REVIEW
Counterpointing the functional role of the forebrain and of the brainstem in the control of the sleep–waking system

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SUMMARY This paper reviews the lifetime contributions of the author to the field of sleep–wakefulness (S–W), reinterprets results of the early studies, and suggests new conclusions and perspectives. Long-term cats with mesencephalic transection show behavioral/polygraphic rapid eye movement sleep (REMS), including the typical oculo-pupillary behavior, even when the section is performed in kittens prior to S–W maturation. REMS can be induced as a reflex. Typical non-rapid eye movement S (NREMS) is absent and full W/arousal is present only after a precollicular section. The isolated forebrain (IF) rostral to the transection exhibits all features of W/arousal and NREMS [with electroencephalographic (EEG) spindles and delta waves], arousal to olfactory stimuli, and including the appropriate oculo-pupillary behaviors. These features also mature normally after neonatal transection. REMS is absent from the IF. After deprivation there is NREMS pressure and rebound in the IF, but the decerebrate cat only shows pressure for REMS. Most IF reactions to pharmacologic agents are within expectations, except for the tolerance/withdrawal effects of long-term morphine use which are absent. In contrast, these effects are supported by the brainstem (i.e. seen in the decerebrate cat). In cats with ablation of the telencephalon, or diencephalic cats, delta waves are absent in the thalamus. EEG thalamic spindle waves are seen triggering S for only 4–5 days after ablation. Therefore, true NREMS is absent in chronic diencephalic cats although pre- and postsomniac behaviors persist. These animals are hyperactive and show a pronounced, permanent insomnia; however, a low dose of barbiturate triggers a dramatic REMS/ataypical NREMS rebound. Cats without the thalamus (athalamic cats), initially show a dissociation between behavioral hyperactivity/insomnia and the neocortical EEG, which for 15–20 days exhibits only delta and slower oscillations. Fast, low-voltage W rhythms appear later on, first during REMS, but spindle waves and S postures are absent from the start, such that these cats also display only atypical NREMS. Athalamic cats also show barbiturate-sensitive insomnia. Cats with ablation of the frontal cortices or the caudate nuclei remain permanently hyperactive. They also show a mild, but significant hyposomnia, which is permanent in afrontal cats, but lasts for about a month in acaudates. The polygraphic/behavioral features of their S–W states remain normal. We conclude and propose that:

(a) the control of the S–W system is highly complex and distributed, but is organized hierarchically in a well-defined rostro-caudal manner; the rostral-most or highest level (telencephalon), is the most functionally complex/adaptative and regulates the lower levels; the diencephalic/basal forebrain, or middle level, has a pivotal role in inducing switching between S and W and in coordinating the lowest (brainstem) and highest
levels; (b) W can occur independently in both the forebrain and brainstem, but true NREMS- and REMS-generating mechanisms exist exclusively in the forebrain and brainstem, respectively; (c) forebrain and brainstem S–W processes can operate independently from each other and are preprogrammed at birth; this helps understanding normal and abnormal polygraphic/behavioral dissociations in humans and normal dissociations/splitting in aquatic mammals; (d) NREMS homeostasis is present in the IF, but only REMS pressure after deprivation persists in the decerebrate cat; (e) the thalamus engages in both NREMS and W; (f) insomnia in diencephalic cats is the result of an imbalance between antagonistic W- and S-promoting cellular groups in the ventral brain (normally modulated by the telencephalon); (g) the EEG waves, which are signature for each S–W state, appear to truly drive the concomitant behaviors, e.g. a hypothetical human IF could alternate between behavioral NREMS and W/arousal/awareness; (h) a role for REMS is to keep the individual sleeping at the end of the self-limiting NREMS periods. The need for accelerating research on telencephalic S–W processes and downstream control of the lower S–W system levels is emphasized.

**KEYWORDS** forebrain–brainstem interactions, functional anatomy, regulation, sleep–waking

1. **INTRODUCTION**

For a long time, encompassing the 1940s through the 1970s, the emphasis regarding the functional anatomy of sleep–waking (S–W) was on the dominant role of the brainstem. The notion that the forebrain has little or no participation in S–W control was particularly striking for W and was supported by a double set of converging findings: (i) the report that the forebrain separated from the brainstem by a transection at mesencephalic level and observed in acute conditions, ceased to display the electroencephalographic (EEG) rhythms of W (Bremer, 1935); and (ii) the discovery of the ascending arousal influence of the brainstem reticular formation (e.g. Moruzzi and Magoun, 1949; Nauta and Kuypers, 1968). But this concept also included the regulation of S. The discovery of rapid eye movement sleep (REMS) and the intensive investigation of its neuroanatomical bases, together with the reports of sites in the brainstem controlling non-rapid eye movement sleep (NREMS), particularly the raphe nuclei (e.g. Jouvet and Renault, 1966) and the nucleus (n.) of the solitary tract (e.g. Bonvallat and Allen, 1963; Magnes et al., 1961; see Vertes and Koehn, 1997), contributed heavily to sway caudally the interest of S researchers. The findings, starting in the early sixties, that an isolated forebrain (IF), also called ‘cerveau isolé’, permanently separated from the brainstem, in physiologic conditions and in a performing animal, could indeed display EEG and behavioral manifestations of S–W, were first disputed (Jouvet, 1972) and thereafter, frequently ignored, even in recent reviews that still maintain that the IF cannot support S–W alternation (e.g. Saper, 2000). However, in more recent years, the role of the reticular formation in W/arousal has at least been challenged (Denoyer et al., 1991), and the findings on the S-promoting function of the raphe n. have been reinterpreted with the participation of these nuclei in NREMS regulation practically being negated (e.g. Arpa and de Andrés, 1993).

In this essay, within the context of the extant literature, we review and discuss the lifetime work in this author’s laboratory using cats, and then we attempt to present a balanced view of the control of S–W by the entire encephalon. As will become clear, many of the results that we collected years ago can now be reinterpreted in the light of the large amount of knowledge accrued since those days. Moreover and importantly, this approach has led us to new conclusions and potentially useful hypotheses that as we expect, may contribute to enhance the understanding of S–W regulation as well as to advance the field.

Ascending from the lower brainstem to the neocortex, the headings of this paper critically examine the role of each caudorostral encephalic level, studied in relative isolation, in the functional control of the three main states of S–W, i.e. W, NREMS, and REMS, with drowsiness (D) included as a separate state whenever necessary. The states are defined by standard behavioral, EEG, ocular-pupillary and electrooculographic (EOG), electromyographic (EMG, neck muscles), and electrocardiographic (EKG/respiration) patterns. However, this is not an exhaustive review of the literature (which is why, e.g. often precedes a reference when others are not been quoted).

In all our experiments efforts were made to minimize suffering and to use a minimal number of animals. The procedures used for experiments conducted before 1971 were reviewed and approved at the Departmental level [J. Hopkins or Santiago (Chile), Medical Schools] as Institutional Review Boards were not available at that time. Thereafter, all procedures were approved by the UCLA Chancellor’s Committee for Animal Research (or equivalent Review Board). Moreover, as in the majority of our animals the neocortex was either absent or disconnected, it was estimated that these subjects could react to but not perceive pain.
2. THE BRAINSTEM AND THE DECEREBRATE ANIMAL

2.1 Wakefulness

The level of wakefulness and behavioral performance of these animals is strictly proportional to how much brainstem tissue is left caudal to a transection (in the coronal plane). In chronic bulbar cats with a section between the rostral medulla and the lower edge of the pons (Fig. 1a), and in midpontine cats, with a section just caudal to the n. locus coeruleus, Siegel et al. (1986), described that, within a fluctuating decerebrate rigidity, quietness dominates in these animals, but interspersed there are brief periods of arousal (1–3 min), called phasic activation. The latter periods course with increased EMG activity, EKG, and respiration rates, and in the quiet animal, can be elicited even by light tactile stimuli. Occasionally these cats, which usually are in a recumbent position and cannot get up, may move vigorously thereby rolling and changing sides. Neuronal unitary firing, recorded from the n. gigantocellularis, clearly increases during arousal/motor activation. The authors viewed these periods as a crude, primitive waking/arousal behavior alternating with periods of rest akin to D. As by the third week post-transection behavioral activation showed marked periodicity, the authors concluded, in addition, that the medulla can generate ‘ultradian periodicities similar to those which underlie the REM non-REM alternation’ (in intact cats).

A more typical and integrated W behavior is displayed by cats with a section at mesencephalic level (Fig. 1c,d). We reported the following findings in cats with a high transection just rostral to the third and fourth cranial nerve nuclei (Fig. 1d), which allows monitoring oculo-pupillary behavior using a hollow eye cylinder inserted in the orbit (Berlucchi et al., 1964), or in animals with a midcollicular section (Fig. 1c; Villablanca, 1965b, 1966a), and surviving up to 3 months (a mean of 48.3 days). At 15 or 31 days following the section these cats spend most of the time awake (75 and 55% time, respectively). In prolonged observations, the cats may be found crouching, sitting, standing, walking, or attempting to climb their cage walls. In the precocious animals, the eyelids are open, the nictitating membranes retracted, and the pupils fully dilated (Fig. 2 in Villablanca, 1966a). The eyeballs are either motionless and centered or exhibit slow, conjugated movements. During W, these cats as well as kittens growing with a midcollicular transection (Villablanca et al., 2001; see Rapid eye movement sleep, b), are highly sensitive to sound and touch, which elicit head orienting or a tendency to groom the touched side. Touching their face often induces avoidance head movements or semidirected ipsilateral avoidance paw movements. Handling, e.g. introducing a tube for gastric feeding, frequently triggers spitting, hissing, growling, and even biting. In a more specialized study we showed that classical conditioning of the eye blink with auditory discrimination can be obtained in these animals (Norman et al., 1977). During W, EEG activity recorded from the mesencephalic and pontine reticular formation shows a stereotyped fast activity pattern consisting of low-voltage (<50 μV) fast waves (5–25 Hz) undistinguishable from that recorded in an W intact control cat. The EMG ranges from moderate to highly active depending on the animal’s level of motor activity.

Figure 1. Schematic drawing of a midline sagittal section of a cat’s brain to illustrate the main transection levels discussed in this essay. (a) Spinomedullary: spinal cat, caudally; isolated encephalon, rostrally. (b) Medullary-pontine: bulbar or medullary cat, caudally. (c) Mesencephalic intercollicular (or lower in the midbrain): low mesencephalic or pontine decerebrate cat, caudally; low isolated forebrain, rostrally. (d) Mesencephalic precollicular: high mesencephalic decerebrate cat, caudally; high isolated forebrain, rostrally. (e) Telencephalon removed: diencephalic cat. The cerebellum is spared in all cases. In the athalamic cats only the thalamus is removed bilaterally. Cc, corpus callosum; Fx, fornix; Hypoph, hypophysis; Ic, inferior colliculus; Lc, nucleus locus coeruleus; Mb, mammillary bodies; Och, optic chiasm; Pyr, pyramid; Rn, red nucleus; Sc, superior colliculus; third, fourth, fifth, and sixth, represent the cranial nerves.

Compared with the findings in cats with lower transections, the results in high midbrain cats suggest that the integrity, behind the section, of most of the cholinergic reticular core, is necessary for true W behavior.

### 2.2 Drowsiness and NREMS

If undisturbed, the motor activity of the decerebrate cat decreases, the W posture tends to collapse and, eventually, the animal lies down in a random position. The eyelids and the nictitating membrane close and the pupils exhibit fluctuating myosis. The eyeballs show slow, often dissociated movements, tending to an inward and downward rotation. The EKG and respiration rate slow down. This state is quite labile as it is easily reversed by exteroceptive stimuli, specially auditory; however, there is a slight tonicity to it, i.e. the stimulus intensity required to reverse the oculo-pupillary pattern is greater when the pupillary diameter is small and has been maintained for a longer time. Nevertheless, myosis never becomes extreme (fissured or slit pupils) and is not tonically maintained for long periods (15–30 min), as described for NREMS in intact cats (Berlucchi et al., 1964). During these quiescence periods the EMG activity attenuates but there are no changes in the brainstem EEG.

Indeed, the slow, high-voltage waves which are recorded from the brainstem of intact cats during NREMS are no longer seen after brainstem transection, as also documented by others (e.g. Jouvet, 1962). This is not due to incapacity of the intrinsic brainstem circuitry to support higher voltage EEG activity, as ponto-geniculo-occipital (PGO) waves are generated in the brainstem circuitry to support higher voltage EEG activity, as Jouvet, 1962). This is not due to incapacity of the intrinsic brainstem circuitry to support higher voltage EEG activity, as also documented by others (e.g. Villablanca and Riobo´, 1970; see 4.2).

In our original description (Villablanca, 1965a), we cautiously called the above set of events the ‘variable myosis stage’ of S, but interpreted it as the ‘synchronized or slow wave S of intact cats’, now NREMS. We have revised that interpretation (Villablanca et al., 2003) and concluded that \textit{NREMS cannot be sustained by the brainstem} in decerebrate animals. The reasons are: (i) behaviorally, this is a transient, labile state which is more similar to quietness of the medullary cat or to D of the intact animal than to true NREMS, this is epitomized by the unstable nature of the pupillary myosis as well as by the absence of somnic and postsomnic NREMS postures; (ii) spindles and slow waves are absent in the truncated brainstem and we now conceive of these events as truly triggering and maintaining D and NREMS, respectively (see 5.2); (iii) the periods of quiescence in our mesencephalic cats are behaviorally very similar to those in Siegel’s medullary animals. In the latter cats, single unit activity of reticular neurons only slows down during these periods, albeit without any specific patterning, and we predict that the same would occur in our mesencephalic cats. This absence of true NREMS agrees with the fact that there are not brainstem structures, which can actively sustain NREMS within the brainstem. Indeed, it appears that the only brainstem site currently accepted as NREMS-promoting is the area of the n. tractus solitary (e.g. Golanov and Reis, 2001; Vertes and Kocsis, 1997). However, this site appears to have, not a local, but an ascending effect mediated by either solitarily projections to hypothalamic areas, or indirectly, via the reticular formation. The latter site most likely consists of the medullary cerebral vasodilator area suggesting (Golanov and Reis, 2001) that the role of the solitarius area may be to link visceral activities with S-W and other related events.

