Elementary Central Nervous System Arousal

84

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Contents

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Abstract

Wakefulness is a state that an animal can consciously sense internal drives and external stimuli and actively respond to environment. Fundamental endeavors such as finding food and avoiding predators require conscious and wakeful behaviors in order to improve chance of survival. Elementary CNS arousal drives shift between states of sleep and wakefulness, to orient an animal towards important stimuli and to maintain wakefulness in the absence of important external stimuli. Specific motives and incentives explain why an animal does one thing rather than another. Arousal is modulated by circadian, homeostatic, executive/cognitive, emotional, and environmental factors, which can be simply summarized as internal drives and environmental pressures. Both conceptually and experimentally, we know that an animal's level of arousal can be variable across minutes, days, seasons, and years. These variations are associated with behavioral characteristics such as mood, feelings, temperament, and overall cognition. While arousal's role in promoting sleep or wakefulness is one of its most obvious, and well-studied, effects on behavior, the more subtle shifts between quiet waking, alertness and attention are equally important, with potential clinical manifestations.

Keywords

Acetylcholine • Adenosine • Basal forebrain • Brainstem • CNS arousal • Dopamine (DA) • Electroencephalography (EEG) • Electromyography (EMG) • Functional magnetic resonance imaging (fMRI) • Glutamate and gamma-aminobutyric acid (GABA) • Histamine (HA) • Hypocretin • Hypothalamus • Neuropeptide S • Noradrenaline/norepinephrine • Serotonin • Thalamus

Brief History

Diffuse, global controls over brain arousal have long been recognized in clinical neurology, as their damage leads to disorders of consciousness. In the first half of the twentieth century, brain arousal was thought to be triggered and maintained by

the influx of sensory information. This viewpoint was superseded by two relevant lines of research that developed in relative isolation. One involved the "ascending reticular activating system," a system which comprises neuronal cell bodies in the brainstem reticular formation that have diffuse ascending axonal projections to the forebrain and which plays a key role in modulating levels of arousal and attention. The other system involves hypothalamic centers involved in controlling the expression of both sleep and motivated or goal-oriented behaviors essential for the survival of the individual (e.g., appetitive behaviors associated with hunger and thirst) and survival of the species as a whole (e.g., sexual and parental behaviors).

The first line of research gained traction through the classical experimental studies by both Bremer and the team of Moruzzi and Magoun who demonstrated that stimulation of the reticular activating system evoked changes in electroencephalogram (EEG) recordings from anesthetized cats. In these studies, electrical stimulation of the midbrain reticular core or the central thalamus changed EEG activity from a resting-state pattern to an awake-state pattern, with diffuse thalamocortical projections being implicated in the changes. Confirmation of this anatomical pathway from the midbrain reticular core to the central thalamus was later provided by detailed electrophysiological and anatomical studies. Subsequently, the thalamus, which was originally thought to play a simple role as a relay of information, has been shown to be critical to the state of an animal.

The second line of research, which focused on the hypothalamus, linked stimulation of regions within the hypothalamus to basic survival behaviors (e.g., aggression, sex, and food intake), the CNS arousal necessary to support these behaviors, and the control of the sympathetic and parasympathetic autonomic nervous system (ANS). Likewise, the experiments of Nauta showed that large lesions of the posterior hypothalamus resulted in a sleeplike behavioral state. Convergence of these two lines of research has since been established with the posterior hypothalamus having been shown to have important reciprocal projections with brainstem-arousal-associated nuclei. Since these early lines of research, major technical and theoretical breakthroughs in almost all neuroscience domains have revealed much about the identity and structure-function organization of neural systems underlying (a) the sleep–wake cycle; (b) arousal, alertness, and attention; and (c) motivation and emotion.

Elevated Generalized CNS Arousal (GA) Refers to a Continuum of Behavioral States Marked by Increases in Motor, Sensory, and Emotional Responsiveness

An organism has to be aroused in order to fulfill its normal biological requirements for life. While specific motivational states can help account for why a human being or other animal does one behavior rather than another, generalized CNS arousal (GA) contributes to the initiation of all behaviors (Fig. 1).

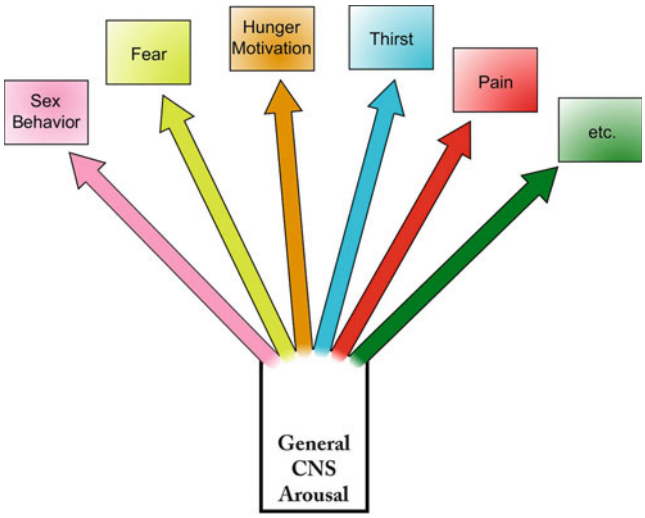

Fig. 1 Any given behavior that has a biological motivation depends both on generalized arousal and on a more specific kind of arousal (e.g., sexual arousal for sex). Without a sufficient level of generalized arousal, the specific arousals cannot be acted on (Adapted from Pfaff 2006)

The requirement for the concept of GA seems obvious as an abstraction, but it needs to be concretely defined in order to be tractable for experimental study. The definition should reflect that CNS arousal is universal across vertebrates, that it incorporates multiple brain systems (sensory, motor, and emotional), and that it is a constant throughout an animal's life. As such, the definition should embrace the evolutionary conservation of such a fundamental system; and it cannot be limited by particular, temporary conditions or measures. For example, the definition cannot be confined to explaining responses to only one stimulus modality, nor should it be limited to reflex responses to environmental stimuli. Therefore, the following has been proposed as an *operational definition* that is intuitively satisfying and that leads to precise quantitative measurements:

A more aroused animal is:
*(S) More alert to **S**ensory stimuli from all modalities;*
*(M) Highly predisposed to express **M**otor behavior;*
*(E) More reactive **E**motionally.*

with respect to a non-aroused, drowsy, or sleeping animal.

All three components – sensory alertness, motor activity, and emotional reactivity – can be measured with current technology and can therefore differentiate changes in arousal both within and between animals. This approach allows CNS arousal to be treated as a physical variable that can be explained with contemporary electrophysiological and genetic techniques. Operating requirements for GA systems are stated in Table 1.

Table 1 Operational requirements of generalized arousal

Feature	Definition
Lability	"Hair triggered"; rapid, not sluggish
Sensitivity	The system can respond to small changes in input in a state-dependent manner
Convergence	All sensory stimuli activate the same set of arousal subsystems, which, in turn, activate each other
Divergence	Activation of the cerebral cortex, autonomic nervous systems, and endocrine organs to initiate behavior
Robustness	The system does not fail; survival of the organism depends on adequate arousal

CNS Arousal Is Reflected in Quantitative Electrophysiological, Brain Scanning, and Behavioral Measurements

While behavior, as summarized above, is the most important physical manifestation of arousal, other types of measurements are useful as well. Electrical activity across the cerebral cortex corresponds with behavioral activation. Cortical electrical activity is measured by various methods, including electroencephalography (EEG) and local field potential (LFP) recordings, each of which results in consistent profiles of brain activity states through slow-wave sleep to attention. Likewise, as it correlates to the activity of local regions of the brain, changes in regional blood flow can be detected by fMRI and can be utilized to predict cortical arousal. Additionally, increased activity in the sympathetic pathways of the autonomic nervous system (ANS), which leads to physiological changes such as increased muscle tone, heart rate, and respiration, signals cortical arousal and is frequently followed by behavioral activation. Measurements focusing on ANS functions yield consistent results for determining brain activity levels. While we know many of the biological mechanisms behind various measurement methods, and they support our operational definition, behavior is the bottom line to assess arousal.

Electroencephalography (EEG)

Cortical EEG is recorded from the surface of the scalp, and its waveforms result from large numbers of synchronous postsynaptic responses in local populations of neurons beneath the scalp electrode. Large pyramidal neurons at the cerebral cortex are the main contributors to EEG waves. When multiple concurrent synaptic events happen, the EEG waveform has large absolute amplitude at that instant. If the numbers of neurons involved in an event decrease, or if the timing of events becomes less correlated, the absolute EEG amplitude decreases. The fluctuations in amplitude over time have distinct characteristics, most notably in the periodicity of amplitude fluctuations, depending on the behavioral state of the animal. High-frequency (20–80 Hz; also called fast oscillations), low-amplitude activity is

