suggestions are supported by experimental results, others are still speculative but, as will be briefly discussed, they can be addressed experimentally.

I. *The sleep-waking cycle and neural activity.*

One of the most significant events in the study of sleep was the discovery that brain activity does not switch off during sleep (29, 105), contrary to what had been a common belief for a long time (27, 80, 99). Since then, a large number of studies has demonstrated that, in most of the brain areas examined, average firing rates of neurons do not differ conspicuously between sleep and wakefulness (see e.g. 107). On the other hand, especially during slow-wave sleep, patterns of activity may be quite different than during wakefulness, as exemplified by the transition from low-voltage fast-activity rhythms ("desynchronization") to high-voltage slow-activity rhythms ("synchronization"). The consequences of such different patterns of activity are striking: most notably, external signals are not transmitted far into the brain (see e.g. 85a, 107), and they are not incorporated into ongoing, intrinsic patterns of brain activity (e.g. 62). As a result, the brain is relatively isolated from the external world.

II. *Activating systems.*

The demonstration of major changes in patterns of neural activity at the transition between sleep and waking prompted the search for the underlying neural mechanisms. The notion of an ascending reticular activating system was put forth in 1949 by Moruzzi and Magoun (71), following their discovery that electrical stimulation of brainstem reticular formation resulted in the replacement of high amplitude slow waves by low amplitude fast waves. This switch from slow synchronization to desynchronization they called "activation". The concept of brain activation implied a diffuse and tonic readiness to respond on behalf of cortical circuits, which would subserve alert consciousness and behavioral arousal.

Since then, the notion of an activating system has been substantiated by lesion and recording experiments. Specific groups of neurons in the brainstem show tonic changes in activity several seconds before the transition from sleep to waking and vice versa, consistent with their role in cerebral activation (refs. in 106, 107). The attempt to more precisely identify the ascending activating system has led to the recognition of at least five neurotransmitter-specific cell groups that may be part of it: cholinergic neurons in the basal forebrain and in the dorsal pontine tegmentum (laterodorsal and pedunculopontine nuclei); noradrenergic neurons in the locus coeruleus; serotonergic neurons in the raphe dorsalis; dopaminergic neurons in the substantia nigra and ventral tegmental area; and histaminergic neurons in the

tuberomammillary hypothalamus. Other cell groups, especially in the mesencephalic reticular formation, which are as yet not identified neurochemically, will probably have to be added to this list.

Many studies have clarified the cellular basis for such an activating influence. Several of these systems, for instance, are able to alter the activity and excitability of cortical and thalamic cells by blocking a K^+ conductance which is active at rest and keeps these neurons hyperpolarized into a burst firing mode, as well as by shifting the voltage-dependence of the hyperpolarization activated cation current I_h (refs in 63, 64). Although several other mechanisms are also involved, the overall result is that of switching thalamic and cortical neurons from a hyperpolarized, burst mode of firing, in which they are relatively impervious to external signals, to a more depolarized, tonic mode, in which the transmission of external signals becomes more reliable (reviewed in 63, 64).

Despite these advances at the cellular level, several issues remain open. For example, REM sleep is not easily placed within this framework, since it is associated with cortical activation and with behavioral sleep (48). In addition, lesion and pharmacological experiments suggest that the ascending activating system cannot be identified with any one of the neurochemical systems mentioned above in isolation (47). A relatively clear distinction can be made between tonic and phasic patterns of firing of these systems. However, the cellular or system consequences of these two modes of firing are not yet clear. Finally, it has been suggested that the transition between sleep and waking may be primarily a local rather than a global phenomenon (54). Irrespective of these open issues, the discovery that an increase in firing of at least some of these systems plays a causal role in the transition between synchronized states of sleep and desynchronized waking remains one of the unifying foundations of integrative physiology.

III. *The sleep-waking cycle and gene expression.*

It has recently become clear that neuronal responses to changes in their environment include a variety of mechanisms spanning a period of time much longer than the changes in neuronal firing revealed by classical neurophysiological approaches. Second messenger systems activate protein kinases that phosphorylate specific proteins. Phosphorylation patterns are tightly controlled by a system of phosphatases, giving rise to a dynamic equilibrium which is not unlike the one between excitatory and inhibitory interactions. Changes in second messengers and in phosphorylation patterns can extend from tens of milliseconds to minutes and can influence both neuronal excitability and the subsequent activation of genes. The activation or deactivation of the expression of specific genes can occur in a matter of hours, and in some cases in a matter of minutes, through the action of transcription factors molecules that are able to bind to specific regulatory segments of DNA.