### 2.3 Rapid eye movement sleep

Jouvet (1962) and ourselves (Villablanca, 1965b, 1966a) were the first to report the presence of REMS in cats with a transection in front of the pons. Moreover, on the one hand, Siegel et al. (1986) confirmed absence of REMS in cats with a section caudal to the midpons; while, on the other hand, behavioral and polygraphic signs of REMS are absent in the IF following a section rostral to the pons (see 3.1). At more than 50 years since the discovery of REMS, there is now extensive information regarding the specific nuclei, synaptic connectivity, and neurotransmitters in the rostral pons and caudal mesenccephalon which participate in REMS control (for reviews, see e.g. Reinoso-Suárez et al., 2001; Siegel, 1994). Here we will only discuss selected contributions, mostly from our laboratory, which have shaped our own view of this behavioral state.

(a) Oculo-pupillary behavior is an integral component of REMS in high mesencephalic cats (Villablanca, 1965b, 1966a). When undisturbed the variable myosis of the quiescent cat progresses and becomes extreme (slit pupils), the eyeballs rotate downward and inward, the muscle tone decreases with drooping of the head, and quickly reaches total atonia with silence of the neck EMG. Bursts of clonic muscular activity appear over the entire body, but particularly in the face, originating REM (as also recorded in the EOG), as well as minute fluctuations in pupil size. Typical PGO waves appear in the pontine (but not mesencephalic) reticular formation and tend to group in clusters with a density and amplitude that appear to be proportional to the amount of the phasic peripheral muscular events. The postural atonia is closely linked to PGO waves as the head is seen to often fall in a stepwise manner at PGO onset. Altogether, the REMS episodes of the precollicular cat are indistinguishable from those in intact cats. The arousal threshold to exteroceptive stimulation is increased during REMS such that moderate handling, in adult cats, or even inserting a subcutaneous needle electrode in decerebrate kittens (Villablanca et al., 2001), does not wake the animals up, and the intensity of electrical stimulation of the reticular formation needs doubling in order to awake an REM sleeping decerebrate cat (Villablanca, 1966a). Quantitatively, REMS occupies 8.9 and 11.4% of the recording time in adult and in developing decerebrate cats, respectively. These values are not different from those in our intact control cats (11.0%).

(b) If the midbrain is transected in kittens prior to REMS maturation (Valatx et al., 1964), i.e. before 40 days of age, all the behavioral and polygraphic REMS features develop normally and following a similar time table to that in intact
littermates (Villablanca et al., 2001). Thus, REMS appears to be genetically programmed to develop independently from forebrain influences.

(c) An important feature is that REMS can be triggered as a reflex. In our adult decerebrate cats the introduction of the tube for gastric feeding often induces REMS (Villablanca, 1966a) and a similar effect is elicited by cutaneous and proprioceptive stimuli (Jouvet, 1965). In our mesencephalic kittens, slow, repetitive sounds almost regularly trigger REMS. Therefore, REMS has a striking reflex component which may be important to understanding the physiologic meaning of this state. Moreover, this phenomenon is reminiscent of cataplexy, a key narcolepsy symptom. Indeed, human cataplexy can be triggered by emotions as well as other stimuli (Guilleminault et al., 1976), and in dogs, by feeding (Mittler et al., 1974). Thus, cataplexy may be interpreted as a REMS component which is no longer under normal downstream control, i.e. pathologically disconnected. It has been recently shown (Yamuy et al., 2004) that activation of hypothalamic orexin/hypocretin neurons (see 3.2 (b)) enhances excitability and promotes discharges of spinal motoneurons in cats; as these hypothalamic cells are largely lost in human narcolepsy (see Section 7) and as their axons are interrupted in midbrain-transected cats, the Yamuy et al. finding has probably provided the cellular basis for the ‘reflex component’ of REMS in decerebrate animals and in narcolepsy.

(d) Also interesting is the stabilizing effect of REMS upon the EKG arrhythmia that is seen in about 25% of our mesencephalic cats and in 50% of the kittens, but that is canceled during REMS (Fig. 7, in Villablanca et al., 2001). This effect is puzzling as in intact cats the heart and respiration variability is enhanced during REMS (e.g. Rowe et al., 1999); perhaps the latter is due to a descending forebrain influence (eliminated by the transection). This cardiac effect of REMS is reminiscent of the strong downstream stabilizing inhibitory effects of REMS upon hypoglossal and spinal cord motoneurons (Yamuy et al., 1999), and of similar but ascending activating effects of REMS upon the ECoG in athalamic cats (see 6.3). All of these features suggest that REMS effects are mediated by highly secured and strong synapses. Furthermore, in terms of ascending effects, it has been shown that during REMS the discharge rates of the ascending reticular formation pedunculopontine and laterodorsal cholinergic neurons markedly increase above W levels (Steriade et al., 1990), What is the meaning of these REMS features (see 4.4)?

(e) After selective, non-stressful, REMS deprivation in adult mesencephalic cats, a strong pressure (propensity) to enter REMS develops, however, the normal ensuing REMS rebound of intact cats is absent (de Andrés et al., 2003). Moreover, pharmacologically induced (morphine) REMS deprivation renders similar results (see 4.2). These data strongly suggest that: (i) pressure and rebound are two different components of the recovery process after REMS deprivation; (ii) these components are controlled via different mechanisms, with rebound requiring prosencephalic participation, while the brainstem suffices to sustain REMS pressure; (iii) as true NREMS is not present in decerebrate cats, at least the pressure component of REMS recovery cannot be deemed to depend on the previous occurrence of NREMS (Villablanca et al., 2003), as some authors have proposed (Benington, 2002; Benington and Heller, 1999) (see Section 7).

(f) The random pattern of PGO waves discharge seen in intact cats changes into a grouped-bursts pattern in mesencephalic and neodecorticate animals (Gadea-Ciria, 1977); the author’s interpretation was that the cortex regulates the patterning of PGO sequencing.

3. THE PERMANENTLY ISOLATED FOREBRAIN

Studies in the early sixties (Batsel, 1960, dogs; Villablanca, 1962, cats) first showed that the forebrain permanently separated from the brainstem by a high or low midbrain transection displays alternating fast, low-voltage activity (or ‘desynchronized’) and slow, high-voltage activity (or ‘synchronized’) electrocortical (ECoG) patterns similar to those seen in intact animals during W and S, respectively (to be called fast and slow activity, respectively, for the sake of brevity). This discovery has been replicated in cats (Bakuradze et al., 1975; Batsel, 1964; Berladetti et al., 1977; Corpas and de Andrés, 1991; Hobson, 1965; Naneishvili et al., 1975a; Serkov et al., 1966; Slozarska and Zernicki, 1973), rats (Gandolfó et al., 1985; Hanada and Kawamura, 1981; Zernicki et al., 1984), and monkeys (Massopoust et al., 1968). Since 1960 and until now (e.g. de Andrés et al., 2004), we have defined abundant behavioral, EEG, and neuropharmacologic properties of the IF leaving little doubt that the ECoG manifestations originally described correspond to physiologic S and W.

3.1 Wakefulness

In all the above studies, long periods of sustained ECoG fast activity were observed in the IF of all animals, but with variable delays after midbrain transection. In our cats brief epochs of fast, low-voltage ECoG rhythms, interrupting spindling and slow waves alike, are seen as soon as by the second day after transection. The duration of these periods progressively increases so that between days 7 and 10 protracted epochs of fast waves within the range of 50 µV and 10–25 Hz are present, and after 12–15 days, these periods may last up to many hours (range, 30 min to 8 h), occupying as much as 50–65% of the recording time. Seven- to 11-day delays are also reported for the onset of sustained fast rhythms by other investigators working with cats and dogs. In monkeys, Massopoust et al. (1968) reported that after precollular transection there was only a brief period (2–10 min) with slow ECoG waves which was followed by a longer lasting fast wave activity. They interpreted this pattern as the normal ECoG arousal seen much later after transection in other species. However, the condition did not last more than about 25 min and changed into a slow wave pattern, coincident with a terminal drop in blood pressure. Indeed, with such an unstable physiology the meaning of this finding is difficult to
evaluate. Relatively short recovery intervals of 1–4 days were reported in rats. Thus, the animal’s species appears to be an important factor in the delay of arousal recovery by the IF. Other intervening factors are: the amount of mesencephalon which remains connected to the IF, type and duration of the anesthesia, and quality of the surgery (e.g. additional brain damage, preservation of local circulation, method used for the transection; see Villablanca, 1972).

The reasons for the delayed onset of fast ECoG activity in the IF have not been investigated. As the transection suddenly disconnects the forebrain from specific (except for olfactory and visual) and non-specific (reticular formation) ascending afferences, the consequences can be equated to the depression of spinal cord functions, or spinal shock, following transection. Moruzzi (1972) proposed that the W structures of the posterior hypothalamus are more heavily depressed by shock due to ascending deafferentation than the S-promoting diencephalic sites (see 3.3); time after-the-lesion would compensate for this imbalance. Our findings in the isolated hemisphere of the cat (Villablanca, 1967), support the neurologic shock hypothesis. Following disconnection of a cerebral hemisphere from the diencephalon (see 4.2 (a)) and at least for 4 days after surgery, the ECoG shows isoelectric epochs, lasting up to 8 s; moreover, even more days must pass before cholinergic drugs become effective in modifying the ECoG.

Other features of the IF ECoG arousal that have been reported by us and others are:

(a) Appropriate behavioral events occur concomitantly with fast ECoG rhythms. The eyes become centered in the orbit and the pupils are dilated, best seen when the light reflex is blocked (Fig. 2; Villablanca, 1966b). Olfactory stimulation (e.g. canned sardine presented in front of the animal’s nostrils) during ECoG spindles, evokes both fast EEG activity and the corresponding ocular behavior (Fig. 3). The influence of olfaction is powerful as it is seen even in the acute IF (Arduini and Moruzzi, 1953). However, removal of the olfactory bulbs or blinding does not preclude the display of fast EEG rhythms (also reported by Batsel, 1964), indicating that the latter is an intrinsic property of the forebrain. In our cats visual stimulation did not change slow wave activity; however, visually elicited arousal was reported in low mesencephalic cats and rats (Slozarska and Zernicki, 1973; Zernicki et al., 1984).

(b) Fast, low-voltage arousal rhythms mature on time (40–45 days of age) in kittens with midbrain transection performed prior to development (Valatx et al., 1964) of EEG and behavioral S-W (Villablanca et al., 2001). Sprouting of axons which could bridge the transection in these kittens (Jouvet, 1972) is out of the question as the histologic gap was wide, the opposing surfaces were gliotic at necropsy, and in one animal almost the entire midbrain had been removed (Villablanca et al., 2001).

(c) Fast ECoG activity is not blocked by a catecholamine synthesis inhibitor (Marcus and Villablanca, 1975) which dispels the argument (Jouvet, 1972) that circulating catecholamines released behind the transection are responsible for the IF arousal.

(d) The hippocampal EEG theta (ø) rhythm persists in the IF (Olmstead and Villablanca, 1977) and can be seen as early post-transection as the animal recovers from anesthesia. This finding was replicated in the acute IF of rats and cats (see Gottseman et al., 1989), indicating that forebrain sites can generate this rhythm independently from the brainstem. Moreover, the amount of ø time in the IF more than doubles (83.7%) that of intact cats, at the expense of both hippocampal synchrony and desynchrony. Generally, while fast activity is present in the ECoG, ø waves interrupted by brief episodes of desynchrony are seen in the hippocampus. Theta oscillations have been recognized as an important feature of REMS (see Vertes and Kocsis, 1997), but their abundance in the IF suggests that this association may not be too important. However, it has been proposed that these oscillations participate in other forebrain-based functions including memory encoding and retrieval, learning rate and sensorimotor integration (see Cantero, 2003). Concomitantly, other forebrain sites of origin have been found/proposed to generate ø, particularly the hypothalamus (e.g. Vertes and Kocsis, 1997; Wilson et al., 1976), as well as independent sites in the human neocortex (e.g. Cantero, 2003), prompting the comment that ø may contribute to cognition. Finally, the finding that these rhythms are present so soon after transection supports the neurologic shock hypothesis for the delay of ECoG arousal; i.e. the hypothalamic and thalamic sites involved in IF arousal suffer the deafferentation impact across fewer synapses compared with the hippocampus which, being for the most part several synapses away, is removed from the direct impact of neural shock.

Based on the above findings, the capacity of the long-term IF to display an EEG and behavioral arousal in all similar to that in intact animals is undisputable today. The interesting but still pending question is what is the meaning of this property; but this is discussed in 4.3. However, in this context, it is also interesting that from the stand-point of neurotransmitter, the status of the IF is more akin to the state of the brain during REMS than during W; this is because in both conditions the monoaminergic ascending pathways originating in the n. locus coeruleus or raphe nuclei are either at a very low level of activity (REMS) or are interrupted in the IF; except perhaps in low midbrain transections in which a small segment of the rostral raphe may be spared rostrally (see 3.3).