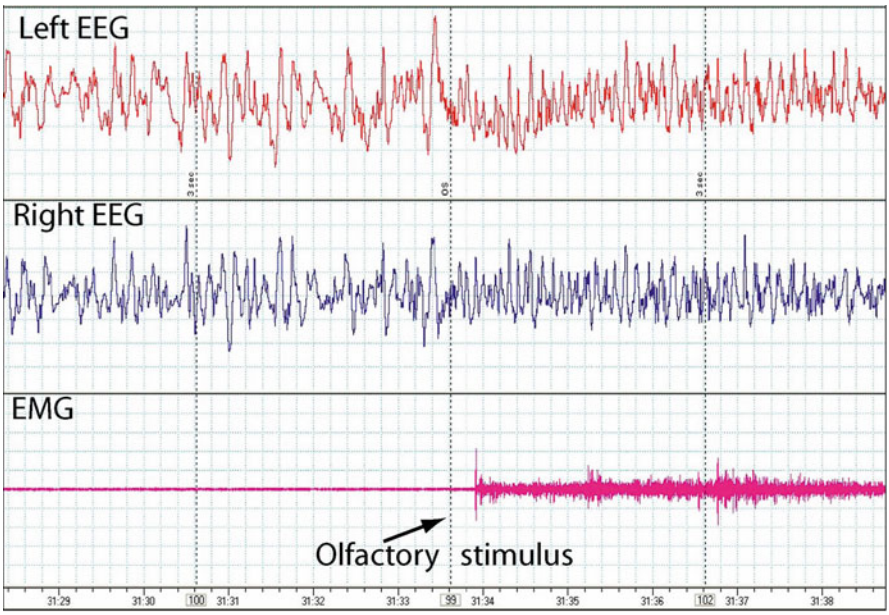

Fig. 2 Simultaneous electrophysiological recordings of the electroencephalogram (*EEG*) from the *left* and *right* cortices and neck muscle electromyogram (*EMG*) in a mouse. Experiments were conducted in the mouse's home cage during the light part of the daily cycle, so that baseline activity would be steady and quiet. Upon presentation of a novel olfactory stimulus, the EEG changed from high-amplitude/low-frequency waves, which are associated with rest, to low-amplitude/high-frequency waves, which are associated with higher CNS arousal. Concurrently, the mouse contracted its neck muscles, consistent with increased axial muscle tone (Adapted from Pfaff 2006)

primarily associated with behavioral wakefulness and attention, whereas low-frequency (0.5–15 Hz), high-amplitude waveforms are associated with sleep and rest (Fig. 2). Current literature suggests that high-frequency oscillations facilitate the encoding and decoding of information during perceptual and cognitive processes, while low-frequency oscillations facilitate memory formation, plasticity, and restoring cell homeostasis.

Electromyography (EMG)

EMG can be used to predict arousal level by measuring muscle fiber electrical activity, which reflects the extent of total neural input received. Regarding the importance of motor behavior expressed by an aroused organism, preparation of muscles for movement is another generalized effect conceivably observed during sleep-to-wake transition and varying degrees of arousal. It has been extensively reviewed that muscle tonus is highest in alert waking, reduced in drowsy state, and further inhibited at stages of deep sleep. The primary contributors to muscle tone

adjustment appear to be glutamatergic neurons. Motor neurons receive glutamatergic input from axons descending from rhythmogenic nuclei of brainstem regions regulating arousal states.

Functional Magnetic Resonance Imaging (fMRI)

Changes in the regional blood flow and blood oxygenation in the brain are closely linked with local brain activity. By measuring relative blood oxygen levels across brain regions, fMRI can predict the level of general arousal. Unlike EEG recordings, fMRI prediction of arousal is based on local activation in defined brain regions which have been shown to be activated by arousing stimuli. Several different studies have reported discrete brain regions whose activities are directly or inversely correlated with reported arousal experience. For instance, activity in the dorsolateral, prefrontal, and superior parietal cortices (which are also crucial loci for attention) is correlated with overall high-frequency, low-amplitude EEG activity, which marks arousal and attention. Likewise, changes in thalamic and frontomedial activity correlates with self-reported arousal levels.

Local Field Potentials

The recording of local field potentials is an electrophysiology-based method achieved by placing very thin electrodes into the brain and measuring electrical potentials in the extracellular space of a confined region. The recorded aggregate transmembrane currents from neuronal assemblies predict the activation of a particular region. Unlike EEG, which sums the electrical activity across large swaths of the cortex, LFPs are summed across smaller brain regions. Although many reports have suggested that LFP measurements are extremely local, different distances of detection have been reported from 200 μm to 5 mm. Such differences are suggested to be related with the diverse cellular composition of different brain and varying measurement locations.

Behavior

Behavioral measurements comprise the most fundamental method for assessing an animal's state of arousal. In humans, arousal can be assessed by personal report. However, for animals, various behavioral tests can be utilized in order to determine the extent of their arousal. In our lab, the GA assay measures behavior continuously across weeks or months with 20 ms resolution.

Hyperactivity is one of the most apparent features of an aroused animal. Keeping with the operational definition of arousal, a highly aroused animal is more predisposed to express voluntary movements. Therefore, quantification of motor behavior serves as a strong method to detect the level of arousal in many animals. Both infrared laser-based movement detection tools and running wheels are used to

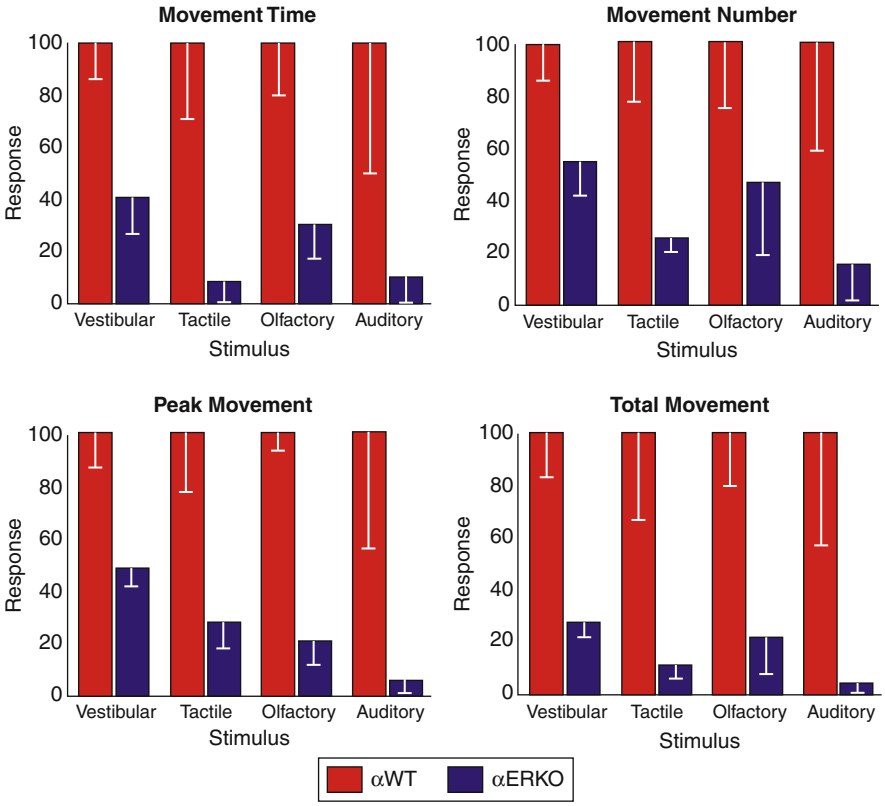

Fig. 3 The operational definition of arousal allows CNS arousal to be treated as a physical variable. Presented are data which, by measuring alertness to sensory stimuli and motor activity, show that knocking out the alpha estrogen receptor gene (αERKO) produces mice which have decreased arousal relative to wild-type mice (αWT) (Adapted from Pfaff 2006)

yield quantitative data. Criteria such as total number of movements, vertical and horizontal activity, and total distance traveled are frequently considered aspects of motor behavior (Fig. 3). Overall, highly aroused mice are expected to express more home cage activity compared to less aroused ones.

Increased sensory responsiveness is another feature of an aroused animal. Even though one stimulus set may dominate the others, during a high arousal state, senses from all modalities are more likely to be consciously processed when compared to a drowsy state or sleep. Operationally, a highly aroused animal is expected to more intensely respond to a sensory stimulation relative to a low-arousal animal. For example, highly aroused mice demonstrate increased behavioral reactivity toward an odorant. When mice are exposed to the olfactory stimulus benzaldehyde, the highly aroused ones express more motor activity toward the source of stimulus compared to controls. This data suggests that generalized arousal increases sensitivity and reactivity to bottom-up sensory stimuli.

An animal's level of general arousal is also associated with its level of emotional responsiveness. In terms of emotional responsiveness, a highly aroused animal is more predisposed to show behaviors related to fear, anxiety, or attraction. For example, in order to quantify the emotional aspect of arousal in mice, tests like elevated T-maze or light–dark transition are frequently utilized. Highly aroused animals are predisposed to express less risk taking and to be fearful of exploratory behavior, both of which are considered high-anxiety phenotypes. In an elevated T-maze, highly aroused mice spend more time in closed arms, in which they feel safer, and are hesitant to enter open arm sections. Similar anxiety-related behaviors have been reported for light–dark transition tests. Highly aroused mice are predisposed to spend more time in dark sections and have a higher latency to first entry of the light section of the test box.

Effects of GA on emotional responsiveness are documented in human subjects as well. As one example, male subjects aroused via physical exercise or watching a comedy movie or a violent video were much more emotive with their opinions regarding affection toward a female performer. This type of illustration could be repeated in virtually all aspects of human behavior.

Neuroanatomical Components of CNS Arousal Systems Have Been Described

Multiple studies indicate that CNS arousal is mediated by diverse neuronal circuits with different neurotransmitter identities (Fig. 4). The diversity of arousal-related nuclei may serve multiple purposes, including redundancy against failure and fine tuning to behavioral needs. First, the separate origins and neurochemistry among different pathways should allow some pathways to survive and function even when others are compromised. All these systems can respond to different subsets of stimuli and distribute their excitation widely. Such organization is consistent with each of them serving the common goal of brain arousal.