Considering the typical duration of sleep-waking states and the time constants of their regulation, it is plausible that cellular events of the kind described above may be subject to significant modulations in the course of waking and sleep.

However, whereas the notion that firing patterns change in the brain during different sleep-waking states is well established, the possibility that gene expression may also be subject to widespread and systematic alterations in the context of physiological sleep and wakefulness has rarely been considered (see, however, 35, 49). A few studies examined overall changes in RNA content (79) or synthesis (33, 34, 117) as well as overall changes in protein synthesis (13, 15, 74, 89, 98). More recent studies have investigated changes in the expression of specific genes after relatively long periods of total sleep deprivation (55) or after selective deprivation of REM sleep (86). Only one study (92) has attempted to look systematically at mRNA expression after 24h of sleep deprivation, with an emphasis on the homeostatic mechanisms of sleep.

1. *Immediate early genes.* - We have recently started a systematic investigation of changes in gene expression in the brain during sleep-waking states. Initially, we have examined the expression and distribution of immediate early genes (IEGs) such as *c-fos* and *NGFI-A*. These encode for a particular class of transcription factors that are induced by many extracellular stimuli and that need first to be transcribed and translated themselves, before they can affect the expression of other genes. Thus, IEGs are typically the first genes to be turned on or off in the chain of events that leads to changes in the expression of other genes. Their protein products have specific DNA binding domains (such as AP-1/TRE binding sites or zinc fingers) by which they act as nuclear "third messengers". For instance, the Fos (Fos, Fos-B(Long), Fos-B(Short), Fra-1, and Fra-2) and Jun (Jun, Jun-B, and Jun-D) transcription factors form hetero- (fos-jun) or homo-dimers (jun-jun) with a unique ability to affect the expression of target genes. It should be noted that IEGs have generally been considered an indirect but convenient markers of neuronal activation. For instance, the mapping of IEGs expression has been used to identify at the cellular level specific cell groups thought to be concerned with the regulation of sleep (68, 69, 101, 102, 120, 121). On the other hand, the use of antisense oligonucleotides targeted at IEGs indicates that IEGs can act as transcription factors *in vivo* and produce functional and behavioral consequences (22). In our studies, the underlying hypothesis was thus that the activation or deactivation of IEGs during the sleep-waking cycle might be an early event heralding and possibly triggering specific changes in the pattern of expression of other genes in many brain regions, including the cerebral cortex and the hippocampus (20, 21, 22, 82, 83, 84, 112).

The results of these and other studies have demonstrated that mRNA and protein levels of *c-fos* and *NGFI-A* are dramatically modulated by sleep and wakefulness (20, 21, 22, 36, 78, 82, 83, 112). For instance, a series of experiments has examined the expression of IEGs over the entire rat brain under conditions of spontaneous sleep and waking. A group of rats (S-L) was sacrificed during the light hours at the end of a long period of sleep. A second group (W-L) was sacrificed under similar conditions, except that during the last half hour the animals had been spontaneously awake. A third group (W-D) was sacrificed during the dark hours after a long period of continuous wakefulness. *In situ* hybridization and

immunocytochemistry were employed to assess mRNA and protein levels. It was found that *c-fos* and NGFI-A expression in several brain areas was increased in W-L and more markedly in W-D rats with respect to S-L rats. These areas included cerebral cortex, caudate-putamen and neighbouring areas, hippocampal formation, medial and lateral preoptic areas, some thalamic nuclei and, in the brainstem, superior and inferior colliculi, central gray, dorsal raphe, cuneiform nucleus, locus coeruleus, parabrachial nucleus and pontine nuclei. Especially in W-D rats, there was an increase in both mRNA and protein levels for both *c-fos* and NGFI-A. An example of such modulation of IEGs expression during spontaneous sleep and wakefulness is shown in Fig. 1. A parallel series of studies has indicated that after a few hours of sleep deprivation during the light hours, induced by gentle handling in order to minimize stress, patterns of IEGs expression were remarkably similar to those observed after spontaneous wakefulness (21, 82), suggesting that such patterns are associated with waking *per se*, rather than with circadian or other factors.