It would be of interest to investigate if the IF can also display the faster rhythms in the gamma frequency range (< 30 Hz), which have been described more recently, as these oscillations have been implicated in the arousal underlying high alertness and attentiveness (Steriade, 2000; Steriade et al., 1996).

3.2 Non-rapid eye movement sleep

This is a highly interesting behavioral and EEG state in the IF, and yet it has received little attention outside de Andrés (Corpas and de Andrés, 1991) and our own laboratories. In long-term IF cats, lasting epochs of slow, high-voltage ECoG waves are regularly seen (Figs 2 and 3). At the beginning of a recording session the ECoG usually exhibits a fast activity pattern. Sooner
or later, within a range of minutes to 1–2 h, there is a progressive EEG slowing with typical spindle bursts appearing and alternating with slower waves of increasing voltage. Within 5–20 min, the slower activity reaches a maximum voltage (100–200 μV) and delta-range (1–4 Hz) frequency, with the spindles fading and then almost disappearing. In hindsight, the slow oscillations of <1 Hz described in intact animals long after our experiments (Steriade et al., 1993) are also present during these epochs (Fig. 4a; Villablanca, 1965a; Villablanca et al., 2001). Therefore, the slow activity of the IF is indistinguishable from that in intact cats during NREMS. An intriguing feature of the NREMS periods in the IF is that they almost invariably end in a spontaneous, abrupt manner suddenly giving way to ECoG fast activity (also reported by Batsel, 1960, 1964). In adult cats the duration of the slow activity periods with the above features fluctuates within the mean of 61 ± 4 min (range, 30–120 min in 49 measured periods), which is greater than values in intact cats (Sterman et al., 1965; Ursin, 1968); however, the duration of the NREMS episodes was not systematically measured in the IF. These stereotyped episodes recur periodically within long recording sessions and alternate in a cyclic but ultradian sequence with the fast activity periods. The total amount of NREMS fluctuates between 35 and 50% (adult cats; Villablanca, 1965a) and 38.9% (kittens; Villablanca et al., 2001) of the recording time, which is well within the range reported by us (e.g. Villablanca and Salinas-Zeballos, 1972) and by others (Sterman et al., 1965; Ursin, 1968) in intact cats. In rats, which show circadian S–W alternation (while cats are polycyclical), the IF recovers circadian rhythmicity 4–9 days after transection (Hanada and Kawamura, 1981).

In cats with the oculomotor nuclei third and fourth in front of the transection, the ocular and pupillary behavior follows the time course of the slow activity periods (Fig. 2), as reported in intact cats (Berlucchi et al., 1964). Concurrent with the initiation of spindling, the pupils begin fluctuating while progressively decreasing in size. Myosis increases with progression of slow activity and the pupils become fissured later on, at the time of full presence of delta waves, remaining constricted for the duration of the period. Simultaneously the eyeballs converge medially and move downward, as in intact cats during NREMS. The pupils dilate and the eyeballs recover a centered position as soon as the NREMS ECoG switches into fast wave activity.

As mentioned in 3.1 (a), olfactory stimuli readily reverse spindling at the beginning of slow activity periods. As the
The graded progress of the ECoG and behavioral events during the NREM periods of the IF as well as the gradual loss of effectiveness of olfactory stimulation in reversing the slow activity as it becomes fully developed, are an excellent demonstration of the active physiologic nature of the NREMS state. In fact, we now view this interesting progression as a reflection of NREMS pressure. We also believe that it would be impossible to NREMS-deprive the IF of the adult cat using olfaction, as this modality progressively loses effectiveness, during the course of an NREMS period, as the only exteroceptive stimulus capable of awakening the IF. Fortunately, the IF can be NREMS-deprived pharmacologically, using morphine, and it is important to note that, by the end of the drug effect, there is a robust NREMS rebound (Corpas and de Andrés, 1991). Therefore, in contrast to the brainstem (decerebrate cat) which cannot independently support REMS rebound (de Andrés et al., 2003), the forebrain can independently sustain both S pressure and rebound, which means that it can control its own S homeostasis.

The above collective information shows that the forebrain, independently from ascending brainstem influences, possesses the mechanisms which are needed and sufficient to initiate and maintain the ECoG and behavioral manifestations of NREMS. Moreover, our results in kittens demonstrate that the structures involved are preprogrammed to take independent S–W control early in life.

3.3 Structures which support S–W in the isolated forebrain

It is reasonable to assume that the same forebrain structures which support S–W in the intact brain should play a similar role in the isolated forebrain. The long-term isolated forebrain shows periods of slow high-voltage waves of non-rapid eye movement sleep (NREMS) which can be interrupted by olfaction, but only to some extent. Recordings are from the somatosensory (Ss C) and auditory (Au C) cortices, and from the pontine reticular formation (Pont R F). In (a) the tracings show spindle waves (drowsiness), which are interrupted by olfactory stimulus (sardine) of 30 s duration (on–off, at arrows). At the beginning of (b) the EEG now shows continuous NREMS which is only partially interrupted by a longer (5 min 30 s) stimulus. In (c) the same stimulus fails to alter the EEG. Stimuli were presented 7, 20, and 45 min after the start of a non-rapid eye movement period that ended spontaneously and abruptly after lasting for 93 min as shown in the inset at the bottom. The eyes exhibited the changes illustrated in Fig. 2, as the section was behind the oculomotor nerves. The pontine EEG shows a monotonous low-voltage fast pattern [interrupted during the course of the 93 min by periods exhibiting pontine (geniculate-occipital) waves of rapid eye movements sleep; see text]. Modified from Villablanca (1965a).
role in the IF. Since the insightful observations by Von Economo (1930) of encephalitis brains during the early 1900s epidemics, abundant evidence points to a key role of the hypothalamus in S-W control. Participation by the basal forebrain area was suggested later on (Nauta, 1946) and is fully recognized now (see below). Today we have abundant

Figure 4. Sleep-waking matures independently in the brainstem and in the isolated forebrain, even when the midbrain transection is performed prior to patterns development. As a rule, at any given time point sleep-waking of the isolated forebrain and decerebrate animal are dissociated from each other. Polygraphic recordings from a 55-day-old kitten with a midbrain transection (midcollicular) performed at age 27 days. (a) A sudden, spontaneous ending (at the middle of this epoch) of neocortical high-voltage slow activity which is followed by low-voltage fast activity. The interruption was interpreted as a typical, abrupt termination of an electrocortical slow activity period (non-rapid eye movement sleep, see 2.2.) followed by waking. Throughout this epoch the decerebrate animal was behaviorally awake as shown by visual inspection, active electromyogram (EMG), and absence of the pontine (geniculo-occipital) waves of rapid eye movement sleep. Note a slower recording speed (calibration bar) than in (b). (b) Continuous neocortical high-voltage slow activity in the isolated forebrain indicating non-rapid eye movements sleep. The decerebrate kitten was in rapid eye movement sleep during the first half of this epoch as shown by visual inspection, the pontine (geniculo-occipital) bursts, and inactive EMG, but spontaneously woke up in the middle of the epoch (EMG activation preceding ‘head up’). Note that none of the bodily and polygraphic changes of the decerebrate animal induced substantial change of the non-rapid eye movements sleep EEG pattern of the isolated forebrain. As sleep-waking mechanisms were immature at the time of transection, this figure also illustrates the preprogramming of these events. Fr Cx, frontal cortex; Par Cx, parietal cortex; Occ Cx, occipital cortex; Pons, pontine reticular formation; EMG, electromyogram of the neck muscles; EKG, electrocardiogram. Modified from Villablanca et al. (2001).
cellular and neuromodulator/neurotransmitter information about these sites. Very briefly:

(a) S-promoting sites

These are located in the anterior hypothalamus and basal forebrain and include the galaninergic ventrolateral preoptic area (VLPO; e.g. Saper et al., 2001; Sherin et al., 1996) and, more medially, the GABAergic anterior preoptic area (POA, e.g. McGinty and Szymusiak, 2001). Within the basal forebrain, the main contributors are GABAergic neurons of the magnocellular n. (e.g. Szymusiak, 1995), which overlap with W-promoting cells in the same area (see below). These cellular groups modulate S–W via projections to the brainstem (e.g. McGinty and Szymusiak, 2004; Saper et al., 2001; Steininger et al., 2001) and perhaps, also via the thalamus (Asanuma and Porter, 1990; Gritt et al., 1998). Recent data (e.g. Gong et al., 2004) show that in rats, neurons of the median preoptic n. increase activity during sustained W while decreasing firing during sustained S; moreover, GABAergic cells in this n. and also in the VLPO, increase their activity during S deprivation. This strongly suggests that these cellular groups are involved in S homeostasis and that, therefore, they are most likely the substrate for NREMS pressure and rebound in the IF. As the S deprivation was total in these experiments, it is also likely that the identified neurons might also be responsible for ‘permitting’ REMS rebound to occur in intact animals (thereby explaining the absence of rebound in our decerebrate animals, see 2.3 (e)). As it will be reviewed later on, thalamic nuclei are also involved in promoting S; these include the n. reticularis (Steriade et al., 1985), medialis dorsalis and anterior ventralis (Lugaresi et al., 1986).

(b) Waking-promoting sites

These include: (i) posterior hypothalamic cells located mainly in the histaminergic tuberomammillary n. (TMN; e.g. McGinty and Szymusiak, 2004; Saper et al., 2001); (ii) orexin/hipocretin neurons in the perifornical and lateral hypothalamus (e.g. Burlet et al., 2002; Siegel, 2004); and (iii) cholinergic neurons of the basal forebrain (e.g. Jones and Milethaler, 1999; Szymusiak, 1995). These cellular groups project diffusely to the neocortex and downstream to the mesopontine sites (see below). The thalamus also plays a role in ECoG activation/arousal as will be discussed in Section 6.

Lesion and stimulation experiments support the role of the above structures in S–W in the IF. Thus, in the IF of the cat, on the one hand, electrical stimulation of the posterior hypothalamus and of the basal forebrain induces fast ECoG rhythms (Bakuradze et al., 1975; Berladetti et al., 1977) and cholinergic (Sakai et al., 1990) stimulation of these areas induces arousal. On the other hand, lesioning the posterior hypothalamus in rats enhances IF slow activity, while a similar lesion in the preoptic area (POA) increases the amount of fast wave activity (Nakata and Kawamura, 1986; Naneishvilli et al., 1975b). Moreover, there is evidence that the S-promoting influence of the basal forebrain still persists in the IF (Obál et al., 1979).

3.4 Three additional comments regarding the IF are pertinent at this point

First, we believe that the adult cat IF presents a unique advantage to study NREMS, i.e. as NREMS is almost entirely shielded from afferences (except olfactory), it can run its entire course in an uninterrupted and stereotyped manner, thereby offering more stable conditions when compared with intact animals.

Secondly, current brain imaging studies based on energy metabolism measurements generally fail to show any active nature of NREMS (e.g. see Braun et al., 1997; Maquet, 2000, in humans; Ramm and Frost, 1996, in cats), either at onset or during maintenance [although the decrease in glucose utilization rates is greater during state 3–4 (deeper) than during stage 2 (lighter) in human NREMS, at least in the thalamus; Maquet et al., 1992]. This was explained (see Maquet, 2000) on the basis that, particularly in thalamocortical sites, membrane hyperpolarization is the dominant electrophysiologic state of neurons during delta waves S (see 5.2) and that, during this condition, energy utilization is minimal when compared with states involving high levels of synaptic transactions (e.g. W).

However, this assumption is debatable (see Ackerman et al., 1984). But, if there are diencephalic sites that actively initiate and maintain NREMS (see 3.3, 3.5), why are they not revealed by imaging studies? A likely explanation is that these structures are so small that they are beyond the level of spatial resolution that the imaging techniques currently possess. In fact, the ventral forebrain sites mentioned in 3.3 are not even included in the list of structures which were examined in the cited imaging studies. An additional problem in the interpretation of energy metabolism studies is the participation of prosencephalic sites, particularly cortical ones, in overlapping imager/sensorimotor activities during REMS or NREMS dreaming (Braun et al., 1997; Maquet, 2000). At least this latter problem is obviated in the IF, so that coupling this model with now available micro PET techniques, could be very effective in future mapping studies of S–W processes.

Studies of c-fos gene expression are also proving informative. Reports (see Cirelli and Tononi, 2000) indicate that during NREMS, c-fos induction is low throughout the brain, but with one important exception, i.e. the VLPO, where there is a positive correlation between the number of c-fos positive cells and the S percent during the preceding hour (Sherin et al., 1996). An additional group of c-fos expressing neurons has been found in the POA (median preoptic n.) and in the VLPO (Gong et al., 2004).