Second, although these pathways have global effects across the brain, they are not identical. Their dominant regions of termination in the forebrain, and their greatest points of functional impact, provide opportunities for separate manipulation. For example, noradrenergic terminals are denser in the posterior regions of the cerebral cortex (except occipital) than in the anterior regions. Correspondingly, as the posterior cortical regions are more devoted to sensory processing than to motor behavior, noradrenergic pharmacology has been tied most closely to alertness and attention. In contrast, dopaminergic pathways travel more anterior toward the forebrain, terminating in regions tied to motor control and motivation. In this case, their neuropharmacology shows that they are crucial for voluntary behaviors toward salient stimuli. The emotional valences, and therefore behavioral leverage, of different ascending systems may also differ. For example, the ß-adrenergic ventral circuit from the locus coeruleus generates signals to report stressful stimuli to induce exploratory behavior and causes attention shift, whereas the dopamine system in the ventral tegmental area signals upon rewarding stimuli to induce

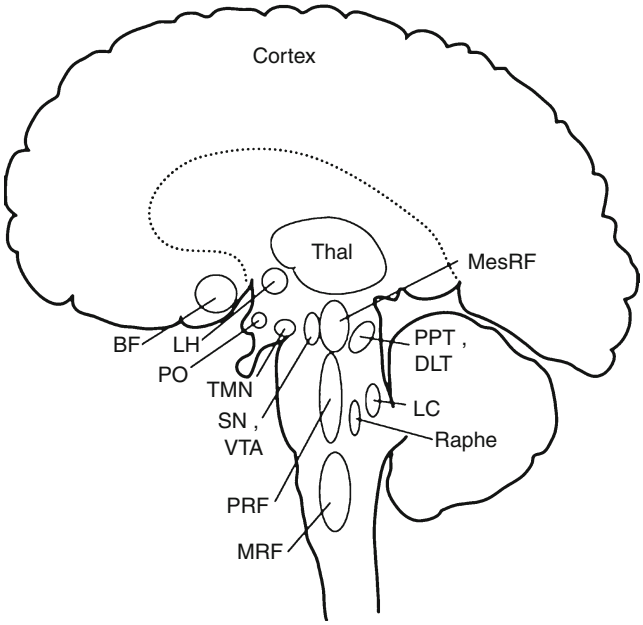

Fig. 4 The nuclei which predominantly drive changes in arousal state are clustered in the brainstem. Their projections tend to ascend toward the forebrain following either a low road to the basal forebrain (*BF*) or a high road toward the thalamus. Ultimately, both pathways modulate the activation of the cerebral cortex (Adapted from Jones 2008)

motivation and maintain vigilance. Likewise, control of mood and sensory responsiveness depends on serotonergic pathways ascending from the raphe nuclei at the brainstem.

Brainstem

As an evolutionary conserved region of the brain, the brainstem contains multiple nuclei to facilitate and regulate basic survival functions, such as respiratory and cardiac activities, as well as more complex functions like locomotion, consciousness, and sleep regulation. Along the brainstem, several of these nuclei interconnect to constitute ascending arousal systems via cholinergic, monoaminergic, and glutamatergic pathways through projections to the thalamus and basal forebrain (Fig. 5).

A "prime mover" in brainstem-arousal systems is found in the large reticular neurons of the nucleus gigantocellularis (NGC) in the medulla, just above the spinal cord (Fig. 6). These neurons have widespread ascending axonal projections, and they respond to a wide variety of sensory stimuli (Fig. 7). We are producing NGC-like neurons from embryonic stem cells (Tabansky et al., in progress) and are working to discover their normal developmental trajectories, which appear to include expression of the Nkx6.3 and Chx10 genes.

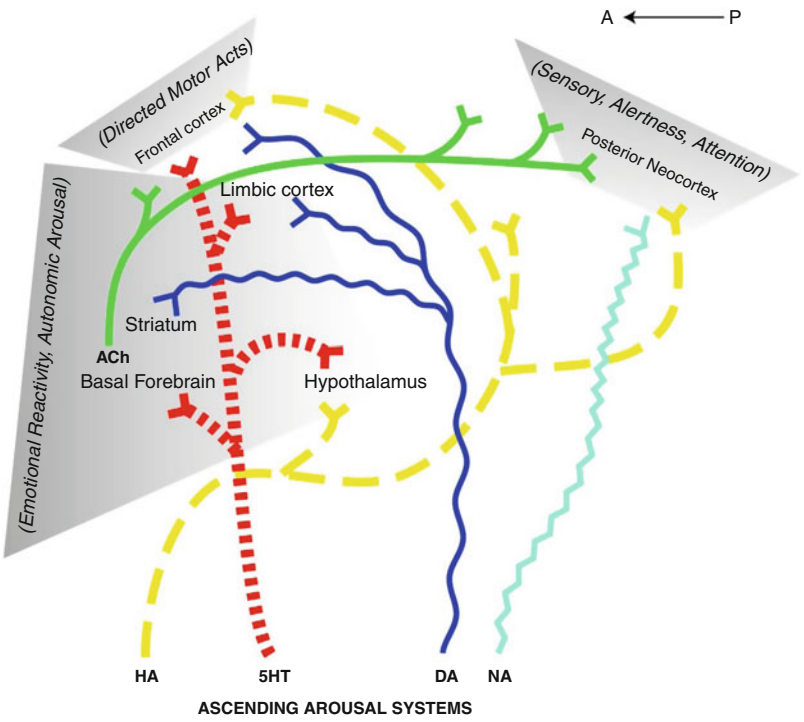

Fig. 5 Each brainstem system is biased toward unique efferent targets, which is functionally associated with that system impacting one aspect of arousal. Despite this, there is a wide overlap among the targets, which emphasizes the redundancy among these critical brainstem systems (Adapted from Pfaff 2006)

An important component of the ascending activation system consists of two cholinergic structures: the pedunculopontine nucleus and the laterodorsal tegmental nucleus. They are primary brainstem structures that project to the thalamus and induce thalamocortical activation. Neurons in these nuclei are most active during wakefulness and REMS, two states which are associated with high cortical activity. In contrast, the activity of these nuclei is significantly diminished during slow-wave sleep. They contribute to the more dorsal component, the "high road" to cortical arousal.

A second set within the ascending arousal system comprises many nuclei which project through the basal forebrain to activate cortical regions. This is the more ventral component, the "low road" to cortical arousal. These nuclei possess different neurotransmitter identities and connections, thereby imparting genetic and anatomical resilience to this arousal system. Monoaminergic neurons of the locus coeruleus, serotonergic neurons of the raphe nuclei, and dopaminergic neurons of the ventral periaqueductal gray are several of these nuclei which will be discussed in later sections.

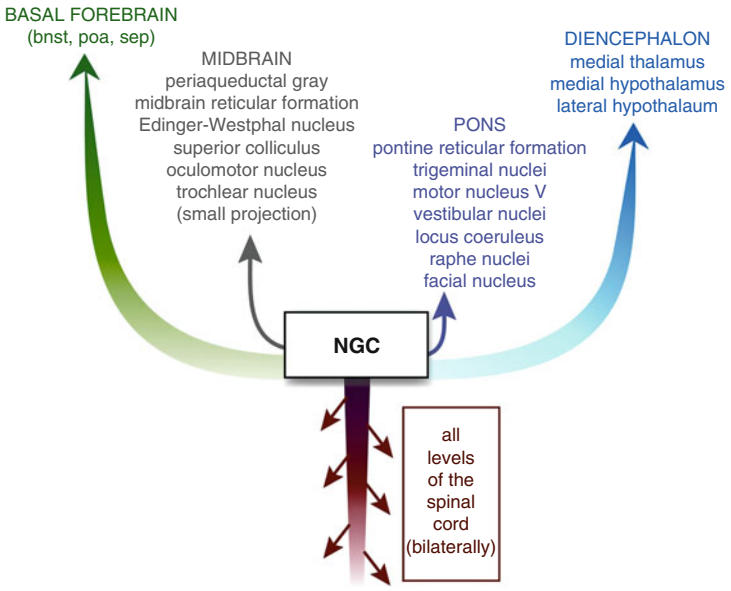

Fig. 6 Medullary reticular nucleus neurons have significant ascending and descending projections to arousal-related nuclei. These ascending projections reach regions of the midbrain reticular formation that are essential to consciousness, portions of the nonspecific thalamus, and basal forebrain sites important for modulating neocortical activity (Adapted from Pfaff et al. 2012)

Overall, the ascending activation system has an excitatory role over cortical function and involves multiple nuclei and circuits. These excitatory nuclei are regulated via reciprocal interaction with the ventrolateral preoptic area which is the primal actor in circadian rhythm.

Thalamus

Located at the center of the brain, the thalamus organizes widely distributed network activity across the brain. Integration and transmission of relevant information acquired from different regions of the brain is maintained via thalamocortical loops. Apart from inputs descending from the cortical regions, the central thalamus receives projections from the brainstem. The activity of the brainstem projections closely correlates with salient stimuli and rapid change in arousal levels. Neurons at the central thalamus are most active in conditions with high cognitive demand, stress, and other conditions associated with low hedonic state. Many nuclei within the central thalamus have an essential role on vigilance, alertness, attention, working memory, and motor preparation. Increased blood flow to several thalamic nuclei is strongly associated with high levels of arousal and behavioral performance for tasks requiring vigilance and sustained attention.