The modulation of the expression of certain IEGs during physiological sleep and waking becomes particularly interesting when considering the abundant evidence that IEGs are among the genes most clearly associated with a role in long-term changes in synaptic efficacy. For instance, several paradigms that induce long-term potentiation (LTP) in the hippocampus in awake animals also induce the expression of Fra proteins, of mRNAs and proteins in the *jun* family, and of NGFI-A mRNA and Krox-20 and Krox-24 proteins (50, 51). Note that, in anesthetized animals, induction of LTP is less effective and, correspondingly, the induction of IEGs is less marked, except for NGFI-A mRNA. NGFI-A and Krox-20 are the IEGs that seem to be more closely correlated with LTP in the hippocampus (1, 119). Several learning paradigms, including discrimination learning (111), olfactory learning (14), escape tasks (18), two-way passive avoidance (76, 77) in rats, barpressing tasks in mice (41); discrimination learning (3), one-trial passive avoidance (4, 93), exposure to rich environments (4) in chicks, and exposure to specific song patterns in birds (67) have been associated with changes in the expression of one or the other class of IEGs. In *Aplysia*, it has been shown that the binding to DNA of the protein product of an IEG (C/EBP) is required for serotonin to induce long-term facilitation in synaptic responses (2).

2. *Late genes.* - The finding of a dramatic change in the expression of IEGs during the transition from sleep to waking opens the possibility that other, late genes may be in turn activated or deactivated in an orchestrated way. Several potential target genes of IEGs have been proposed, such as prodynorphin, tyrosine hydroxylase, nerve growth factor, thyrotropin-releasing hormone and cholecystokinin (46). At present, possible variations in the expression of these genes during the physiological sleep-waking cycle have not been investigated. It is known, however, that tyrosine hydroxylase mRNA levels increase after 24h of sleep deprivation (86). There is also some initial evidence that the expression of a few other genes may also be modulated in relation to sleep and wakefulness (e.g. refs. 75, 84, 87, 114). Neuner-Jehle *et al.* (75) showed that both the mRNA and the protein levels of neurogranin are altered after 24 hours of sleep deprivation. It has been suggested

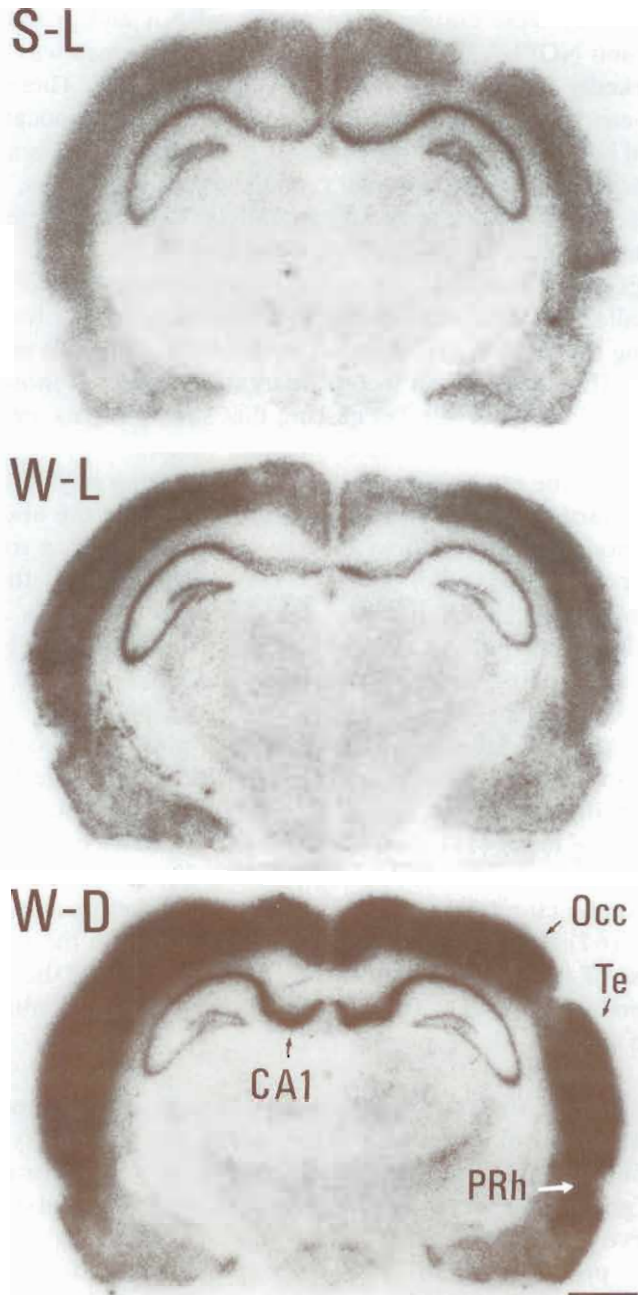


Fig. 1 - Changes in NGFI-A mRNA expression during spontaneous sleep and wakefulness.