Thirdly, we were impressed by the reliably abrupt ending of the NREMS episodes in the IF and have avidly searched for explanations. This is puzzling as, in the absence of any apparent external arousal stimuli, this switch-like event must be triggered internally. Perhaps the model which can best explain this phenomenon at this time is the hypothalamic ‘flip-flop’ or monosynaptic ‘sleep switch’ hypothesis, best presented by Sap et al. (2001), but supported by others (see McGinty and Szymusiak, 2001, 2004). This view is based on the role of
the hypothalamic/basal forebrain S-promoting and W-promoting sites reviewed above plus the following considerations: (i) neurons of the VLPO/POA project to the posterior hypothalamus and inhibit W-promoting cells, particularly in the TMN (Sherin et al., 1998); (ii) the hypothalamic sites project to the brainstem structures involved in arousal and REMS regulation, including the mesopontine reticular formation (cholinergic; arousal/W), the n. locus coeruleus (noradrenergic), and the raphe n. (serotonergic); neurons of the latter two monoaminergic nuclei discharge profusely during W but tend to silence during REMS (see 2.3 for references); (iii) there are inhibitory connections between collaterals of the brainstem ascending arousal-related neurons and the S-promoting VLPO/POA hypnogenic cells (e.g. Jones et al., 1977; McGinty and Szymusiak, 2001, 2004; Saper et al., 2001), as well as in between ascending hypothalamic projections and cells in the basal forebrain (e.g. Cullinan and Zaborszky, 2001; España et al., 2001). Therefore, S initiated by VLPO/POA activation would inhibit W-promoting downstream sites, which, in turn would remove inhibition from VLPO/POA neurons thereby facilitating S onset. In contrast, activation of the W-promoting side of the ‘switch’ mediated by the W/arousal components of this circuit, would inhibit S-promoting sites thereby leading to W/arousal. Due to this connectivity the ‘switch’ appears well poised for fast action (McGinty and Szymusiak, 2000). Although the pontomesencephalic excitatory components of the flip-flop are absent in the IF, we propose that the hypothalamic sites suffice to promote the S–W alternation seen in the IF while at the same time accounting for the quick NREMS–W transition. Lesions or reversible inactivation of the posterior hypothalamus cancel the insomnia that follows POA lesions in intact cats (Sallanon et al., 1989), and this also supports our suggestion.

3.5 Rapid eye movement sleep
This state does not occur in the IF as: (i) we have never observed fast ECoG rhythms coinciding with pupillary myosis, which is the rule during REMS in intact animals; (ii) rapid eye movements are no longer recorded/seen in the IF (with the third and fourth nerves attached); (iii) PGO waves are no longer observed in the dorsal lateral geniculate body after separating this n. from the pons (Hobson, 1965).

As mentioned, our basic findings in the IF of midbrain-transected animals have been replicated in at least 10 laboratories around the world and in four mammal species. Based on the above evidence we propose that the premesencephalic forebrain contains the minimal neural substrates not only for behavioral and electrophysiologic W/arousal, as described in 3.3, but also, perhaps for awareness. A clue to the latter assertion is first provided by the arousal response of the high (precollicular) IF to olfaction. The behavioral repertoire increases as lower structures are preserved with the IF. Inclusion of the third and fourth nerves allows integration of ocular movement pupillary behavior with the ECoG rhythms, both during W and NREMS patterns, and during their transitions. And a slightly lower section, as in pretrigeminal animals (a pontine section just in front of the fifth nerve n.), dramatically enriches performance of the IF, so much so that habituation of the arousal response, classical (e.g. conditioning of the pupillary reaction) and instrumental paradigms (e.g. conditioning of the vertical eye movements) are possible (e.g. Ikegami and Kawamura, 1981; Zernicki, 1986). Animals with yet a lower section, i.e. at the medullary–spinal junction, yielding an isolated encephalon or ‘encephale isolé’ (Fig. 1a), are hard to maintain in stable physiologic condition and, therefore, to our knowledge, they have rarely been tested behaviorally (Ikegami and Kawamura, 1981). However, and this is well known, the isolated encephalon readily displays all S–W states, including REMS (e.g. Bon et al., 1980; Foutz et al., 1975). As for the spinal cord behind this section, we do not know of studies in spinal animals that report any S–W-related alternation.

4. OTHER FEATURES OF THE MIDBRAIN-TRANSECTED ANIMALS AND THEIR IMPLICATIONS

4.1 Forebrain–brainstem interactions
As a rule, the EEG patterns of the IF are dissociated from the EEG and behavior of the decerebrate cat. For example, delta activity of the IF may concur either with PGO waves and REMS (Fig. 4b), or with the low-voltage background brainstem EEG and behavioral W of the decerebrate animal (Fig. 4a). But there are exceptions. Stimuli likely to activate a NREMS pattern in the IF are: repeated manipulation of the animal involving strong proprioceptive stimulation, vigorous spontaneous movements (e.g. walking, defecation, micturition, sneezing), and REMS episodes with strong, widespread muscle twitching. The magnitude of these couplings is age-dependent. For example, in kittens, about 23.0% of the cases when REMS episodes occur during ongoing delta waves of the IF, these waves end suddenly, coinciding with intense muscle twitching followed by abrupt behavioral arousal. In contrast, in adult cats under similar circumstances, only about 12.0% of the cases REMS episodes alter ongoing slow waves periods in the IF, and there is only a brief interruption in delta oscillations. Similarly, in the kittens, strong spontaneous motor activation will arouse an NREMS pattern in the IF 77% of the times, while in adult cats, strong motor activity cancels ongoing NREMS only 56% of the times, revealing, once again, enhanced neuroplasticity in the kittens. We explained this age-dependent differences (Villablanca et al., 2001) on the basis of our work showing enhanced postlesion structural, cerebral metabolic, and behavioral neuroplasticity in neonatal-cerebral hemispherectomy cats when compared with adult cats (Villablanca and Hovda, 2000).

Many humoral factors are known to be active during S–W alternation (e.g. Krueger et al., 1998) and some have been touted as important hypnogenic factors (e.g. melatonin; Shorat et al., 1998); however, the fact that S–W of the forebrain and the
brainstem are uncoupled after anatomical separation, strongly suggests that humoral factors do not play a very effective role in controlling S. Perhaps systemic changes produced by strong motor/proprioceptive/internal activation of the decerebrate animal, including fluctuations in respiration, blood pressure, and body temperature, might better account for the occasional S–W interactions between the IF and the decerebrate animal.

### 4.2 Effects of drugs

We studied these effects first to validate the physiologic integrity of the IF and, later on, to investigate the site of action of selected drugs. In general, most forebrain effects of the drugs persist in the IF after transection; but, in contrast, few of their behavioral and EEG actions remain in the decerebrate animal.

(a) **Atropine** (Villablanca, 1966c). In intact cats, systemic administration of this prototypical anticholinergic drug in doses ranging from 0.2 to 0.8 mg kg\(^{-1}\) (i.v., remote injection), induces slow high-voltage ECoG waves that, in a dose-dependent manner, tend to mimic the NREMS pattern. At the higher 0.4–0.8 mg kg\(^{-1}\) dose range this slow ECoG rhythm becomes continuous, but is uncoupled from the animals’ behavior. In fact, the animal is fully aroused, or awakes from NREMS upon injection of the drug, and also in a dose-dependent manner, becomes hyperactive to the point of making EEG recording difficult or impossible. In the IF, the ECoG effects are very similar such that at the highest dose (0.5 mg kg\(^{-1}\)), even prolonged olfactory stimulation, which at the smaller doses would cancel a drug-induced slow ECoG activity, ceases to be effective. In intact cats the slow EEG activity is also reflected in the recordings from the pons; however, the drug-induced slow waves are entirely absent in decerebrate animals (Villablanca, 1966c). There is no motor hyperactivity in decerebrate cats, which suggests a forebrain site of action for this effect.

(b) **Eserine** (Villablanca, 1966c). In doses ranging from 0.04 to 0.05 mg kg\(^{-1}\) (i.v., this typically cholinergic drug induces a low-voltage fast activity ECoG pattern mimicking the EEG associated with behavioral arousal in intact and IF cats. When injected during NREMS, eserine interrupts the episode in both intact and IF cats (although it does not interrupt REMS). With the higher dose, there is a moderate behavioral activation of intact and decerebrate cats alike, but there are no effects upon the brainstem EEG (Villablanca, 1966c).

The effects of the above two drugs were further studied in cats with a permanently isolated hemisphere (Villablanca, 1967). In these animals the right hemisphere was separated from the diencephalon by penetrating the corpus callosum and aspirating tissue lateral to the thalamus. In chronicity the isolated hemisphere shows a monotonous ECoG consisting of a background of slow, low or medium voltage waves with random high-voltage slow waves and a few spikes. This predominant slow wave pattern is enhanced by atropine (0.02–0.04 mg kg\(^{-1}\), i.v.) and suppressed by eserine (0.04–0.06 mg kg\(^{-1}\), i.v.) such that the ECoG approaches the morphology of the NREMS and W ECoG patterns, respectively, of the attached contralateral control hemisphere. Thus, the behavioral-ECoG dissociation described in intact cats under a high dose of atropine can be explained as a lack of arousal effects upon the ECoG in a neocortex undergoing a strong, direct anticholinergic influence. Eserine, undoubtedly, also has a direct effect upon the neocortex. Therefore, the distinct possibility that other central-acting pharmacologic agents may have a similar direct, masking cortical effect, should be considered when trying to identify the central nervous system (CNS) site of action of other drugs.

Undoubtedly, in all the above cases atropine and eserine were acting directly upon muscarinic cholinergic receptors. As mentioned in 3.3, an important cholinergic input to the neocortex originates in basal forebrain neurons and the activation of this pathway induces fast, low-voltage cortical waves. Therefore, it is not surprising that atropine, in a dose-dependent manner, blocks the EEG consequences of behavioral arousal (intact cat) or the arousing effects of olfactory stimulation (IF). Moreover, this supports the notion (see 3.3) that the basal forebrain is a substantial contributor to the behavioral/EEG arousal in the IF. Understanding the effects upon the brainstem/decerbrate animal might be more complex. It has been reported (Baghdoyan et al., 1964) that cholinergic stimulation (carbachol microinjections) of the midbrain and medulla reticular formation elicits W and motor activity, whereas REMS-like behavior is enhanced with injections in the pons but suppressed by injections in the midbrain and medulla. With our i.v. injections reaching all three sites simultaneously, we observed that the cholinergic (eserine) effects were overall dominant.

(c) **Adrenaline** (Villablanca, 1966c). In intact and in decerebrate cats, adrenaline (0.005 mg kg\(^{-1}\), remote i.v. injection) produces arousal/behavioral activation both during NREMS (including a shifting to fast ECoG rhythm) and REMS in intact cats, or during REMS/drowsiness in decerebrate cats. In contrast, the drug does not change NREMS episodes of the IF. The latter suggests a brainstem site of action for adrenaline, which fits well with the effects of interrupting ascending catecholamine projections from the n. locus coeruleus in these cats, as well as with the absence of adrenergic sites in the forebrain (Jones and Yanh, 1985).

(d) **Harmaline.** This drug is a serotonin uptake inhibitor, with powerful hallucinogenic effects, which has clinical and anthropological interest (Naranjo, 1967). Threshold doses of harmaline (2.0–3.0 mg kg\(^{-1}\), i.v.) produce similar behavioral effects in both intact and decerebrate cats (Villablanca and Ribó, 1970). The effects consist of intense motor activity, generalized tremor, postural and gait abnormalities, REMS suppression, and a number of autonomic CNS events. The EEG is also quite similar in both intact and midbrain-transected cats. In the ECoG there are 3–7 Hz high-voltage (up to 300 \(\mu\)V) waves which wax and wane in bursts of variable duration. In the brainstem, 10–15 Hz, 50 to over 200 \(\mu\)V rhythmic waves occur in trains of up to 6 s, coinciding with bursts of muscle tremor. Therefore, one outstanding result is that descending forebrain influences are not needed for the expression of most of the behavioral and brainstem EEG effects of the drug. Our brainstem results were...
confirmed (see Llinás and Volkmd, 1973) in studies of the olivocerebellar system.

(e) Pentamethylenetetrazol. In intact cats a threshold dose of 8 mg kg\(^{-1}\) (remote i.v. injection) triggers a typical grand mal tonic-clonic seizure simultaneous to a generalized ECoG epileptic high-voltage hyper-synchrony discharge (Villablanca, 1966d). In midbrain-transected cats, the IF exhibits a typical EEG grand mal attack [replicated by Walker et al. (1984) in the acute IF] and, if the III and IV nerve nuclei are connected to the forebrain, the eyes show tonic–clonic manifestations. In contrast, the decerebrate animal shows only a tonic epileptic seizure with absence of pontine EEG epileptic discharges (Villablanca, 1966d), dramatically illustrating the independence of the forebrain from brainstem processes following transection.

(f) Amphetamine. The typical stereotyped head movements seen after administration of this drug (0.5–4.0 mg kg\(^{-1}\), i.p.) in intact cats, are still present in decerebrate animals with a precollicular transection, but are suppressed after a low midbrain section (Marcus and Villablanca, 1974). This certainly indicates that the mesencephalon is essential to support the stereotypic behavior.

(g) Opiates. In intact cats, a single, small dose of morphine (0.5–3.0 mg kg\(^{-1}\), i.p.) produces a typical, rich behavioral response that includes autonomic manifestations, discrete complex movements, and a protracted S suppression (de Andrés et al., 1984; Villablanca et al., 1982, 1984). There is a pronounced NREMS rebound with a minor REMS rebound. Following midbrain transection, the discrete movement component is no longer present and it is replaced by unspecific motor activation (de Andrés and Corpus, 1991). This is not surprising as the complex behavioral response is also eliminated after removal of the caudate nuclei (Villablanca et al., 1982). There is suppression of NREMS in the IF with a strong rebound by the end of the drug’s effects (Corpas and de Andrés, 1991); in contrast, there is no rebound after REMS suppression in the decerebrate animals (as in 2.3(e)). Finally, we have just reported that, surprisingly, the decerebrate animal but not the IF shows tolerance and withdrawal (postnaloxone challenge) manifestations following chronic morphine administration (de Andrés et al., 2004). This points to a preeminent role by the brainstem in the long term behavioral effects of opiates which may help understanding the ingrained nature of opiate addiction.