Widespread reciprocal connections of the central thalamus with other brain regions facilitate its role in multiple arousal pathways. For instance, the thalamus

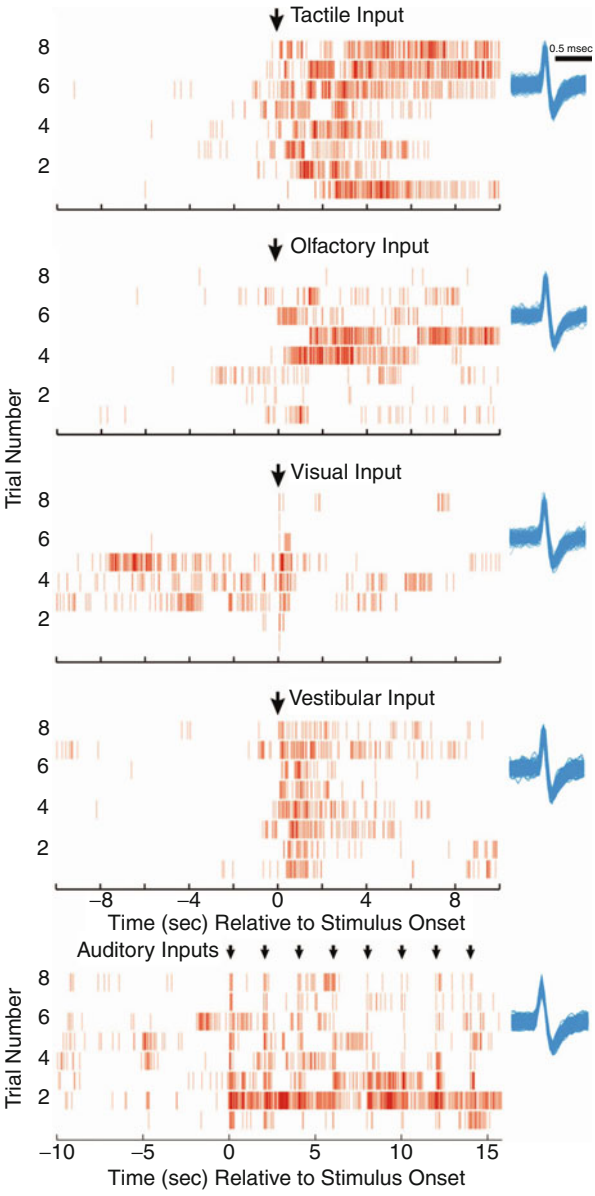

Fig. 7 Neurons in the medullary gigantocellular nucleus are hypothesized to be powerful drivers of state change in general arousal. A recording electrode was placed in the medullary gigantocellular nucleus of a rat and, and after the rat had been motionless and resting (negative time values), the rat was presented with stimuli from five different sensory modalities. Stimuli from each sensory modality were presented eight times (eight rows in each raster plot) and the stimulus was presented at approximately Time 0 (*black downward arrows*); in the auditory domain, stimuli were presented eight times in each of the eight rows (*black arrows*). Each hash mark (*red*) represents the occurrence of an action potential. Responses of this single neuron to tactile, olfactory, vestibular, and auditory stimuli were obvious. Responses to the visual stimulus were present, although the middle rows show some of the intrinsic firing variability of these neurons. An overlay of all of action potentials (*cyan*) for a given raster plot is shown to the *right* of each figure, showing that all action potentials were from the same neuron (Adapted from Martin et al. 2010)

has strong interactions with regions involved in planning and decision making such as frontal eye fields, the supplementary motor area, and the anterior cingulate cortex. It is also connected with the posterior cortical association areas to facilitate top-down decision making and polysensory integration.

The thalamus receives inputs from arousal-related brainstem nuclei. Cholinergic inputs from laterodorsal tegmental nucleus and pedunculopontine nucleus innervate

the thalamus along with norepinephrinergic inputs from locus coeruleus and glutamatergic inputs from midbrain reticular formation.

Hypothalamus

Located ventral to the thalamus, the hypothalamus is comprised of multiple nuclei and is involved in a broad range of functions. Along with its significant role in regulating CNS arousal, many more functional properties have been identified such as sexual dimorphism, feeding behavior, thermoregulation, and cardiovascular regulation. With respect to arousal, the hypothalamus contains nuclei which are reciprocally interacting with components of the ascending arousal system to tune arousal level and also regulate sleep–wake transitions.

The perifornical area of the lateral hypothalamus constitutes an important arousal regulatory region in the area. These neurons project to other hypothalamic nuclei, basal forebrain, and cholinergic neurons at the brainstem, as well as norepinephrinergic neurons at the locus coeruleus and histaminergic neurons of the tuberomammillary nucleus. Widespread connections of hypothalamic orexinergic neurons with various key arousal nuclei suggest that they have an important role in regulating generalized arousal.

The ventrolateral preoptic area (VPOA) is another important hypothalamic region with strong regulation over sleep and arousal. Sleep–wake cycles are primarily regulated via reciprocal interaction between VPOA, orexinergic neurons of the hypothalamus, and the excitatory nuclei at the brainstem. Neurons at the VPOA are most active during deep sleep and highly active during REMS.

Basal Forebrain

The basal forebrain is a cortical region located at the ventral part of the cortex, rostral to the hypothalamus. There are three major divisions of the basal forebrain: medial septum, diagonal band, and nucleus basalis. Along with serving as a gate between the ascending activation system and higher cortical areas, the basal forebrain mediates a broad range of cognitive functions such as episodic memory retrieval, attention, and execution. Age-onset cognitive deficits are associated with basal forebrain dysfunction.

Many different components of the arousal system use the basal forebrain to relay signals in order to communicate with the cortex. Most important are the cholinergic neurons that project to the cortex. Inputs to the basal forebrain include glutamatergic parabrachial, norepinephrinergic locus coeruleus, serotonergic raphe nucleus, and dopaminergic periaqueductal gray neurons from the brainstem. All such systems converge onto cholinergic, GABAergic, and glutamatergic neurons at the caudal basal forebrain.

Apart from having a broad range of incoming inputs, the basal forebrain synapses onto many different brain regions. Among the subdivisions of the basal

forebrain, the medial septum primarily projects to the hippocampus, whereas the diagonal band projects to multiple areas including the hippocampus, anterior cingulate cortex, and Broca's area. The nucleus basalis of the basal forebrain has excitatory projections to the neocortex and amygdala.

Behavioral States Comprise the Fundamental Measures of GA

High Brain Activity States

Wakefulness

Although wakefulness is not a unitary state, it is generally defined as the state when an animal can actively react to the environment. Wakefulness is electrophysiologically characterized as when an animal has increased cortical activation (fast oscillations) and increased postural muscle tone. Intracellular recordings from chronically implanted cats demonstrate that the first sign of transition from slow-wave sleep (SWS) to either wakefulness or rapid eye movement sleep (REMS) is the abolition of the long-lasting hyperpolarizing potentials in the thalamus and cortex. Upon waking, experiments with humans have shown that the transition to an aroused state is almost instantaneous, as determined by lack of disorientation, intact memory, and accuracy at performing tasks, while the transition to an attentive state, as determined by decreased reaction time, ranges in duration from minutes to hours (sleep inertia).

The abolition of long-lasting hyperpolarization at the cortex and thalamus is mediated, in part, by acetylcholine inputs from the brainstem and basal forebrain which decrease the hyperpolarizing potassium currents in the thalamus and cortex. This, in turn, changes the firing of thalamocortical neurons from slow rhythmic bursting activity to predominantly tonic firing, which is associated with fast oscillations and increased transmission of signals from the periphery. One of the consequences of the transition to tonic firing and fast oscillations is that it increases the rate by which the nervous system can sample the external world, thereby allowing quicker responses to a dynamic environment.

Alertness

Alertness is fundamentally a bottom-up attention-related process by which environmental stimuli trigger brainstem-arousal systems and eventually facilitates cortical excitability. An alert animal knows that an event has occurred and the central nervous system is grossly primed to filter and process that particular set of stimuli. Alertness and associated forebrain and cortical arousal are mediated by several ascending pathways with distinct neuronal components that project from the upper brainstem near the junction of the pons and the midbrain. One pathway innervates the thalamus, and the second extends into the posterior hypothalamus and forebrain. Key cell populations of the ascending arousal pathway include cholinergic, noradrenergic, serotoninergic, dopaminergic, and histaminergic neurons located in the pedunculopontine and laterodorsal tegmental nucleus, locus coeruleus (LC), dorsal

and median raphe nucleus, and tuberomammillary nucleus. Activity at the LC is the primary initiator of alertness. LC activity results in the release of noradrenaline at its target sites, including the frontal and parietal cortices. The LC shows high rates of tonic firing during alertness and exploratory behavior. This signaling increases the overall excitability and activation of high cortical areas, so information from all senses can be considered and filtered accordingly. An alerting response is often followed by an orienting response to the alerting stimulus.

Orientation

Orienting is usually thought of in the spatial domain and involves an animal focusing on a restricted area while relatively attenuating everything outside of that area. Functionally, orienting increases the signal-to-noise of the attended target. Events that occur within an oriented location are responded to more rapidly, are associated with greater neural activity, and can be reported at lower thresholds. The process of orienting requires disengagement from what the animal was attending to, changing the target, and maintenance on the new target. This activity is associated with the parietal lobe, the prefrontal cortical regions, and the pulvinar nucleus, and the response is modulated by acetylcholine.

Cholinergic projections from the basal forebrain are likely key contributors to acetylcholine function during orienting. The cholinergic projections of the basal forebrain inhibit the thalamic reticular nucleus, by which, in turn, these cholinergic projections serve indirectly to disinhibit the thalamus. Likewise, axons projecting from the basal forebrain may also synapse at muscarinic receptors on inhibitory cortical interneurons, thereby regionally disinhibiting cortical projection neurons.

Attention

Attention refers to the moment-to-moment optimizing of CNS processing to fit the demands of a situation. The adjustment of activity across cortical structures is controlled by bottom-up brainstem reticular core activity and top-down signals from frontal regions involved in monitoring demands on effort and vigilance. Although there is no universal physiological signature of attention, it is associated with increases in high-frequency oscillatory activity in the 20–80 Hz range of the EEG. One of the basic foundations of attention is that an animal has limited processing capacity at any one time and, due to homeostatic cell mechanisms, cannot use any one processing system indefinitely. As such, the role of attentional systems is to modulate when the brain is at heightened activity and which processing systems are activated. Cognitive studies have shown that increasing activity within one processing system produces widespread interference with most other cognitive operations. Correspondingly, functional magnetic resonance imaging studies have shown that during tasks that require a high attentional load, unattended salient stimuli are not represented at higher cortical levels.