The distribution of NGFI-A mRNA is shown for S-L, W-L and W-D animals in corresponding brain sections. S-L rats were sacrificed during the light hours at the end of a long period of sleep. W-L rats were sacrificed under similar conditions, except that during the last half hour the animals had been spontaneously awake. W-D rats were sacrificed during the dark hours after a long period of continuous wakefulness. CA1, field CA1 of Ammon's horn; Occ, occipital cortex; PRh, perirhinal cortex; Te, temporal cortex. Bar is 2 mm.

that neurogranin may be part both of the Ca^{++} /calmodulin and of the protein kinase C signal pathways. Neurogranin has been shown to localize in dendritic spines and it has been proposed that it may be implicated in synaptic function (53).

In a study by Pompeiano *et al.* (84), it has been demonstrated that mRNA levels of Ca^{++} /calmodulin-dependent protein kinase II (CaMKII) are also regulated by sleep and wakefulness. CaMKII is the principal protein of the postsynaptic density, strategically positioned to sense Ca^{++} influx and to phosphorylate synaptic channels. It has been associated with physiological and behavioral plasticity in both vertebrates and invertebrates (refs in 103). This kinase is necessary for the induction of LTP in the rat hippocampus. Knockout mice lacking the α subunit of this kinase were deficient in LTP and in some learning paradigms. It has also been shown that CaMKII α mRNA undergoes rapid changes in activity-dependent conditions (8, 16, 40). Given the involvement of CaMKII in LTP, kindling, and learning, the observation of changes in its expression in specific brain areas during spontaneous and forced waking suggests potential differences in synaptic functioning during different arousal states.

Despite these positive results, determining the validity of the above-mentioned hypothesis will ultimately require systematic attempts at detecting differences in gene expression across physiological sleep-waking states. Recent studies in our laboratory employing reverse transcription mRNA differential display (60) aim at the simultaneous and near-exhaustive comparison of patterns of gene expression across multiple sleep-waking conditions and multiple individuals. This approach should make it possible to determine whether there are characteristic constellations or “complexes” of genes expressed specifically during wakefulness or during sleep, respectively, to describe the time course of such expression, and to examine whether such complexes may have a meaningful functional counterpart.

IV. Value systems.

The occurrence of marked and diffuse changes in the expression of IEGs and other genes during the sleep-waking cycle, raises the question of what are the mechanisms involved. As for the transition between sleep and waking at the electrophysiological level, it is possible to consider both local mechanisms and diffuse humoral or neural factors. Local factors, such as the different dynamics of Ca^{++} entrance into cells during slow, synchronized activity vs fast, desynchronized activity may have far-ranging consequences on many cellular processes, including gene transcription. Theoretical reasons suggest however that, at least during waking, there should be mechanisms capable of coordinating changes in activity/excitability as well as in gene expression simultaneously over many cortical areas. Just as it is essential that the entire brain be activated during an orienting reaction in order more efficiently to respond to salient signals, so it is important that changes in gene expression, ultimately leading to modifications of synaptic efficacy and to forms of long-term memory, be diffusely and synchronously enabled in all the relevant brain areas.

We suggest the hypothesis that certain neurochemical systems with diffuse

projections that had previously been considered as substrates for electrophysiological activation during the sleep-waking cycle may act to coordinate not only changes in activity and excitability but also changes in gene expression and possibly in synaptic plasticity. Neuromodulatory systems with diffuse projections capable of signaling events of evolutionary significance to vast regions of the brain have been called “value systems” or “saliency systems” (32, 90, 113) with reference to the notion of value introduced by Gerald Edelman (28) in an attempt to understand brain functioning from a selectionist perspective. According to this perspective, evolution has provided organisms with several means to sense and signal to the entire brain the occurrence of behaviors having adaptive value and thereby to modulate changes in neural activity and plasticity so that the organism may appropriately adapt to them. In synthesis, the key characteristic of value systems is that of allowing salient events on a global or organismic scale to affect local changes in neuronal activity or plasticity. More specifically, value systems are defined as local groups of neurons that: i) have diffuse projections; ii) display tonic firing that may correlate with long-lasting behavioral states; iii) display phasic firing which is triggered by salient intrinsic or extrinsic events, at first innately, then via acquired connections; iv) diffusely release neuromodulatory substances, by which they v) globally affect neural activity and excitability, both tonically and phasically; vi) globally affect plasticity, both tonically and phasically; vii) different combinations of value systems may be activated under different conditions and may have differential effects on the activity and plasticity of target cells. While this is not the place to discuss and document these various properties of value systems, the available evidence shows that this definition applies well to diffuse projections systems capable of releasing neuromodulatory substances, in particular to the cholinergic, noradrenergic, serotonergic, and histaminergic systems. In the present context, it should be underlined that one of the most clearly documented aspects of the physiology of most of these systems is the marked and sustained change in their mean firing rate in the transition between waking and sleep (noradrenergic system: 6, 19, 31, 42, 95; cholinergic system: 26, 94, 95, 96, 106, 109, 110; serotonergic system: 66, 88, 115; histaminergic system: 91, 116).