Therefore, it is important to emphasize that the midbrain-transected animal model has proven highly useful in separating forebrain from brainstem effects of drugs with complex and widespread CNS effects. Other authors have also used the model to screen sites of CNS drug effects (e.g. Horn et al., 1998; Kadzielawa and Widy-Tyszkiewicz, 1970; Sakai et al., 1990).

4.3 Relevance of the midbrain-transected animals for the understanding of S–W pathophysiology in humans

The extensive body of information collected in midbrain-transected animals demonstrates that brain W/arousal is mediated by brainstem as well as forebrain sites and that these structures can operate independently of each other. In contrast, control of S is sharply divided between the two main S states: while only the forebrain can comprehensively control NREMS, the brainstem alone can only sustain REMS. A practical implication of this organization, is that in the intact brain, there is potential for uncoupling between rostral and caudal S–W processes. For this reason, the S–W dissociations often seen in normal, borderline, and pathologic conditions (e.g. see Guilleminault et al., 1976; Mahowal and Schenck, 1999), are not at all surprising. For example, somnambulism can be thought of as a sleeping forebrain in a waking body, and cataplexy as a waking brain on a sleeping body. In addition, the striking dissociations that normally exist in some aquatic mammal and avian species (see Rattenborg et al., 2000) can also be understood on the basis of an S–W system with the intrinsic capacity of shifting control between dissociable subsystems. Moreover, it has been reported that S–W ECoG patterns also recover in an unilateral IF obtained by first performing a unilateral precollicular transection and then sectioning the cerebral commissurae (Berlucchi, 1966). This would help to explain the remarkable dissociation in dolphins; these animals can sleep with only one hemisphere at a time while still maintaining navigation (Mukhametov, 1984).

In terms of neurologic syndromes that may potentially disconnect the forebrain from the brainstem, as in our cats, chances are that midbrain or upper pons lesions very rarely result in a ‘clean transection’ across the entire brainstem in humans. However, and to start with the truncated brainstem, it is known that the human brainstem pathologically disconnected from the forebrain can display REMS, such that the typical postural decerebrate rigidity, which is present in many of these cases, dramatically melts away during REMS periods (Jouvet et al., 1961; Schott et al., 1972). In addition, patients with chronic functional/anatomical decortication or decerebration often display a persistent vegetative state syndrome which exemplifies the full W/arousal of our high mesencephalic cats, to the point of simulating awareness (e.g. Adams et al., 2000; Plum, 1991).

Regarding patients reminiscent of our IF animal model, numerous cases have been reported to show ECoG patterns closely resembling those of normal W and NREMS (for references see Plum, 1991; Schott et al., 1972; Westmoreland et al., 1975). These are usually long-term unconscious patients with lesions of varied etiology. In some cases, as in our cats, a progress from a slow wave pattern at the onset of symptoms to a late fast wave ECoG pattern may occur (e.g. Obrador et al., 1975). Often the low-voltage component falls within the alpha rhythm pattern of 15–50 \(\mu\)V waves at 8–10 Hz, such that some authors have spoken of an ‘alpha coma’ (Westmoreland et al., 1975). It has been proposed that the alpha rhythm is related to sensory processing and preparatory cognitive processes (Basar and Schürman, 1996). The pathologic anatomy of the lesion in these cases is always complex involving, in various combinations, lesions of the mesencephalon, pons, thalamus, subthalamus, and hypothalamus. Although most patients are described as unconscious or comatose, in these early cases, a thorough analysis of the rostral forebrain could not be
appropriately conducted, due to the unavailability of effective brain mapping techniques, and therefore the presence of arousal or perhaps even awareness, could not be ruled out. But at present, not only has the number of such cases increased dramatically due to prolonged survival, but sophisticated imaging technologies can be applied. This issue is not only of theoretical importance, but has practical implications as well. For example, following an extensive brainstem lesion, can we always be sure that the patient has lost awareness, partially or entirely, just because she/he appears to be clinically unresponsive, in a state of coma, or in a persistent vegetative state (for syndromes definitions, see Adams et al., 2000)? And, what are the implications for prolonging life or selecting therapies?

A ‘locked-in’ syndrome patient exemplifies the condition of a hypothetical IF patient in the future. In such individuals there are bilateral lesions of the descending motor pathways at the level of the ventral pons, with variable invasion of the mesencephalic pedunules and brainstem tegmentum (e.g. Lundervold et al., 1956, Plum, 1991). The ascending somatosensory pathways are preserved, dorsally. As expected, these patients are quadriplegic and mute such that they are easily pronounced unresponsive and unaware (Bauer et al., 1979). However, there is variable preservation of eye ball and lids movements allowing patients to communicate; moreover, the ECoG rhythms are usually normal and, during fast activity, the patients are alert. But, as Markand (1976) has remarked, if the physician fails to examine eye movements the patient may be deemed comatose. We could envision a similar scenario for a hypothetical IF patient, unless imaging studies were promptly applied and revealed that the ECoG indeed underlies a hypothetical IF patient, unless imaging studies were promptly applied and revealed that the ECoG indeed underlies normal/quasi normal functional brain conditions (and as eye movements cannot be commanded in the IF).

A few recent reports (e.g. Menon et al., 1998; Schiff et al., 2002; Tommasino et al., 1995) support the possibility of a futuristic aroused-aware IF patient. Notable are findings by Schiff et al. (2002). They studied five patients using clinical neurology, video-EEG monitoring, PET, MRI, and magnetoencephalography. All cases were complex involving combinations of forebrain and brainstem lesions. Therefore, it is safe to assert that each of the cases involved a more comprehensive, diffuse, and overall destructive damage (i.e. ‘catastrophically injured brains’ in the authors’ own words) compared with our straightforward, clean transections. Surprisingly, in all cases the authors identified an ‘unexpected cerebral activity’ that showed ‘partial preservation of brain function that correlates with isolated behaviors in chronically unconscious brains’. Moreover, the authors commented that preserved, but covered forebrain processing, ‘provides a possible window onto the functions of isolated modules in the human brain’. Within this context we would suggest that the permanently IF is a large, important functional ‘module’ that, although it is as yet undetected in humans, could be revealed today with far reaching physiological, diagnostic, and management benefits.

Another promising approach, according an optimistic review (Kotchoubey et al., 2002), is the study of event-related evoked brain potentials.

5. THE DIENCEPHALIC CAT

In this animal model the telencephalon is removed bilaterally, but the thalamus, hypothalamus and basal forebrain are not surgically damaged (Villablanca and Marcus, 1972). The ablation is followed by thalamic degeneration, and this opens a window into the S–W role of the thalamus as the degeneration progresses during the early postsurgical days. In long-term adult-hemispherectomized cats, we reported that the volume of the ipsilateral thalamus decreases by 60% and that this atrophy is due to widespread neuronal loss (Villablanca and Hovda, 2000). In the ventrobasal complex (Villablanca et al., 1986), the cell packing density of the largest neurons (100–1000 pm^2 cross-sectional area) decreases by 82%, and the size of the remaining neurons shrinks by 35%. The number and size of smaller neurons (50–100 pm^2) remains unchanged due to cell survival and also to a size shift of the larger neurons that only shrunk but do not die. In the same nuclei and following unilateral neocortications, Carreras et al. (1969) reported that chromatolytic cellular changes (large and medium size projection neurons) appear by the second postlesion day and quickly progress, and by day 7 about 60% of the cells are abnormal. The actual decrease in neuron counts starts by the 10th day, and a steady state is reached in about 180 days (a 62–67% decrease).

5.1 Wakefulness

Diencephalic cats are usually up and walking by the second day postsurgery. They are extremely active but placing them in a circular corral with smooth walls and padded floor, precludes damage to the head and paws. Initially, they show obstinate progression and can walk continuously for many hours (a record 17 h was noted). Hyperactivity decreases by 20–30 days, at least in terms of walking, but the cats remain restless throughout their survival (five cats lived for 66–207 days; two cats were sacrificed at days 10 and 15 due to self-inflicted cranial damage). The W behavior of diencephalic cats is certainly more complex than that of midbrain cats to the point that some hints of awareness can be observed. In long-term cats olfactory stimuli elicit intense sniffing and searching, such that the animal can locate a can of fish placed as far as to 3–4 yards away, can walk toward the food, and even initiate eating (as also witnessed and reported by Wyrwicka (1987)). They are exquisitely responsive to auditory stimuli which induces oculo/pupillary arousal, perking of the ears and orientation of the head and body. Other complex behaviors often seen were: using a fore paw and head avoidance to reject a feeding tube or an eye piece, fragmentary grooming behavior of soiled fur, stopping/turning around when reaching a wall, or even touching the wall with the whiskers, or reaching the edge of a table, selecting a soft pad rather than staying on the uncovered floor for resting/sleeping, congregating with other diencephalic cats (see Villablanca and Marcus, 1972). During W the electrothalamogram (ETHG) shows a low-voltage (<25 μV), fast (usually >10 Hz) pattern similar to that seen in intact cats (Fig. 5).
5.2 Non-rapid eye movement sleep and drowsiness

Starting by the second day, D and NREMS behaviors are identifiable. Drowsiness was defined as the cat sitting or laying down quietly, occasionally crouching, with the eyelids partially closed, the nictitating membranes protruding variably, the pupils reduced in diameter (albeit to no less than 5 mm), but fluctuating, and with the eyeballs exhibiting slow, often dissociated movements. NREMS was characterized by the animal laying down, curled up, or in a random position, with the eyelids and nictitatings relaxed, the pupils smaller than 5 mm in diameter and diminished fluctuations, and the eyeballs rotating inwardly and downwardly. Pre- and postsonnic behaviors become more stable after a month. At that time, and by the end of a S period, cats show the typical limb-stretching, back-arching and even yawning of normal cats.

Figure 5. There is a two stage time course of sleep–waking in the long term diencephalic cat: thalamic sleep spindles are present for up to 5 days postablation while only low-voltage ‘wavelets’ persist thereafter during atypical non-rapid eye movement sleep (see 5.2). (a) Electrothalamogram and the electromyogram (EMG) of a cat 3 days after removal of the cerebral hemispheres. Sleep spindles appear at about 10 s into this epoch and, concomitantly, the head rapidly falls (drop of EMG activity) as the cat begins sleeping. Arrows indicate change in paper speed. (b) Recording taken 21 days after surgery. At the beginning the animal is behaviorally in atypical non-rapid eye movement sleep state, but wakes up upon presentation of olfactory stimulation (between arrows), as shown by activation of the EMG and by visual inspection. At this time spindles are no longer present, but the thalamic EEG shows a slight increase in voltage (‘wavelets’, first and third tracing) as documented by the increased output of a voltage integrator (Int, second tracing). Upon arousal the thalamic EEG becomes of very low voltage and the output of the integrator is flat. R Thal, L Thal, are a bipolar combination of thalamic electrodes in the right (R) and left (L) thalamus (two electrodes on each side separated by 2 mm). Pont R F, recording from the pontine reticular formation which shows no change (except for the movement artifact). Modified from Villablanca and Marcus (1972).
This NREMS state is more labile in diencephalic than in intact cats, such that auditory stimuli (tones) that will arouse the cat during D, are uneffective during NREMS; however, by only slightly increasing the frequency or intensity of the stimuli, arousal and ETHG fast activity are induced in diencephalic but not in intact cats. Therefore, a truly tonically maintained NREMS behavior cannot be demonstrated in these cats.

Spindle waves are present in the ETHG but only for about 4–5 days after surgery (Fig. 5a). They occur in strict parallel with the onset of D, which during this early period is abrupt; i.e. as the animal stops ongoing activity, spindle bursts appear at a relatively frequent rate while the head falls pari passu. Thus, spindles appear to directly trigger behavioral D and, hence, are more than just an epiphenomenon (moreover, in intact cats Hongo et al., 1963, reported coincidence between S spindle bursts in the neocortex and attenuation of discharges of muscle spindles). Between the third and fifth days spindles decrease in voltage/frequency and by the 5th–6th day they are absent. Beyond this time point, and having lost its EEG signature, D is no longer the typical state of intact cats, and its onset and end are hard to determine. These observations confirm, in behaving animals, the thalamic origin of spindle oscillations (e.g. Anderson and Manson, 1971; Villablanca and Schlag, 1968) and clearly point to their functional role in the initiation of NREMS. Spindling is also independent of ascending influences as we (Villablanca and Schlag, 1968) and others (e.g. Kellaway et al., 1966) have shown that spindles can still be recorded from the acutely decorticated thalamus after a midbrain transection. At the cellular level, spindles arise from a cycle of events in which the n. reticularis thalami neurons inhibit thalamocortical cells, eliciting rebound bursts and re-excitation of the reticularis neurons (e.g. Steriade et al., 1985; Von Krosigk et al., 1993). Consequently, spindles are abolished when thalamic neurons are disconnected from the n. reticularis (Steriade et al., 1985). As mentioned, these large thalamocortical projection neurons quickly suffer structural retrograde/anterograde degradation following telencephalectomy. Therefore, with these cells rendered non-functional in a gradual fashion, it is easy to understand the early and progressive fading of spindle waves in diencephalic cats.

In sharp contrast with the early presence of S spindles, delta waves (1–4 Hz) and the slow oscillations (0.5–1 Hz), are never seen again in diencephalic cats, not even in our earliest recordings, on the second postsurgical day. Interestingly, however, we observed that during the time course of the atypical behavioral NREMS there is some slowing down of the ETHG coupled to a limited increase in voltage, with ‘wavelets’ reaching close to 50 μV (Villablanca and Marcus, 1972). Using a voltage integrator, one can determine that these small waves do indeed boost the output by several times in comparison with that during W (Fig. 5b). Auditory or olfactory stimuli cancel this ETHG activity and restore behavioral arousal.