While it is known that attention is not a unitary phenomenon, the categorizing of attention mechanisms into different domains, and the biological processes supporting each domain, is still being debated. Studies have revealed that constitution and

maintenance of attention are strongly regulated by norepinephrinergic system. Two modes of activity have been observed in locus coeruleus (LC) neurons, a nucleus which mediates the trade-off between alertness and attention. Phasic firing of the LC is associated with decision making, attention, and vigilance toward salient sets of stimuli. Phasic to tonic firing is observed during disengagement and explorative behavior. As salience of stimuli wanes (either because of satiety or removal of reward), the firing pattern of the LC also shifts from phasic to tonic. The Yerkes–Dodson law states that maximum performance is achieved with intermediate levels of arousal; insufficient arousal will fail to initiate behavior, while excessive arousal will cause distraction. Balance between different states of arousal and constitution of attention appear to be mediated by the firing pattern of the LC. These firing patterns are being modulated by forebrain executive systems which are predominantly affected by dopamine. Two frontal nuclei, anterior cingulate cortex and orbitofrontal cortex, are the main structures mediating the firing pattern of LC neurons. Orbitofrontal cortex mainly predicts positive valance of the stimulus. Its activation predicts hedonic value of expected reward and therefore has a strong effect on regulating motivation and voluntary behavior. The anterior cingulate cortex however codes for negative valance, and its activity predicts the level of expected burden. The anterior cingulate cortex is recruited by a diverse range of cognitive demands and is activated with increasing cognitive load across a wide variety of tasks. Combinatorial effects of these executive structures on LC firing pattern determine whether to attentively engage in particular task to maximize exploitation or disengage from any task and began exploration for salient stimuli.

Low Brain Activity States

Slow-Wave Sleep

Electrophysiologically, slow-wave sleep (SWS) is defined by the presence of slow oscillations (0.5–1 Hz), delta activity (1–4 Hz), and spindles (7–15 Hz), with the latter two rhythms often superimposed on the slow oscillations. The different oscillations are the product of both intrinsic channel dynamics at the cellular level and synaptic interactions among the thalamus, the reticular nucleus of the thalamus (RE), and the cortex at the network level. While the generation of each rhythm is beyond the scope of this chapter, each rhythm is associated with increased hyperpolarization at the thalamic level and long hyperpolarizations followed by long depolarizations (during the slow rhythm) at the cortical level. Hyperpolarization at the thalamus is secondary to cessation of activation from brainstem-arousal systems and to prolonged bursting by afferent GABAergic RE neurons, with the latter mechanism being the most pronounced during RE-generated spindle rhythms. This hyperpolarization largely prevents any sensory signals from relaying through the thalamus from the periphery. At the cortical level, neurons do not fire action potentials during the hyperpolarization phase of the slow oscillation. In contrast, during the depolarization phase, cortical neurons can fire at gamma frequencies (20–80 Hz).

Drowsiness/Somnolence

The state of drowsiness reflects decreased transmission of peripheral signals from the thalamus to the cortex. It is associated with increases in bursting activity and prolonged hyperpolarization, in isolated thalamocortical neuron populations. As such, drowsiness can be considered a sleeplike state within local neuronal assemblies. Accordingly, local neuronal circuits may be under homeostatic modulation in a use-dependent manner.

Anesthesia

General anesthesia is a drug-induced reversible condition which defines a range of behavioral and physiological states associated with decrease or absence of voluntary movement and sensory responsiveness, as well as with steadiness of autonomic processes such as heartbeat, respiration, and thermoregulation. Low-frequency, high-amplitude activity progressively increases as the patient is induced into a deeper anesthetic state.

Anesthesia is frequently induced by drugs which elevate the inhibitory GABA signaling in the brain. Administration of small dose of drugs such as propofol, barbiturate, or etomidate induces the anesthesia process. These molecules bind to GABA receptors and increase inhibitory signaling throughout the brain. Additionally they block AMPA receptors which are crucial factors in excitatory glutamatergic signaling and potassium channels. As the dose increases, the patient advances into paradoxical activation, which is associated with purposeless movements and euphoria–dysphoria along with decreased cerebellar metabolism. As the treatment increases, the patient begins to lose signs of consciousness such as response to oral commands, eye tracking, sensory responsiveness, reflexes, and muscle tone. In this stage, a patient's EEG pattern is similar to the comatose/vegetative state, and metabolic activity in the brain is severely decreased.

Several Genes and Neurochemical Systems Drive CNS Arousal

Each neurochemical system that contributes to the regulation of elementary CNS arousal implies at least four types of genetic contributions: those genes whose products synthesize the neurotransmitter, genes for receptors, genes for reuptake, and genes whose products break down that neurotransmitter. Overall, it is important to understand that there is no single molecular neurobiological system responsible for the activation of behavior, but multiple and functionally overlapping systems.

Brainstem systems that control CNS arousal and behavior tend to ascend toward the forebrain following either a "low road" or a "high road." The low road system comprises the ventral pathways – which are the evolutionarily older of the two pathways – that impact (at least) the cholinergic neurons of the basal forebrain. These neurons, in turn, release acetylcholine in the cerebral cortex. The "high road" system comprises the dorsal pathways – which are more recently evolved among vertebrates and especially well developed among primates – that impact the

nonspecific thalamic nuclei. Brainstem systems can be distinguished by their efferent targets although they show significant overlap. The systems that drive changes in arousal are best organized according to the predominant neurotransmitter synthesized in each nucleus.

Noradrenaline/Norepinephrine

There are seven distinct nuclei that synthesize noradrenaline (NA) with the locus coeruleus (LC) being the largest and most crucial in terms of regulating arousal. NA neurons are the most active during wakefulness, especially during active wakefulness, show low activity during SWS, and cease firing during REMS. Activity of NA neurons serves to depolarize systems involved in waking and to hyperpolarize systems involved in promoting sleep. Likewise, drugs that inhibit NA action facilitate sleep onset. Noradrenergic neurons send substantial projections to the frontal and parietal cortices and are widely interpreted as supporting sensory alertness, while projections to the other forebrain structures, including the amygdala, help control cardiovascular, visceral, and neuroendocrine functions. Furthermore, NA neurons send diffuse projections to the spinal cord, where they can modulate motoric processes (Fig. 8). This fact feeds the idea that hindbrain cell groups supporting arousal are not simply divided into those that just ascend and those that just descend – some cell groups project to both forebrain and spinal cord nuclei. Returning to the operational definition of general CNS arousal, NA can influence sensory, motor, and emotional responsiveness. Amphetamines, which act at noradrenergic and dopaminergic synapses, likely have their arousing effects via the noradrenergic and

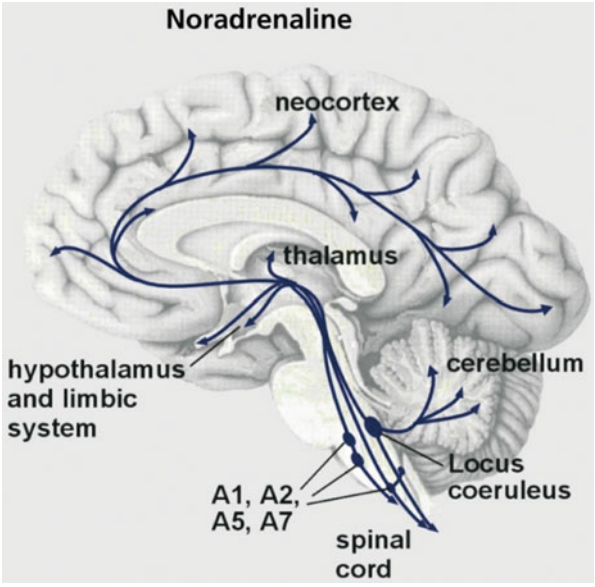

Fig. 8 Noradrenergic neurons project from the brainstem to multiple cortical and limbic regions along with the thalamus, cerebellum, and spinal cord. These projections are responsible from mediating arousal, attention, and mood. Motor responses as well are partially regulated via projections descending to the spinal cord (Adopted from Fuchs and Flugge 2004)

dopaminergic systems' diffuse stimulation of the frontal cortex; in contrast, the rewarding effects of amphetamines are likely linked to noradrenergic synapses in the nucleus accumbens. Despite the clear links between arousal and the NA, chemical lesioning of the NA does not result in coma; there are redundant arousal systems.

By and large, sensory afferents do not project directly to the LC. The inputs which must account for the LC's integrative powers are those from the medullary and pontine reticular formation in the hindbrain. These regions integrate inputs from a variety of sensory modalities in a generalized fashion and feed the LC as a nodal point in ascending arousal circuitry. Among them, stress-related and autonomic-related inputs must be of special importance. Transmitters involved include noradrenaline itself, serotonin, and excitatory amino acids such as glutamate. Importantly, not all of the inputs ascend from the periphery toward the forebrain. The LC also receives descending inputs from regions involved in sleep and other circadian behaviors, including a small preoptic area devoted to sleep, the dorsomedial hypothalamus, and the suprachiasmatic nucleus. It also receives descending inputs from limbic-associated regions including the amygdala and the bed nucleus of the stria terminalis.

Neurons of the LC have been shown to have different firing patterns which give rise to various behavioral outcomes. Phasic LC activation is observed due to task-related decision processes and facilitates top-down goal-oriented behaviors and to help optimize task performance. Whereas, tonic activation of LC neurons is associated with disengagement from the ongoing task due to loss of perceived valance and subsequent engaging in explorative behavior. Maximal discharge of LC neurons is associated with highly aroused conditions, including stress. Correspondingly, genetic ablation of the LC showed a decrease in anxiety in mice.

Examples of how arousal mechanisms can have valences assigned to their eventual behavioral impacts come from the actions of adrenergic transmitters in the forebrain. Adrenaline and noradrenaline participate in generalized arousal of the CNS. However, as Stone et al. have pointed out, α-1 adrenoreceptors function both in positively motivated approach activities and in behavioral inhibition. The ability for adrenergic transmitters to evoke seemingly opposite behaviors is based on the regional activity of the transmitter. Adrenoreceptors mediating adrenergic effects at neuroanatomic sites such as the lateral hypothalamic area, the nucleus accumbens, and the piriform cortex activate positive, approach, behavioral explorations. However, those receptors in sites such as the paraventricular hypothalamus and the central nucleus of the amygdala are associated with fear, stress, immobility, and signs of depression.