What is the experimental support for the hypothesis that changes in the activity of such systems during the sleep-waking cycle may be responsible for changes both in neural activity/excitability and in gene expression? As we have seen above, the evidence that the levels of several neuromodulators influences the activity and excitability of neurons is by now quite convincing. Experiments directly addressing the role of neuromodulatory systems with diffuse projections with respect to changes in gene expression during waking and sleep will certainly be required to support the present hypothesis. Some circumstantial evidence has been accumulating, however, indicating that the transcription and translation of certain genes depends upon the level of acetylcholine, noradrenaline, and serotonin. Neuropeptides and nitric oxide, which can be co-released with these substances, may also play potent and specific roles in regulating gene expression. Much of this evidence concerns the influence of neuromodulators on the expression of IEGs. For instance, several studies have demonstrated that the administration of cholinergic antagonists re-

duce the basal or experimentally induced expression of IEGs in the brain. Pirenzepine, a muscarinic antagonist acting on M1 receptors linked to phosphatidyl inositol biphosphate hydrolysis and activation of protein kinase C, abolishes Fos induction in the brain (45). More generally, muscarinic agonists induce a complex pattern of IEGs expression in the brain and muscarinic antagonists prevent it (44, 45). Recent studies have also indicated that the level of activity of noradrenergic locus coeruleus cells may be important for IEGs expression in many brain areas. The injection of the α_2 -adrenergic antagonist yohimbine increases c-fos immunoreactivity in the rat cerebral cortex (11, 39). After local unilateral infusion of the neurotoxin 6-hydroxydopamine into the locus coeruleus, c-fos induction by yohimbine or stress is strongly reduced in the ipsilateral but not contralateral frontal, cingulate, and piriform cortices, suggesting that c-fos can be involved in the effects of noradrenergic transmission in the nervous system (108). In addition, it has been shown that high basal expression of NGFI-A in the cortex requires not only a degree of sensory stimulation but also the presence of a noradrenergic tone (9). Finally, several direct or indirect agonists of serotonin promote the expression of Fos protein in various brain areas (58, 59). Conversely, serotonin antagonists (57) or lesion of the serotonergic system (10) prevent such effects. It should be kept in mind that the combination of different receptors activated, or the degree of activation of the same receptor, can influence which IEGs are expressed. For instance, a low level of activation of NMDA receptors is sufficient to induce Krox-24 in the hippocampus, while higher levels are required by other IEGs. Thus, it is plausible that specific patterns in the expression of IEGs, and consequently of late genes, may be triggered by different combinations or amounts of neuromodulators released. This is significant in view of the different combinations of neuromodulators released during waking, nonREM sleep, and REM sleep, and of the different levels of neuromodulators released during tonic vs phasic firing.

V. *Gene expression, plasticity, and the functional consequences of sleep.*

Above, we have briefly summarized some evidence supporting the following propositions: i) the expression of IEGs, and possibly of other genes, changes dramatically between waking and sleep; ii) the activity of neuromodulatory systems with diffuse projections also changes dramatically between waking and sleep; iii) the activity of these systems influences the expression of IEGs and possibly of other genes. On the basis of this evidence, the hypothesis has been proposed here, that the activity of these systems controls the changes in gene expression occurring during the sleep-waking cycle. The effects of neuromodulatory systems on gene expression should also be considered in the light of further evidence indicating that iv) the activation of these systems is important for the occurrence of plastic phenomena during development, in the adult, and in several experimental preparations. For instance, neuromodulators such as acetylcholine, noradrenaline, serotonin, dopamine, and histamine influence the induction of LTP both in slices of the hippocampus and of the cortex (refs. in 5, 17). *In vivo* approaches (70, 97, 118)

have led to the conclusions that sufficient levels of certain neuromodulators are needed for physiological and behavioral plasticity to occur in the adult animal. Several lines of evidence, summarized in (65) show that pharmacological agents interfering with their action may impair learning and memory. Finally, the intactness of the cholinergic (7, 37), noradrenergic (7, 52, 100), and serotonergic (38) system is essential for developmental plasticity, e.g. for ocular dominance formation.