Two types of delta waves have been reported. The first type is synaptically generated in the neocortex and then propagated to the thalamus by corticothalamic neurons. The second type is non-synaptic in nature and is generated intrinsically by the interplay of two currents of thalamocortical neurons (see Steriade, 2001; Steriade et al., 1993). Accordingly, it has been reported that these oscillations can be recorded from thalamic neurons in acute cats after decortication (Curró Dossi et al., 1992). Therefore, the simple explanation for the immediate loss of delta waves in our cats is that they were of the first, synaptic type, and that therefore they did not withstand cortical removal. The slower 0.5–1 Hz waves, are also generated in the neocortex (Steriade et al., 1993), which explains why they were also lost in our cats. Regarding the thalamic ‘wavelets’ during atypical NREMS, we can only speculate that they might be remnants of the thalamic-intrinsic delta waves. Perhaps the large thalamocortical neurons that only shrink after decortication (Villablanca et al., 1986), are the cells that still sustain the mildly higher voltage wavelets seen during atypical behavioral NREMS in diencephalic cats. Finally, at this point we still do not have a cellular explanation for the high-voltage discharges triggered by metrazol in these cats (5.4 (c)).

From the functional stand point, it has been proposed (Steriade et al., 1991) that, as the cortically generated delta thalamic oscillations which prevail during late and deep S stages are triggered at more negative neuronal membrane potential than during light S, there is a progressive hyperpolarization of thalamocortical neurons that leads to the deepening of behavioral S and, thereby, to profound NREMS. Indeed, according to the authors, this neuronal hyperpolarization would block ascending transmission, thereby inducing a functional forebrain disconnection from the outside world. Given the close linkage between spindle waves and behavior that we observed during the early survival days in diencephalic cats, we concur in that these delta waves and the slower corticothalamic oscillations, most probably underlie and maintain the NREMS behavioral state in intact animals. Consequently, in the absence of these rhythms in diencephalic cats, the NREMS manifestations are rendered fragmented, and at best, weak.

Still, substantial components of NREMS behavior are preserved in diencephalic cats, particularly the postural components. As mentioned, the number of smaller thalamic neurons does not change much after hemispherectomy, and therefore, these may be the cells that are responsible for sparing S behavior in these animals. Altogether, the collective above-discussed information, has led us to revise (Villablanca et al., 2003) our original interpretation that diencephalic cats have a true NREMS state (Villablanca and Marcus, 1972). We now believe that, once the spindles are gone, typical NREMS is absent in the diencephalic cat and that what remains is an incomplete, fragmented, and atypical NREMS state, and this is what we refer to below whenever we write about NREMS in these cats.

5.3 Rapid eye movement sleep

Diencephalic cats exhibit REMS with all the typical behavioral and polygraphic features seen in intact animals. While S spindles are still present, small amplitude pontine PGO waves and muscle atonia precede the end of spindling by several
seconds; thereafter PGO waves become progressively larger and within 15–20 s they are grouped into complex bursts. By the end of REMS, spindles may reappear prior to reactivation of the EMG and cessation of the PGO bursts. By the time spindles are no longer present, REMS occurs following NREMS and cancels the EThG ‘wavelets’ present at the time.

Besides the above qualitative S–W changes, diencephalic cats show impressive quantitative shifts. Throughout their survival these are markedly insomniac animals (Villablanca and Marcus, 1972). We scored the mean amount (SD) of REMS and NREMS during twelve 24 h sessions across 6 months. Mainly behavioral criteria, and particularly the highly sensitive oculo-pupillary behavior, were used to define S–W states, but polygraphic recordings were also employed representing about 10% time of each session during the first month, when these cats are so hyperactive that they are hard to restrict, and about 30% of the recording time thereafter [with no substantial differences between percent values of S–W when behavioral criteria alone [versus behavior coupled with recordings] were employed]. The amount of NREMS/24 h declined progressively from 38% in control cats to 19, 15, 8, and 7% on postsurgery days 5, 10, 20, and 30, respectively; while REMS went from 13.8 (controls) to 0.8, 1.4, 1.5 and 0.3%, respectively, on the same days. For the remaining eight sessions the values for NREMS ranged between 1.9 and 5%, while the values for REMS fluctuated between 0.3 and 0.9% (all with little variability of the SD). Motor hyperactivity was not measured independently from S to W, nevertheless it clearly peaked during the first month and markedly declined thereafter. In contrast, there was a tendency for the amount of S to further decline after the first month.

### 5.4 Effect of drugs

(a) Thiopental enhances EThG spindle bursts while they are still present, but fails to elicit them once they have disappeared. In the long term cat a large amount of S is induced by a small thiopental dose (8.3 mg kg$^{-1}$, i.p., or about 20% of that required to induce S in intact cats), which in control animals depresses S (Villablanca and Marcus, 1972). During a 4-h period after thiopental the cats spend a mean of 51.3 and 33.0% of the time in NREMS and REMS, respectively, in sharp contrast with an average of 6.4% (NREMS) and 0.9% (REMS) during the 4 h prior to drug administration. A re-analysis of these data shows that the differences between the pre- and post-thiopental values are highly significant for both NREMS and REMS ($P < 0.005$, Student’s t-test). We interpret this effect as a strong S rebound, which would indicate that S, and particularly REMS, is spontaneously suppressed in diencephalic cats. The density and frequency of the PGO bursts is markedly increased both after spontaneous and barbiturate-induced episodes (Villablanca and Marcus, 1972, Figs 7 and 8); because this is also the case for REMS rebound after deprivation (e.g. Ferguson and Dement, 1968), this suggests that the rare REMS episodes seen in these animals are indeed break-through events in the context of the spontaneous REMS suppression.

(b) A single, low dose of morphine (1.5–2.0 mg kg$^{-1}$, i.v.) induces first a brief autonomic stage followed by marked behavioral quietness (Villablanca, 1994) that lasts for about 2 h. Thereafter, the cats become hyperactive once again for a period of about 6 h, and their motor activity becomes so vigorous that recordings had to be interrupted. NREMS and REMS are suppressed for about 4 and 7 h, respectively. However, after this time there is a pronounced REMS rebound lasting throughout the second, third and fourth postmorphine days (23.2, 24.4, and 14.9% per 24 h, respectively). NREMS also rebounds, but only through the third day and to a lesser extent compared with REMS. A similar dose in intact cats (de Andrés et al., 1984) suppresses S for about 12 h and elicits a rebound only for NREMS which lasts for about 12 h. Therefore, it appears as if morphine pushes the already marked predrug REMS suppression in these cats to a total blockade. The blockade cannot be sustained once the action of morphine fades and the effect ends with a protracted rebound.

(c) Pentamethylenetetrazol (Villablanca and Ip, 1971) has dose-dependent effects. A representative effect is obtained with a dose (15 mg kg$^{-1}$, i.v.) that is about 50% higher than that required to produce a grand mal seizure in intact cats. This dose induces a tonic-only generalized convulsion which starts about 10 s after the injection and lasts for about 1 min. By the middle of the behavioral attack, slow waves begin to appear in the EThG with an initial frequency of about 2 Hz at 50 μV. Thereafter, the waves progressively increase in amplitude while decreasing in frequency, to reach over 150 μV at < 1 Hz at about 75 s postinjection, at which point they are quite reminiscent of spike-wave complexes, and end suddenly (at about 120 s). This is a puzzling observation because, as described above, high-voltage waves are not seen during the atypical NREMS in these cats.

(d) d-amphetamine (0.25–10.0 mg kg$^{-1}$ i.p.) induces stereotyped head movements which, at the larger doses, is masked by intense motor activity (Marcus and Villablanca, 1974). The hyperactivity becomes so pronounced that, in order to block it, we used the antagonist haloperidol. To our surprise, haloperidol triggered intense catatonia lasting several minutes (J. R. Villablanca and R. J. Marcus, unpublished observation).

We wish to highlight two general S–W consequences of telencephalic ablation. First, the graded deterioration of S function that courses in parallel with the progressive thalamic degeneration (particularly in terms of the EEG effects). This process originates two distinct postlesion periods: a spindle wave period lasting up to 5 days, and a postspindle period lasting for the rest of the animals’ life. Secondly, the dramatic imbalance of S–W control with a marked shift toward W and motor activity. In our view, this imbalance occurs primarily at diencephalic level as a result of the elimination of influences descending from the hemispheres; and secondarily, at brainstem level, particularly in regards to REMS, as a descending consequence of the primary effects. These aspects will be discussed in Section 7.

It is puzzling that, as far as we know, there have been no attempts to replicate the above experiments, in sharp contrasts to multiple replications of our IF results. The literature at the time
of, or prior to, our experiments, in bits and pieces, generally confirms our results; however, those authors used ablations which were similar but not the same as ours, used animals with shorter survival times, or did not focus on S-W. Hyperactivity was a common finding in the older studies in cats (Emmers et al., 1965; Jouvet, 1962; Wang and Akert, 1962) and dogs (e.g. Kleitman and Camille, 1932; Rademaker and Winkler, 1928). Kleitman and Camille reported, in addition, that the nictohemeral periodicity typical of dog’s S, was absent after decortication (Kleitman and Camille, 1932; Rademaker and Winkler, 1928).

From the beginning, there is a marked EEG-behavioral dissociation as high-voltage slow waves dominate the ECoG. Only by 10 days after thalamectomy are epochs of fast activity seen in the ECoG during W, but prolonged periods of W rhythm are not present until 20–25 days postlesion. Therefore, W ECoG activity takes a longer time to reappear in athalamic than in IF cats. The dissociation decreases progressively with time, but a delay between onset of behavioral W and the onset of ECoG fast activity persists through the end of survival, reaching a minimum delay of 10–20 s only after 3 months. These observations strongly suggest that the thalamus has a role in generating fast ECoG activity and the concurrent REMS in athalamic cats. Only slow, high-voltage waves, including delta waves and also (in hindsight) the slower <1 Hz oscillations (Steriade et al., 1993), in all similar to the NREMS pattern seen in intact cats, are present in the ECoG (Fig. 6). Obviously, then, spindles are not necessary for the initiation of NREMS slow oscillations. By the time that ECoG fast activity has reappeared, behavioral D starts with intermittent slow waves in the frontal leads; these waves quickly spread throughout the cortex and increase in amplitude and frequency of appearance to soon become a fully developed NREMS activity. At this time, olfactory stimuli are most effective in arousing these animals, with the acoustic and visual modalities following in that order. However, the effectiveness of the stimuli depends on how long after the onset of NREMS the stimulus is applied and on the degree of high-voltage activity that is present at the time, much in the same way as described in the IF. Also as in the IF, a time is reached when an EEG/behavioral arousal cannot be induced even following prolonged (over 1 min) olfactory stimulation. These findings are interesting because they indicate the existence of NREMS.

6. THE ATHALAMIC CAT

The thalamus was removed bilaterally by pipette aspiration and using a transcallosal approach to minimize cortical damage (Villablanca and Salinas-Zeballos, 1972). Wide exposure of the dorsal thalamus helped to protect adjacent structures as well as to spare damage to regional circulation. Six cats were maintained for a median of 107 days (range 20–189 days). Thalamectomy was practically complete and additional damage occurred in some animals, but this was slight and restricted to portions of the internal capsule, laterally, to the fornix, rostrolaterally, and to the mesencephalic tegmentum, caudally (in different animals). One has to assume that there was some retrograde degeneration of corticothalamic cells, but this was not ascertained. Are athalamic cats the mirror image of diencephalic animals? To some extent they are, but not in all aspects.

6.2 Non-rapid eye movement sleep and drowsiness

Pre- and postsomniac S postures are absent in these cats and this indicates that the thalamus is indispensable to sustain them. When stopping motor activity the cats lie down at random or displaying a distorted crouching position. A further postural relaxation and decline in EMG activity together with the corresponding oculo-pupillary behavior heralds the beginning of NREMS; however, once again, the exact transition point cannot be determined with precision. ECoG S spindles are absent from the very beginning. This, together with the absence of S postures, demonstrates the absence of typical NREMS in athalamic cats. Only slow, high-voltage waves, including delta waves and also (in hindsight) the slower <1 Hz oscillations (Steriade et al., 1993), in all similar to the NREMS pattern seen in intact cats, are present in the ECoG (Fig. 6). Obviously, then, spindles are not necessary for the initiation of NREMS slow oscillations. By the time that ECoG fast activity has reappeared, behavioral D starts with intermittent slow waves in the frontal leads; these waves quickly spread throughout the cortex and increase in amplitude and frequency of appearance to soon become a fully developed NREMS activity. At this time, olfactory stimuli are most effective in arousing these animals, with the acoustic and visual modalities following in that order. However, the effectiveness of the stimuli depends on how long after the onset of NREMS the stimulus is applied and on the degree of high-voltage activity that is present at the time, much in the same way as described in the IF. Also as in the IF, a time is reached when an EEG/behavioral arousal cannot be induced even following prolonged (over 1 min) olfactory stimulation. These findings are interesting because they indicate the existence of NREMS.
pressure, thereby suggesting that NREMS pressure/rebound are not mediated by the thalamus (see 3.3).

Therefore, while S spindles are still present in diencephalic cats, the EEG findings in athalamic cats exactly mirror the results in the former animals in that delta waves are absent in diencephalic cats, while spindles are absent in athalamic cats. As mentioned (see 5.2), this agrees with current studies on the cellular bases of these oscillations showing that delta waves and the slower oscillations persist in the neocortex following acute ipsilateral destruction of the thalamus (Steriade et al., 1993). Furthermore, slow high-voltage waves, albeit fragmented, are also seen in the isolated hemisphere (see 4.2). Indeed, the latter microelectrode studies, as well as those in the thalamus mentioned above (see 5.2), have carried to the cellular level, albeit in anesthetized animals, the basic ECoG and EThG findings reported by us long before in our performing diencephalic and athalamic cats.