Acetylcholine

Brainstem cholinergic neurons are most active during REMS and strongly active during wakefulness, particularly during attentive behaviors. Acetylcholine (ACh) has differential effects on state depending on the physiological context: in the presence of monoamines (DA, NA, 5-HT) and orexin, it leads to attention; in the absence of monoamines, it leads to REMS. Functionally, ACh is associated with

cortical activation, heightened attention, and heightened sensory-motor processing, particularly during taxing conditions (attentional effort). Compared to monoaminergic compounds, acetylcholine has very strong effects on thalamocortical neurons and hence is critical in effecting CNS arousal state. Nerve cells which synthesize acetylcholine are found in three major regions: the pedunculopontine and dorsolateral nuclei of the tegmentum (PPT and DLT), which are located in the brainstem at the border between the pons and midbrain and in the basal forebrain. Through the combined actions of the PPT, DLT, and basal forebrain, ACh affects behaviors associated with arousal through both cortical and limbic activation. In the latter, ACh projections are spread out across the magnocellular preoptic nucleus, the diagonal bands, and the septum. Acetylcholine neurons receive substantial inputs from glutamatergic, cholinergic, noradrenergic, dopaminergic, and histaminergic ascending arousal system neurons.

Acetylcholine-producing neurons in the PPT and DLT project to the thalamus, and to a lesser extent, to the lateral hypothalamus and basal forebrain. Activation of these neurons makes thalamic neurons more sensitive to sensory information and activates the cortical EEG. Bilateral destruction of the PPT and DLT in humans, with neighboring areas damaged following mechanical or vascular accidents, produces a vegetative or comatose state. The functional significance of having two widely separated cell groups producing ACh – in the brainstem and in the basal forebrain, both of which are crucial for arousal – is still unknown.

Dopamine

Neurons that produce dopamine (DA) do not show changes in *mean* firing rate across SWS, wakefulness, and REMS, although they do show peak, burst firing to rewarding conditions, and salient stimuli during wakefulness. While the mean firing rates of DA neurons are stable across states, the patterns of firing of DA neurons are important: tonic activity preferentially activates D2 receptors and phasic activity activates D1 receptors. As the biophysical effects of these receptors at the cellular level can oppose each other, bursting of DA neurons during wakefulness may be critical to active waking behaviors. Dopaminergic axons course from the brainstem toward the forebrain via two predominant routes: From the substantia nigra, they ascend to innervate the striatum, a non-cortical motor control region of the forebrain that also has a broader role in general arousal. A second DA system arises in a loosely formed cell group in the midbrain called the ventral tegmental area (VTA). These DA neurons innervate the phylogenetically ancient cortex called the limbic system, which is known to be important for controls over motivational states and moods.

While the DA system has historically been linked to the phenomenon of reward, recent studies suggest that the system may have broader functionality. A strong line of research from Jon Horvitz at Boston College demonstrated that the salience of stimuli would be the critical requirement for activating DA neurons rather than reward. Put another way, the reward value of a stimulus is just one way for that

stimulus to gain salience. Destroying DA systems markedly slows responses to salient stimuli and leads to the omissions of responses. Continuing on this theme, DA neurons are not necessarily sensitive to reward itself but instead seem to signal anticipations and predictions of future rewarding events. Fluctuations of dopamine levels in nucleus accumbens during rewarded acts are consistent with the following new point of view: DA projections to nucleus accumbens signal excitement and arousal, not simply reward.

When DA is virtually obliterated in mice, these mice show profound changes in behavior. They do not explore either new environments or other mice, and they rarely initiate behaviors. Dramatically, these mice, despite being able to move and having palatable food available, will starve due to decreased feeding behavior. This effect has been localized to DA inputs to the striatum, where it is thought to facilitate glutamatergic signaling from the cortex. In contrast, when the concentration of DA in the synaptic cleft is experimentally elevated, the effected animals have remarkable behavioral hyperactivity in novel environments, making them act similarly to animals on psychostimulants.

The axonal trajectories of DA neurons can be distinguished functionally from those of NA neurons by DA neurons' tendency to synapse in more anterior regions of the cerebral cortex, which are cortical regions that are associated with motor activity. This can be contrasted to more posterior (except for occipital cortex) trajectories of NA axons, which are associated with sensory processing. Notably, some of these DA axons reach the prefrontal cortex where they synapse on neurons which coordinate the left and right sides of the frontal cortex. This is important, in part, because the two sides of the frontal cortex have opposite effects on arousal and mood: heightened activity on the right side is associated with unpleasant feelings in humans, while heightened activity on the left side is associated with positive feelings. Some of these prefrontal cortical neurons project back to the VTA, emphasizing the bipolar, bidirectional feature of arousal systems.

Hypocretin

Hypocretin (also called orexin) refers to a recently discovered set of gene products that are associated with modulating locomotor activity, feeding, and energy homeostasis and have dramatic modulatory effects on behavioral arousal, including CNS arousal and increased muscle tone. Neurons which produce hypocretin are located in the perifornical region of the lateral hypothalamus and in the dorsomedial hypothalamus. While the inputs to the perifornical region of the lateral hypothalamus and dorsomedial hypothalamus have not been fully worked out, these regions receive projections from other hypothalamic nuclei, the dorsal raphe nuclei, the cholinergic A2 group, and the solitary tract nucleus. Both circadian and homeostatic factors regulate the release of hypocretin. Hypocretin neurons project broadly through the nervous system, including projections to the cortex, spinal cord, VTA, dorsal raphe nuclei, substantia nigra, LC, amygdala, and medullary reticular formation. Hypocretin has solely excitatory influences on histaminergic neurons that

are most active during an animal's waking state. Indeed, the firing of orexin-producing neurons may anticipate wakefulness in a sleeping animal by a few seconds. Likewise, delivery of orexin into an animal's nervous system, either intravenously or intraventricularly, increases behaviors associated with wakefulness; in contrast, knocking out orexin in mice leads to narcolepsy.

In vitro studies show that hypocretin-producing neurons can have sustained activity in the absence of synaptic inputs – that is, they would need to be inhibited to cease firing – which can functionally provide these neurons with the ability to maintain an animal's waking state. Hypocretin also interacts with ACh at the cellular level in the brainstem to potentially maintain the integrity of behavioral state. As such, combined ACh and hypocretin activity leads to attention with muscle tone, while ACh alone leads to cortical activation with atonia.

Serotonin

The raphe nuclei of the brainstem are the principal source of serotonin (5-HT) in the CNS. Neurons that produce serotonin are most active during wakefulness, have low activity during SWS, and are quiet during REMS. These serotonergic neurons project to the hypothalamus and to several regions of the limbic system, including the hippocampus, septum, and amygdala. Serotonergic projections from raphe nuclei are strongest in thalamic nuclei that are associated with the limbic system. Serotonergic connections to the cortex are widespread and include the olfactory cortex, the olfactory bulb, and the cortex.

Nerve cells in the raphe nuclei receive significant ascending afferents from adrenergic neurons in the lower brainstem as well as from neurons expressing peptides related to arousal, such as orexin. The raphe nuclei also receive descending inputs from the forebrain, including influences from the limbic system and hypothalamus, and even from the pineal gland. Despite being released at increased rates during wakefulness, serotonin attenuates cortical activation through inhibitory actions on brainstem-arousal systems, including the acetylcholine system. Increased 5-HT concentrations in the CNS are associated with decreases in reactivity to sensory stimulation and with decreased motivation and voluntary behavior. Stimulation of the raphe nuclei causes the cessation of active waking behaviors, such as eating or sexual activity. We can speculate that serotonin signaling is primarily associated with wakeful high hedonic state where the animal has to be awake to experience pleasure, yet not need to behave in order to reach higher hedonic state. According to the operational definition of arousal, serotonin potentiates a low-arousal state, from this point. On the other hand, serotonin is closely linked with disorders such as lethargy and depression which are associated with significant decreases in motivated behaviors. Treatment of these conditions often involves the use of selective serotonin reuptake inhibitors to enhance serotonin signaling throughout the brain to alleviate symptoms. From this point of view, serotonin appears to have a positive function toward increasing arousal. One possible conjecture is that optimal levels of serotonin signaling is essential to

maintain a low activity arousal state, while excessive or insufficient levels of serotonin would result in decreased arousal.

The diversity of molecular structures and expression of the 14 genes coding for serotonin receptors tells us that the logic of their cellular functions will be quite complex. For example, while serotonin can depolarize neocortical cells and enhance their excitability consistent with neocortical arousal, the effects of serotonin at the single neuron level are varied dependent upon the receiving cell. Recording from dissociated prefrontal cortical pyramidal neurons, Carr and Surmeier found that serotonin could inhibit sodium currents in a manner dependent on phospholipase C and protein kinase C, which would reduce excitability.

Glutamate/GABA

Glutamate and gamma-aminobutyric acid (GABA) are, respectively, the predominant excitatory and inhibitory neurotransmitters in the nervous system, and each neurotransmitter has receptors distributed throughout the CNS. As depolarization and hyperpolarization are critical features of CNS arousal, these neurotransmitters play important roles in determining an animal's state of arousal and could be considered the backbone of the entire arousal system. For example, one of the mechanisms by which general anesthetics induce unconsciousness is by decreasing activity at glutamatergic receptors (e.g., ketamine) or increasing activity at GABAergic receptors (e.g., barbiturates).