Considered together, propositions i) to iv) suggest the further hypothesis that changes in gene expression, due to changes in the activity of neuromodulatory systems occurring in relation to waking and sleep, may be of great importance for plastic phenomena. In view of this hypothesis, it seems particularly significant that the genes that have already been shown to be modulated by physiological sleep and waking, i.e. IEGs such as NGFI-A (83) and late genes such as those coding for CaMKII (84), are among the clearest candidates for a role in long-term changes in synaptic efficacy.

If well orchestrated changes in the expression of genes related to synaptic plasticity take place in the transition between waking and sleep, the nature of these changes might lend a sounder molecular basis to longstanding hypotheses about the functions of sleep. For instance, it has been repeatedly suggested that sleep (either nonREM sleep or REM sleep) might subserve the consolidation of synaptic changes occurring during waking (12, 30, 35, 56, 72, 81, 85b, 104). Conversely, it has been claimed that either nonREM sleep or REM sleep might serve the selective elimination, erasure, or cleaning, of "memory traces" (23, 24). It has also been proposed that sleep might promote the selective stimulation of unused synapses (54). A different possibility is that changes in gene expression during the sleep-waking cycle may switch the balance between states favoring an ongoing generation of diversity in synaptic circuits and states favoring their differential amplification as a result of selective events. This possibility is consistent with selectional views of brain function, since the ongoing generation of diversity, providing a rich repertoire for selection, is a crucial requisite for any selectional system, be it evolution, the immune system, or the brain (cf. 28). Hopefully, the identification of "complexes" of genes expressed specifically during wakefulness or during sleep, respectively, will offer a molecular signature that might reveal the functional consequences of different brain states and thereby offer a clue to their functions.

CONCLUSION

This paper proposes that, in addition to generalized changes in neural activity, the transition from waking to sleep is accompanied by generalized changes in the expression of certain genes. It also proposes that such changes may be mediated largely by changes in the activity of "value systems", neuromodulatory systems with diffuse projections that signal salient events. The notion of value systems, which is based on the concept of value in neural selective processes, encompasses the original concept of the "activating system", and extends it to genetic activation. Finally, it is suggested that changes in gene expression between waking and sleep

may represent a functionally important consequence of sleep, in line with Moruzzi's own suggestion that "[...] sleep recovery would be responsible for the stability of the physicochemical properties of those brain structures that are affected by, or contribute to, the plastic changes occurring during the waking state".

At present, some of these suggestions are still speculative and await experimental testing. Several questions will have to be addressed. Is the activity of neuromodulatory systems with diffuse projections, which is generally higher during waking than during sleep, required for changes in gene expression, and possibly synaptic changes to take place? Is the lower level of activity of many of such systems during synchronized sleep an indication that gene expression is decreased or rather that a different pattern of gene expression is triggered? Is the tonic level of activity of these systems fundamental in order to maintain a tonic level of the expression of certain genes? Are phasic changes in the activity of these systems essential in order to trigger the expression of other genes, or to localize such expression to areas of intense, correlated activity at the time that salient events are registered? All these questions can and will be addressed experimentally in the near future. If the overall hypotheses discussed here were to hold true, the notion of brain activation would acquire a wider meaning and new functional implications.

SUMMARY

Moruzzi pioneered the notion of ascending activating systems that were responsible for the electrophysiological activation characterizing the transition from sleep to waking. This paper proposes to extend the notion of electrophysiological activation to the domain of gene expression. Evidence is reviewed indicating that in the transition between sleep and waking there is, together with a change in neuronal firing patterns, a change in patterns of gene expression in widespread regions of the brain. The hypothesis is presented that changes in the activity of neuromodulatory systems with diffuse projections may subserve the diffuse, tonic and phasic activation of both neuronal responses and of gene expression. Finally, the paper discusses the possibility that such changes in gene expression may be of importance for plastic phenomena and for the functional consequences of sleep.

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