6.3 Rapid eye movement sleep

Typical episodes of REMS occur soon after thalamectomy, but as in W, there is also a lasting REMS-ECoG uncoupling. However, it is interesting that ECoG fast activity is consistently seen earlier, by 15 days post-thalamectomy, during behavioral REMS than during W, suggesting a more powerful influence of REMS, compared with W, upon the ECoG. The finding of ECoG fast activity shows that, during REMS, the brainstem can activate the neocortex via an extrathalamic route (activation during W might be assisted by sites in the forebrain; see Structures which support S–W in the isolated forebrain). Once started, the duration of fast ECoG activity also depends on the density of pontine PGO bursts and/or on the magnitude of the high-voltage activity present at the onset of REMS. In each recording session, there is also a delay between the onset of the REMS markers and the beginning of fast ECoG rhythms; this reaches up to a few min by 15–20 days postsurgery, but shortens to seconds after 2 months (Fig. 6).

Athalamic cats are also insomniac, but not to the extent of diencephalic cats. The mean amount of REMS time in the ten 24 h recording sessions (first on day 5 and last on day 180 postlesion) ranges from 1.3 to 3.5% (controls, 13.8%), with little variability of the SD. For NREMS the mean range from 10.7 to 18.5% for the sessions from days 5 through 100, while there is a tendency to further decline during the last four sessions (days 120 through 180) with values of 6.5, 10.0, 8.0, and 5.5%, respectively (controls, 38.0%).

In brief, in terms of REMS, changes are mainly quantitative in both diencephalic and athalamic cats. The effects upon NREMS, however, include in addition, marked qualitative changes which render this state atypical for both animal groups, but in quite different ways. While for the long-term diencephalic cats the main components missing are electrographic, particularly the slow waves marker, for the athalamic animals, slow waves persist and dominate, but the behavioral-postural signs are missing. A common denominator for the two animal models is the absence of D and the concomitant spindles for reasons that, as explained (see 3.2), we now understand well. NREMS and REMS pressure and rebound are preserved in both animal groups.

6.4 Effects of drugs

(a) Thiopental, as expected, does not induce ECoG spindle waves in athalamic cats (Villablanca and Salinas-Zeballos,
1972). Nevertheless, using the same paradigm as in diencephalic cats, a small dose of thiopental (14.2 mg kg\(^{-1}\), i.p.) also produces a strong S rebound in these animals. For REMS the values were a mean of 2.7% in the 4 h prior to administration of the drug versus 13.5% in the 4 h postdrug. For NREMS the values were 13.4% prior and 50.7% after thiopental, respectively. These differences are also highly significant (\(P < 0.005\), Student’s t-test).

(b) The effects of metrazol are no different than in intact animals; i.e. a dose of 10.0 mg kg\(^{-1}\) i.v. in athalamic cats triggers a grand mal behavioral tonic–clonic seizure with the corresponding generalized cortical hypersynchrony discharges (Villablanca and Ip, 1971).

The most dramatic finding in diencephalic and athalamic cats comes from the clinical literature. It is currently widely recognized that extensive atrophy/degeneration of the thalamus, particularly of the nuclei medialis dorsalis and anterior ventralis, is at the core of the fatal familial insomnia syndrome (see Marini et al., 1990). Perhaps the strongest support for our finding of insomnia in athalamic cats comes from the clinical literature. It is currently widely recognized that extensive atrophy/degeneration of the thalamus, particularly of the nuclei medialis dorsalis and anterior ventralis, is at the core of the fatal familial insomnia syndrome (see Marini et al., 1994), a disease that also courses with intense agitation and in athalamic cats. Additional support for this theory comes from the finding that if a small dose of barbiturate is given to cats made insomniac by a POA lesion, there is an REMS rebound similar to, albeit smaller, than the one in our diencephalic cats (Lucas et al., 1980).

We know of only one other paper on the S–W effects of total thalamectomy (Naquet et al., 1965). The ablation was performed via cortical penetration and the cats survived only 2–4 days. Spindle waves were absent from the outset. Behaviorally, the cats exhibited periods of ‘intense agitation’, but the authors did not report EEG/behavioral dissociation (although their Fig. 7 shows a panel with prominent NREMS waves contrasting with a very active EMG). S–W was not quantified, but the authors stated that ‘the phase of sleep with fast activity was very short or may not exist at all’. Authors performing unilateral or selected nuclei lesions reported a reduction of REMS to 5% of recording time (lesion of specific nuclei, Angeleri et al., 1960), or a decrease of both NREMS and REMS (unilateral removal, lesion of n. medialis dorsalis or ventralis medialis, see Marini et al., 1990). Perhaps the strongest support for our finding of insomnia in athalamic cats comes from the clinical literature. It is currently widely recognized that extensive atrophy/degeneration of the thalamus, particularly of the nuclei medialis dorsalis and anterior ventralis, is at the core of the fatal familial insomnia syndrome (see Marini et al., 1990), a disease that also courses with motor, autonomic, and hormonal hyperactivation (Lugaresi et al., 1986). In addition, total insomnia lasting for 72 h was recorded following bilateral stereotaxic thalamectomy in a patient (Bricolo, 1967).

7. INSOMNIA, SLEEP PRESSURE AND REBOUND IN DIENCEPHALIC AND ATHALAMIC CATS

The most dramatic finding in diencephalic and athalamic cats was the persistent insomnia, followed by the surprising discovery that this was not permanent, as just a small dose of barbiturate can trigger impressive REMS and NREMS rebounds. However, there is no insomnia/hyperactivity in decerebrate cats (see 2.3), indicating that the S–W imbalance is created at ventral forebrain sites, including the hypothalamus and the basal forebrain. To understand this imbalance, and based on descriptions in 3.3 and 3.4, we would propose that: (i) the hypothalamic-basal forebrain ‘switch’ is controlled by telencephalic structures; (ii) this control normally facilitates the VLPO/POA S-promoting, inhibitory components of the switch; (iii) removal of the telencephalon disfacilitates this S-promoting process with a resulting disinhibition (or ‘release’) of the posterior hypothalamus-mesopontine reticular formation W-promoting, excitatory side of the switch. The overwhelming consequence of the imbalance would then be behavioral hyperactivity and polygraphic W-arousal dominance with a strong functional suppression of NREMS and REMS. That the latter is indeed a functional imbalance is shown by the dramatic S rebound effect of low doses of barbiturate in diencephalic cats; this drug probably acts by depressing the excitability of the arousal sites (much like the classical barbiturate blockage of the ascending reticular activating system). That dominance of excitatory effects is involved is supported by our experiments using morphine. It is well known that this opiate has strong excitatory effects in cats (e.g. de Andrés et al., 1984; Villablanca et al., 1984), and we have reported (Villablanca, 1994) that a single small dose of morphine produces total and prolonged S suppression as well as behavioral agitation in diencephalic cats. Additional support for this theory comes from the finding that if a small dose of barbiturate is given to cats made insomniac by a POA lesion, there is an REMS rebound similar to, albeit smaller, than the one in our diencephalic cats (Lucas et al., 1980).

But why should there be insomnia in athalamic cats with the telencephalon essentially intact? As discussed above, there is no doubt that the thalamus has an important role in the generation of D and NREMS, and that spindle waves and delta oscillations, respectively, are most likely involved in this function. Thus, total/partial absence of the thalamus most probably underpins the insomnia in athalamic cats and humans with thalamic familial insomnia. Whether the insomnia effect is mediated by ventral diencephalic S–W mechanisms, is not as yet known. In addition, it is possible that some of the telencephalic modulation of the ventral diencephalon (see above) may occur via the thalamus. Regardless, we would still argue that telencephalic sites are the dominant influence in balancing the ventral telencephalon because the diencephalic cats show more insomnia/hyperactivity than the athalamic animals.

The processes for homoeostatic regulation of NREMS are self-contained in the forebrain, as demonstrated by the presence of both NREMS pressure and rebound in the IF and in diencephalic/althalamic cats. As for REMS (see 2.3), only pressure is seen in decerebrate animals after deprivation, showing that the homoeostatic control of this state is only partially located in the brainstem. Obviously, therefore, a ‘permissive’ descending forebrain influence is needed to elicit a rebound. Because REMS rebound can be elicited in diencephalic cats, we have proposed (de Andrés et al., 2003) that this influence descends from the hypothalamus. The thalamus, albeit only residual in diencephalic cats, does not appear to play a direct role, as REMS rebound can also be elicited in athalamic cats. Therefore, it is likely that the same general S–W interactions that we discussed with regard to the putative ‘hypothalamic switch’, also operate to ‘permit’ or to block homoeostatic REMS rebound and, perhaps, the very occurrence of REMS. Prime candidates for this descending regula-
tion are pathways from: (i) the lateral hypothalamus orexin/hypocretine cell groups, which are largely lost in human narcolepsy (see Siegel, 2004); and (ii) the galaninergic neurons in the extended VLPO n. (Lu et al., 2002). The hypothalamic arcuate (infundibular) n. appears to also be involved, at least in REMS rebound (Zhang et al., 1987).

We argued (2.3(e)) that REMS pressure is independent of prior occurrence of NREMS. The evidence is less strong, but still substantial, that REMS rebound is also independent of NREMS. We have argued that a typical NREMS does not exist in dienecephalic cats; however, a strong REMS rebound can be reliably elicited in these animals. For these reasons we are strongly inclined to believe that the presence of NREMS is also not required for the occurrence of REMS rebound (Villablanca et al., 2003), and that therefore, the accumulation of REMS pressure and its actual expression as a rebound, is the result of the absence of REMS per se rather than the consequence of an NREMS effect on REMS homeostasis, as suggested by others (Benington, 2002; Benington and Heller, 1999).

8. THE TELENCEPHALON

8.1 The cerebral cortex

Our experiments that first suggested neocortical participation in S regulation were part of a study of spindle waves generation by the thalamus in an acute cat preparation (Villablanca and Schlag, 1968). We found that destruction of the thalamo-orbitofrontal connections in an otherwise intact hemisphere, abolishes both thalamic and cortical spindles. However, the spindles fully reappear after complete neocorticization, indicating that the effect is not mediated by orbitofrontal-recticular formation connections (French, 1958). We interpreted this result as being due to antagonistic corticothalamic influences upon S spindles generation in which the orbitofrontal cortex would enhance and the rest of the neocortex would suppress spindle production.

Later on we conducted long-term experiments to ascertain if frontal cortical areas do play a role in controlling the amount of S–W (Villablanca et al., 1976b). Such role was suggested by studies on the effects of low-frequency electrical stimulation of frontal cortex sites that reported induction of S in cats (e.g. Alnes et al., 1973; Peñalosa-Rojas et al., 1964). Our cats had bilateral removal of the frontal pole (Villablanca et al., 1976a) in front of A22 (atlas of Reinoso-Suárez, 1961). S–W and motor activity were evaluated by means of eleven 24 h polygraphic recording/observation sessions starting at postsurgical day 5 and continued every 10–15 days for the first 3 months and every 10–30 days for three additional months. A significant, albeit moderate, reduction of REMS is seen when comparing the grand mean session values for the duration of the study between the afrontal (11.5% of recording time) and control (15.5%) cats. This reduction is coupled with a significant increase in W: afrontal (48.8%; control, 37.9%) and only a tendency to a decrease in NREMS. These changes were permanent as the statistical significance persisted through at least the third month. The amount of motor activity was evaluated by scoring epochs of highly active EMG, abundant non-patterned eye movements, and movement artifacts in the records. The grand mean percent time for motor activity, for the duration of the study, also showed a significant increase in afrontal cats (25.1% of the time) compared with control cats (16.5%), and this also was a persistent effect. We do not know of other reports using frontal-lobotomized animals to study S–W control. At least two studies on the effects of prefrontal lobotomy in non-psychotic patients reported either a lasting increase in W time coupled with a decrease in delta sleep time (Hauri and Hawkins, 1972), or a decrease in REMS (Hosokawa et al., 1968) in two lobotomized patients.

8.2 The caudate nucleus

A number of studies reported induction of S and/or motor relaxation following cholinergic (Hernández-Péon et al., 1967) or electric stimulation of the caudate n. in cats (e.g. Lineberry and Siegel, 1971; Parmeggiani, 1962), and in humans and monkeys (Heath and Hodes, 1952). We assessed in cats, if the effects of caudate n. removal would support these results. Bilateral aspiration of the caudate n. was performed through a small cortical midline opening penetrating the gyrus cinguli (area 24) and the corpus callosum, thereby exposing the dorsomedial aspects of the caudate in the lateral ventricle. Cats with almost total caudate ablation and little additional damage were maintained in excellent conditions and indefinitely for the first time (Villablanca et al., 1976a). This procedure does not produce any abnormal, persistent changes in the ECoG, as formerly reported (Kennard, 1943), such that the records were scored as for normal cats. Caudate nuclei ablation also alters the S–W pattern of the cats, but not permanently (Villablanca et al., 1976b). Comparisons of the partial grand means for the 24 h sessions of the first postlesion month show a significant reduction of REMS in acuadata cats (10.8%) relative to control animals (15.5%). Wakefulness significantly increases during this period, from 37.9% of the recording time in control cats to 58.2% in acuadata animals. There is only a tendency to a decrease in NREMS time. This pattern persists through the second month, but the values normalize during the third month and remain non-significantly changed thereafter. Hyperactivity also occurs in acuadata cats and persists throughout the study. The grand mean for motor activity time for all sessions reaches 27.3% for acuadata cats, compared with 16.5% for controls. There is a decline between the first month (31.1%) when compared with the third month (24.0%), but these values are still significantly greater than in the controls. The values stay at significantly higher levels through the end of the study.