The midbrain reticular core is the most effective activating system and large lesions of this area lead to coma. The midbrain reticular core is, in turn, driven by the pontine and medullary reticular cores. The excitatory effects of all of these areas are predominantly mediated by glutamate. One important group of these glutamatergic neurons, the medullary gigantocellular reticular nucleus (mGi), is currently under investigation as having possible "master cells" of general arousal. The mGi is in a position to integrate both ascending sensory information from the periphery and descending information from the forebrain. Electrophysiologically, these neurons increase their firing rate to inputs from multiple sensory modalities and, with regard to tactual inputs, from multiple body regions. In turn, mGi neurons project to arousal-related targets including the basal forebrain, locus coeruleus, midbrain reticular core, and intralaminar nuclei of the thalamus. Their activity has been associated with increased muscle tone and with changes in EEG from slow to fast oscillations.

Two examples of important GABAergic neurons are the projection neurons of the preoptic nucleus and the basal forebrain. The preoptic nucleus receives information about an animal's circadian rhythm from the suprachiasmatic nucleus (whose activity has a wake-promoting effect) and is activated by homeostatic factors, including adenosine (see below). It is a sleep-promoting nucleus that uses GABA to inhibit wake-promoting nuclei, specifically the locus coeruleus, tuberomammillary nucleus, and raphe nuclei. In turn, the preoptic nucleus is inhibited by NA and ACh.

In the basal forebrain, GABAergic neurons are interleaved with ACh neurons, with each hypothesized to have opposing effects. While NA depolarizes the ACh population, it hyperpolarizes the GABAergic population. Interestingly, while the basal forebrain GABAergic neurons are sleep active and can promote sleep, they also receive strong excitatory input from the cortex – this circuitry is hypothesized to mediate top-down attentional mechanisms during taxing situations.

Neuropeptide S

Neuropeptide S is recently identified as an endogenous ligand of an orphan G protein-coupled receptor which is now referred to as neuropeptide S receptor (NPSR). When triggered, NPSR increases intracellular Ca + 2 levels proportional to the concentration of neuropeptide S. In addition to CNS, neuropeptide S and its receptor are expressed in various tissues such as thyroid, salivary, and mammary glands. However, its level of expression in the brain is significantly higher compared to peripheral tissues.

Neuropeptide S is expressed discretely in several arousal-related brain regions. The strongest neuropeptide S expression is in the locus coeruleus area, sensory 5 nucleus, and lateral parabrachial nucleus. Moderate amounts of neuropeptide S are also produced in the dorsomedial hypothalamic nucleus and the amygdala. Unlike neuropeptide S, NPSR expression is widely distributed throughout multiple brain regions. NPSR is abundantly expressed in the anterior olfactory nucleus, dorsoventral endopiriform nucleus, amygdala, precommissural nucleus, paraventricular thalamic nucleus, and subiculum. A lower level of NPSR expression is also reported at the somatosensory cortex, hypothalamus, pons, and medulla. Significant amount of NPRS production is also found in multiple thalamic nuclei that relay signals from the reticular formation to diffuse cortical fields to modulate arousal.

Neuropeptide S possesses paradoxical effects on modulating states of arousal. Neuropeptide S potently modulates wakefulness, motor behavior, and anxiety. Central administration of neuropeptide S increases locomotor activity in mice and decreases REMS and SWS in rats. Its administration restricted to ventral tegmental area also result in hypermotor activity. Such administration also attenuates sleep-promoting effects of inhibitory molecules such as diazepam. It is also further shown to produce anxiolytic effects in mice exposed to different stressful paradigms. Mice treated with neuropeptide S are predisposed to express less anxious phenotypes in the elevated T-maze, dark–light transition, and open field tests. Neuropeptide S also evokes anxiolytic effects by facilitating extinction of conditioned fear responses when administered into the amygdala.

Overall, based on the operational definition of arousal, neuropeptide S has paradoxical effects. On one hand, it increases predisposition to express motor behavior and improve wakefulness. On the other hand, neuropeptide S decreases emotional reactivity and serves as an anxiolytic agent.

Histamine

Histamine (HA) is synthesized in the tuberomammillary nucleus of the posterior thalamus and influences arousal and the sleep–wake cycle. Histaminergic neurons are likely most active during waking, quiet during SWS, and cease their firing during REMS; inhibition of these neurons leads to decreases in wakefulness. The tuberomammillary nucleus receives multiple inputs from brainstem-arousal systems. It sends diffuse, widespread projections to many brain areas, including the cortex, basal forebrain, and brainstem-arousal systems (Fig. 9). By acting through the H1 and H2 receptors, HA neurons excite cholinergic neurons of the basal forebrain, which, in turn, activate the cortex. Additionally, the reciprocal innervation between HA neurons and the sleep-associated ventrolateral preoptic area should help to enforce rapid and powerful state changes between sleep and wakefulness.

Behaviorally, decreasing activity at the H1 receptor has been shown to reduce responsiveness of female mice to external stimuli. Electrophysiologically, the histaminergic system strengthens CNS transmission of afferent, sensory signaling. Despite the above relationships between histamine signaling and general CNS arousal, our understanding of the mechanisms involved in HA's behavioral effects is still quite incomplete. We know that HA can depolarize neurons, making them more able to respond to afferent information, but, compared to the large amount of data on H1 receptors, we know little about H2 and H3 receptor actions. Finally, wiping out HA by itself does not put an animal to sleep. This underlines a main point of this section: redundancy among the neurochemical mechanisms serving arousal prevents system failure.

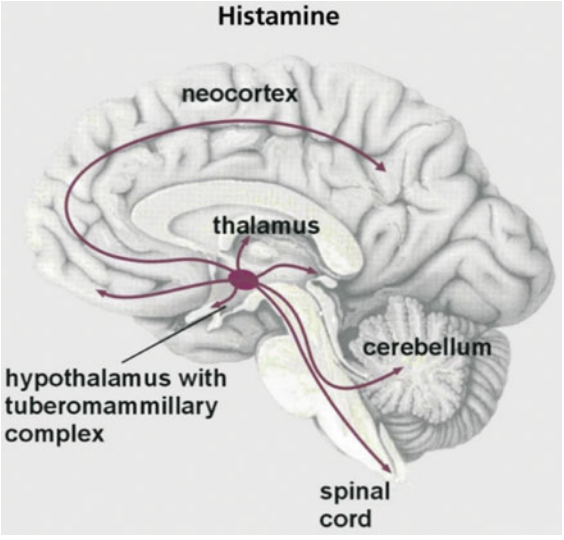

Fig. 9 Histaminergic neurons originate from tuberomammillary complex of the hypothalamus and project to multiple regions of the brain including the cortex, basal forebrain, and cerebellum. Histaminergic signaling is crucial for arousal and sensory responsiveness (Adopted from Fuchs and Flugge 2004)

Adenosine

Adenosine is a metabolite of ATP use and serves as a homeostatic regulator of cell function. As it is related to cell function, it is globally present throughout the brain, and increased concentrations of adenosine have been shown to have sleep-promoting effects. Caffeine, in part, has its wake-promoting effects by inhibiting adenosine 2A receptors. While infusion of adenosine into the basal ganglia results in the onset of sleep, it may also be able to invoke sleep at the level of the hypothalamus and cortex. New theories in neuroscience suggest that adenosine may work at the local level to put isolated populations of neurons into a sleeplike state, as may be apparent during drowsiness.

Redundancy in Genomic Controls Prevents System Failure

A great multiplicity of genes is involved in producing CNS arousal signals. Likewise, these are genes through which CNS arousal can be regulated, by influencing their transcription rate and efficiency. How is the large number of genes arrived at? Consider all of the neurochemicals mentioned in the last few pages. As mentioned, each of them has (a) gene products necessary for their synthesis, (b) gene products encoding their receptors, (c) gene products encoding their transporters, and (d) gene products responsible for their regulated chemical breakdown. Therefore, the numbers of all of the neurotransmitters and neuropeptides we have identified here (plus the others we did not mention for the sake of simplicity and brevity) must be multiplied by at least 4, to begin to estimate the variety of genetic influences.

The diversity of arousal-related nuclei may serve multiple purposes. First, the separate origins and neurochemistries of the different pathways should allow some pathways to survive and function even when others are compromised. The fact that all these systems can respond to many forms of stimuli, and distribute their excitation widely, is consistent with each of them serving the common goal of brain arousal.

Second, even as these pathways have generalized features, they are not identical. Their dominant regions of termination in the forebrain, and their greatest points of functional impact, provide opportunities for separate manipulation. Noradrenergic terminals are denser in the posterior regions of the cerebral cortex (except occipital) than in the anterior regions. Correspondingly, as the posterior cortical regions are more devoted to sensory processing than to motor acts, noradrenergic pharmacology has been tied most closely to alertness. In contrast, DA pathways travel more anteriorly toward the forebrain, terminating in regions convincingly tied to motor control. Correspondingly, their neuropharmacology shows them crucial for motor acts directed toward salient stimuli. The emotional valences of different ascending systems may also differ: For example, the NA system tends to signal stressful

stimuli, the DA system tends to signal positive rewards, and 5-HT function has been implicated in the control of mood.

Third, even though pathways which ascend toward the forebrain have been emphasized here, some very important controls over arousal begin in the basal forebrain and descend. An excellent example is the small group of GABA neurons in the ventrolateral preoptic area (vlPOA). Clifford Saper and his lab at Harvard Medical School have used a wide variety of experimental approaches to demonstrate that these neurons are important for normal sleep to occur. Another example is the suprachiasmatic nucleus (SCN) of the hypothalamus, discovered as a biological clock in mammals by lesion studies. Now, the "nonimage-forming visual system" emanating from the SCN is understood to influence many biological rhythms, including daily changes in arousal.

Specific Example: Elementary Arousal and Sex Behavior

In the literature on mechanisms of motivation, it is clear that the occurrence and forcefulness of any motivated behavior depends on arousal. This section relates how elementary CNS arousal is related to a specific behavior: mating. Using the genomic and biophysical mechanisms of mating as background, we will explore potential "trading relations" between a specific form of arousal (sexual) and generalized arousal.

To approach this issue conceptually, suppose you have an animal that lacks sex hormones situated in a well-controlled experimental environment. You supply the experimental animal with a stimulus animal of the opposite sex for mating and nothing happens. The test animal does not mate. Then, suppose you inject the test animal with an appropriate sex steroid hormone, and retest using the same controlled environment, the same time of day, the same age of test animal, and the same stimulus animal. This time, the test animal mates. In the logical equations which describe behavior, the stimulus and the response have been held constant. Therefore, the sex steroid hormone must have altered another variable term in the equation. That variable is called "sexual motivation."

Even in relatively simple mammals such as mice, motivation to mate plays out in a series of courtship responses. Given an adequate environment, the female will initiate unusually fast and directed locomotion to orchestrate her contact with the male. In fact, an entire series of communicative and locomotor behaviors culminate in approach responses. Social investigation and affiliative behaviors are increased while aggression is decreased. In the female mammal, these courtship and locomotor behaviors depend very much on circulating estrogen levels working through the ER-α receptor. More generally, a wide variety of behavioral assays shows that a female animal's sexual motivation is estrogen dependent. In reference to locomotion, estradiol (which is the predominant molecular form of estrogen) enters the brain and is bound by neurons in the preoptic area, where it excites electrical activity in neurons associated with both locomotor circuits and the initiation of courtship behaviors by female animals.

Effects of elevated generalized arousal on sexual arousal. In both female and male experimental animals, neurochemical manipulations which mimic ascending arousal systems increase sexual behavior. Administering NA to female rats increases the primary female sex behavior, lordosis, and also mediates the ovulatory surge of luteinizing hormone (LH) from the pituitary. Thus, it helps to synchronize the female's sexual arousal with her endocrine preparations for reproduction. Conversely, depleting hypothalamic NA abolishes lordosis behavior and disrupts the LH surge. Noradrenaline's effects in the hypothalamus work through adrenergic α-1b receptors. They originate with NA cell bodies in the lower brainstem, following the "low road" (e.g., from locus coeruleus), and increase activity of both ventromedial hypothalamic cells that control lordosis and preoptic cells which control the locomotion associated with courtship behaviors. Reducing ventromedial hypothalamic neuronal electrical activity, for example, by using a high dose of a selective mu-opioid receptor agonist, correspondingly reduces lordosis.

Other arousal-related transmitters that influence sex behavior are histamine and norepinephrine. These neurotransmitters act by increasing activity in the hypothalamic cells that control the lordosis behavior circuit (Fig. 10). Additionally, acetylcholine biochemistry ties into the mechanisms by which estrogens facilitate female sex behavior. Estradiol treatment increases the activity of the enzymes required for ACh biosynthesis and also upregulates the expression of ACh receptors. Thus, in females, neurotransmitters that increase generalized arousal also increase sex behavior.

In regard to male sex behavior, the focus shifts to the neurotransmitter DA and from the ventromedial hypothalamus to the medial preoptic area. Dopamine increases male sex behavior through at least three functional roles: it increases sexual arousal and the courtship behavior, it potentiates the motor acts of mounting behavior, and it facilitates genital responses to stimulation. Testosterone promotes

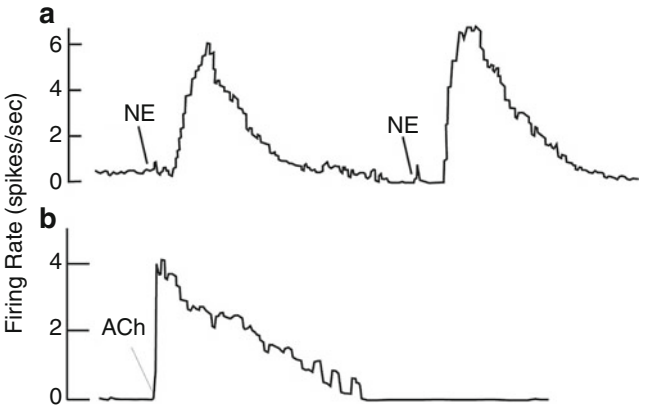

Fig. 10 Neurotransmitters which signal general arousal can elevate electrical activity in ventromedial hypothalamic neurons responsible for sexual arousal and sexual behavior. Recordings here are from ventromedial hypothalamic neurons in female rats in response to (**a**) noradrenaline and (**b**) acetylcholine (Adapted from Kow and Pfaff 1987)

DA release in the forebrain, and this release, in turn, is timed to coincide with actual mating behavior of the male. The impact of generalized arousal on sexual arousal is underscored when considering that erections and ejaculations require complex activation of both the parasympathetic nervous system and the sympathetic nervous system, also intimately involved in CNS arousal. In fact, the paraventricular nucleus, which is a hypothalamic cell group crucial for autonomic nervous system control, shows increased DA concentrations during male sexual activity. Other arousal-related neurotransmitters also tied to male sexual performance include NA, glutamate, and orexin. For example, microinjecting orexin into the preoptic area of male rats potentiates their sex behavior. These close relations between generalized arousal and sexual arousal are not limited to experimental animals. In heterosexual men, sexual interest is strongly suppressed during depressed mood states. Further, in women and men, drugs used to treat decreased libido include those which operate on generalized mood and arousal states.

Effects of elevated sexual arousal on generalized arousal. Sex hormones can modulate gene transcription, membrane biochemistry, and electrophysiology. These hormones bind in regions associated with generalized CNS arousal and modulate general arousal states. Estrogenic hormones accumulate in NA cell groups in the lower brainstem that give rise to major ascending arousal systems. The novel estrogen receptor gene product, ER-β, is found in the midbrain raphe nuclei, which gives rise to serotonin pathways, as well as in midbrain cells that give rise to DA pathways.

A completely different mechanism by which sexual behavior can impact generalized arousal depends on sensory physiology. Sexually significant stimuli from the genitalia, coming into the body over the pelvic and pudendal nerves, send strong signals to the lower brainstem reticular formation's gigantocellular reticular nucleus, which in turn increases activity in the locus coeruleus and the midbrain reticular core, which then activates the entire forebrain.

The molecular biology of sex hormone action at regions associated with generalized arousal further shows how sex hormones contribute to generalized arousal. Prominent among the molecular routes of influence are the ways in which sex steroids increase NA synthesis and effectiveness. Estradiol stimulates gene expression for the enzymes which synthesize NA in the locus coeruleus. In the hypothalamus, estrogens stimulate gene expression for a specific NA receptor subtype, the α-1b receptor, and foster interactions between these receptors and signal transduction pathways in hypothalamic neurons. Likewise, sexual interactions by female rats with males evoke NA release in specific parts of the hypothalamus. It appears that sex hormones increase the signal-to-noise ratio in arousal-related pathways by acting at an entire train of mechanisms – from synthesis through release through receptors through postsynaptic action.

Estrogens work through several other arousal-chemical systems as well. For example, Ingrid Reisert at the University of Ulm, Germany, has found that sex steroids can promote neurite outgrowth in midbrain DA neurons. Estrogens have been shown to affect the amount of time DA can reside in a synapse, increasing the effectiveness of DA at the postsynaptic neuron. Estrogen receptors are also found in

many histaminergic neurons. Indeed, estrogen administration can amplify neuronal responses to HA. Estrogen also increases gene expression for the rate-limiting synthetic enzyme tryptophan hydroxylase and modulates serotonin receptors. Sex hormones desensitize the 5-HT1A autoreceptor, thus facilitating serotonin's synaptic functioning and, probably, heightening mood. Sex hormones also heighten cholinergic function, not only in the hypothalamus but also, importantly, in the basal forebrain.

In summary, sex hormones are able to increase generalized arousal through several molecular and neurochemical routes. While sexual behavior and sexual arousal were used as specific examples to highlight the interaction between generalized arousal and a specific arousal, note that other specific arousals, such as fear, likely both modulate, and are modulated by, generalized arousal systems.

Outlook

Medical significance. Ranging from purely medical problems such as comatose and vegetative states, anesthesiology, and sleep disorders, through problems of mood and motivation, to public health problems such as lead poisoning, disorders of arousal demand a better understanding. Understanding the genetics of arousal, and how arousal-related nuclei of the brain relate to each other, holds the potential to both improve quality of life and to cure diseases of the brain. Severe disorders of CNS arousal are considered in the chapter by Steven Laureys, M.D., in this text.

Further questions. While it is known that elementary CNS arousal, and hence behavioral arousal, has a genetic component, how individual genes and networks of genes relate to the nervous system's activity state and an animal's behavior remains largely unexplored. Specifically, while we know that certain neurotransmitters are involved in modulating an animal's state, the receptors that are activated by an individual transmitter are extensively varied. The challenge for molecular pharmacology is to figure out how the biophysics of multiple receptor subtypes plays into the neurophysiological and behavioral functions. There are at least 14 genes coding for serotonin receptors. What do they all do? Just as striking is the evidence that individual dopaminergic receptor subtypes – D1 versus D2 – have opposite biochemical effects within the same neuron. In fact, even the different isoforms of D2 receptor gene products have distinct functions. Calculating how the differential contributions of all of these histochemical systems and their separate receptor subtypes provides an exciting challenge necessary for a full understanding of how arousal works.

The basal ganglia may play an important role in behavioral arousal. They receive the majority of projections from intralaminar thalamic nuclei, whose activity is closely linked with arousal, and a recent case study showed that stimulation within the basal ganglia could bring an anesthetized patient into an alert state, although with absence of consciousness. How this and other nuclei interact to create or modulate states of arousal remains an important question.

Behaviorally, while there are clearly defined stages of sleep, the stages of wakefulness or arousal are poorly defined. What differentiates states of wakefulness

or is it a continuum? What systems are active during each state or along the continuum? Are there biochemical or anatomically functional switches to move between states or along the continuum? Understanding elementary CNS arousal is the keystone to understanding how and why an animal or human being is able to initiate any behavior at all.

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