The suppressing effect of caudate removal upon REMS (or the W-enhancing effect) lasts only for a couple of months and, hence, its meaning is hard to evaluate. However, these results complement those on the effects of low frequency caudate electrical stimulation cited above. In addition, these results

only partially explain the difference in results between our diencephalic cats and Jouvet’s neodecorticate cats (caudate n. present), as the reduction in REMS in our acaudate cats was only of about 4.0% while REMS was almost entirely suppressed in our diencephalic cats and not at all in Jouvet’s animals. The effects of caudate lesions on S–W had been examined previously only in rats (e.g. Corsi-Cabrera et al., 1975), but their lesions were partial and, more importantly, abundant cortical–subcortical fibers cross the caudate n. in rats and these axons were destroyed by the lesion method employed in those studies.

The above results indicate that both the frontal cortex and the caudate are involved in controlling the level of CNS activation. Thus, caudate ablation resulted in permanent hyperactive cats with a significant decrease in REMS time, which lasted for only 2 months, while the reverse was true for frontal cats where the impact upon REMS was permanent. In Section 7, we postulated the existence of an inhibitory telencephalic system balancing a powerful ventral diencephalic mechanism for W/arousal/motor activity. The above data suggest that the frontal cortex and the neostriatum may be a part of this putative inhibitory system.

The hippocampus is another telencephalic structure that has been implicated in S–W control. Electrical stimulation induces S (Passouant and Cadilhac, 1962), or triggers S preparatory behavior in cats (Parmeggiani, 1962). Conversely, bilateral ablation of the hippocampus (Kim et al., 1975) reduces the total time spent in both NREMS and REMS. Moreover, destruction of the septum (Parmeggiani and Zanocco, 1963) also decreases total REMS time (which might be due to the destruction of hippocampal fibers).

The common denominator in the above results is that ablation of any of the forebrain structures studied tilts the S–W balance towards W, while their electrical stimulation induces S. These observations suggest that the effect of removing telencephalic structures is unspecific and perhaps additive; i.e. obliteration of most of these sites would have a maximum impact, as we have indeed found out in diencephalic cats.

9. A PHYSIOLOGIC ROLE FOR REMS

A consequence of the permanent absence of REMS in the IF is that it becomes a two state organ, W and NREMS. This condition offers a unique clue into the function of REMS. It is clear that S continuity is preserved during any given S cycle in mammals; particularly in species with a circadian rhythm, as in humans, that normally S for many hours at a time. In the absence of REMS this could be accomplished by prolonging NREMS episodes. However, this is not what we see in the IF where the duration of NREMS epochs remains limited because, after each NREMS episode, the IF quickly transits into W. Therefore, W in the IF appears as a sort of ‘default’ state that persistently interrupts the continuity of S. A limited duration of NREMS episodes appears to be the rule as it has been seen in: (a) normative studies in humans (e.g. Rechtschaffen and Kales, 1968), and animals (Ursin and Sterman, 1961), and including values in the control cats in our studies; (b) in all our experiments with brain-lesioned animals, after the early postsurgical stabilization period, NREMS epochs are also self-limiting in duration; (c) during rebound after deprivation; for example, in cats deprived of S for 5–7 days using reticular formation stimulation, the duration of NREMS epochs did not increase, while there was a large increase for REMS episodes (see Lucas, 1975). In addition, a similar effect was reported in humans after a shorter deprivation of only REMS (Beersma et al., 1990). Therefore, this is an important characteristic of NREMS which has been rarely emphasized (e.g. Horne, 2000). Consequently, an obvious conclusion is that, in absence of REMS and as in the IF, normal subjects would wake up every time an NREMS episode ends, thereby fragmenting the S cycle. We propose, therefore, that one important function of REMS is to maintain the species-specific duration of the S cycle by precluding awakening after each NREMS episode.

Can the above hypothesis be tested in intact subjects? Most REMS deprivation studies involve an intervention to awake the animal at the onset of REMS and, therefore, cannot be used to test this hypothesis. Pharmacologically induced REMS deprivation may be of use as the S–W parameters can be examined without directly manipulating W. Human studies of long term REMS deprivation induced by antidepressant drugs are particularly interesting as the most popular drugs act by maintaining high brain levels of serotonin and norepinephrine, thereby also blocking REMS. In general, these studies report what the hypothesis would predict, i.e. partial or total suppression of REMS, S discontinuity, and increased W time, without noting any increases in duration of NREMS periods (e.g. Kupfer et al., 1978; Luthringer et al., 1996; Salin-Pascual et al., 1997; Sharpley and Cowen, 1995). Two of these studies used Venlafaxine, which inhibits the reuptake of both serotonin and norepinephrine, and best illustrate the effects just mentioned in patients with major depression (Luthringer et al., 1996) as well as in normal volunteers (Salin-Pascual et al., 1997). The latter study is of special value for our hypothesis as the authors measured W time (fivefold increase by the last day of the drug) as well as the number of phase shifts (2.5-fold increase), with the advantage that REMS was entirely suppressed in their subjects. Also illustrative is the case of a man living with practically no REMS for 13 years after a shrapnel produced a localized pontine lesion (Lavie et al., 1984; NREMS was reduced to an average of 4 h and 26 min/night and the periods were interrupted by brief lapses of W (regardless his condition, the patient became a lawyer).

A posteriori we found out that a similar role for REMS was previously proposed (Horne, 2000), ‘because non-REM cannot be sustained continuously during sleep’. Horne also suggested that the ‘appearance of REM during S may not necessarily be due to a build up of REM pressure’ and that in the absence of any need for W, REMS then appears by ‘default’. However, current knowledge does not support these premises. The fact is that during a S cycle there are two equally viable choices to...
follow NREMS: W and REMS. For one of these states to be able to co-opt the other there must be a physiologic reason. We suggest that the reason is REMS homeostasis (pressure), which has priority of expression over W because, as argued below, REMS appears to be a stronger physiologic state than W (the important caveat, of course, is that this would not apply if the W system is assisted by arousal stimuli). In 2.3(d) we cited three facts supporting the notion that REMS has powerful ascending and descending synaptic influences. In addition, we were impressed by the findings that (a) in our athalamic cats fast ECoG activity is recovered earlier postlesion during REMS than during W (Wakefulness), and, (b) ontogenetically, REMS matures before NREMS does (e.g. Valatx et al., 1964). It then is quite possible that the synaptic inputs that drive the neocortical neurons during the low-voltage fast rhythm of REMS are stronger than those during W, and that this is what enables REMS to prevail over W by the end of each NREMS epoch.

Important as this proposed role for REMS might or might not be, the hypothesis certainly originates fresh questions. Would nature create a state as complex as REMS for this function alone? Probably not. Perhaps the most paradoxical property of REMS (also called ‘paradoxical sleep’) is that it can have negative and positive consequences for the individual, particularly humans. On the one hand, the reports on effects of anti-depressants mentioned above (and see Horne, 2000), almost routinely state that there were no ‘adverse psychologic effects’ ascribable to REMS suppression (although it is not clear how this conclusion has been ascertained). It is also well known that REMS deprivation alone can improve depressed patients (e.g. Vogel et al., 1980). Therefore, REMS appears to be a depressogenic condition. Moreover, surprising but increasing data suggest that monoamines may help neurogenesis in the hippocampus (e.g. Santarelli et al., 2003), and yet their brain levels are at a minimum during REMS and are boosted by antidepressants. On the other hand, REMS may have other important functions, particularly during development, as highlighted by Rechtschaffen (1998) in his excellent review. And then, why not simply prolong the duration of NREMS episodes? Could the indefinite or repetitive prolongation of NREMS, with its underlying neuronal membrane hyperpolarization, be somehow detrimental for the brain? In this regard, Horne (2000) speculates that NREMS mechanisms ‘may have to recover during sleep and perhaps enter a refractory phase’. However, as discussed in Section 7, it is more plausible that NREMS cessation depends on an active physiologic ‘switch’ mechanism leading to REMS or arousal. Finally, and unfortunately, discussing the role/relevance of REMS beyond what our data permit goes beyond the goals of this essay.

10. THE HIERARCHICAL ORGANIZATION OF THE S–W SYSTEM AND SOME PERSPECTIVES

All systems with widespread physiologic influences upon the body, e.g. the motor and the somatosensory-sensorial systems, possess a highly complex and highly distributed brain functional anatomy. Control of S and W is no exception and, consequently, we suggest that we should rather speak of an S–W system. The question is whether, besides this complexity, there is any integrative organizational model which could be applied to the regulation of the system. Our answer is a resounding yes and the reasons were preliminarily published in 1974 (Dement and Villablanca, 1974). Based on our experiments, which by design progressively disconnected the ‘lowest’ from the ‘highest’ brain structures, we argued that the control of S–W is organized hierarchically, loosely along the lines proposed by H. Jackson for the control of the sensorimotor system in intact or damaged brains (see Evans, 1972; Walsh, 1961). What we have learned since 1974 has only strengthened this viewpoint (we were unaware of a previous paper with somewhat similar concepts; Parmeggiani, 1968). There are two main principles in the Jacksonian conception: hierarchy of organization in the intact CNS, and ‘dissolution’ or deterioration of function in the damaged brain. Hierarchy implies that, as one moves caudo-rostrally, the anatomo-functional levels of control in the CNS evolve from simple to complex, from rigidly organized to flexible and adaptive, and from automatic/reflex to more ‘voluntary’, at the highest level. The concept also implies a dominant control of the lowest by the highest level with the notion of prevalence of inhibition of the descending influences (which, in absence of the higher control, results in a ‘release’ of the lower functional areas; e.g. postural decerebrate rigidity). As Jackson’s concept emphasizes a downstream control, this approach fits very well the organization of the S component of the system. However, we cannot ignore that W is as important as S, and that, given the strong role of the reticular formation core in W/arousal, the ‘ascending’ aspect within the normal organization of W cannot be ignored (as pointed out when discussing NREMS in the IF, 3.1. 3.4(a)). Nevertheless, from the standpoint of deterioration of function, the Jacksonian concept fully applies to both S and W (see below).

Within this view, the Siegel laboratory experiments of the 1980s established that the medulla is the lowest, simplest, control level. In cats with a midpontine transection, or below, only an unpatterned alternation of unspecified quiescence and activity periods exists. But, as discussed in Section 2, in these animals, quiescence is not true S and activity cannot be equated with true arousal. Mesencephalic animals, particularly those with a transection in front of the oculomotor n., exhibit an integrated, persistent, W-arousal behavior, but not signs whatsoever of any awareness (e.g. while I was still inexperienced, a walking decerebrate cat, upon touching my ankle by chance, suddenly clamped on my Achilles tendon!). These animals show typical REMS, only missing the rebound component of recovery following selective deprivation. But, NREMS is entirely absent so that only periods of quiescence persist, akin to D but lacking the S spindles which are the EEG signature of D. Midbrain decerebrate cats are not hyperactive or insomniac, at least in terms of REMS and because D compensates for absence of NREMS; this, coupled with the
finding that S–W in decerebrate animals can occur independently from the forebrain, could deceitfully lead one to believe that there is no suprasegmental control over brainstem S–W. Fitting with the Jacksonian view, there are features of automaticity in S at this level, i.e. REMS can be elicited as a reflex and some complex behaviors may be triggered automatically (e.g. biting, as above). In this context, it is interesting that some REMS features can also be elicited as reflexes in narcoleptic patients and dogs, thereby suggesting that narcolepsy is a condition in which the executive mechanism of REMS is no longer being controlled from a higher, presumably hypothalamic, level.

Diencephalic animals display full W/arousal and even show some behaviors suggesting elementary awareness (see 5.1). NREMS shows progressive ‘dissolution’. For the first 3–5 days, D occurs as in intact cats, but thereafter with the spindle-generating neurons gone, only the remaining S postures suggest the presence of D, but the beginning and end of this state cannot be precisely delimited. Therefore, the disconnected diencephalon, while intact, can still sustain D. In contrast, typical NREMS is absent from the very beginning as delta waves, which are the EEG marker for this state, are never seen again after telencephalecetomy. The pre- and postsomniac postures that persist, are sustained by the residual thalamus, as they are absent in athalamic cats. In brief, in long-term diencephalic animals only an atypical, fragmented NREMS state persists, thereby portraying the progressive ‘dissolution’ of S. REMS persists in these cats but, like the atypical NREMS, it is drastically restricted in duration.

In terms of the motor system, Jackson’s middle level consists of the sensory and motor cortical areas together with their ascending and descending pathways. In the loose application of Jackson’s principles envisioned here, we propose that the diencephalon, jointly with the S–W cellular groups of the basal forebrain, are the middle level for the control of S–W. This level is pivotal as it possesses the switch-like properties, provided by the hypothalamus/basal forebrain, which steer the brain to alternate between S and W. As emphasized in 3.4, the ‘flip-flop’ properties highly impressed us when observing the stereotyped NREMS–W switching of the IF and after learning, later on, that the alternation could be canceled by lesions of the anterior or posterior hypothalamus in the IF (see 3.3). Conceptually, the key role of the hypothalamus has been gaining momentum since the early pioneering proposal of Von Economo (1930). In 1974 we could not invoke structures, pathways or neuromodulators that could play a role in this descending regulation, but that state of affairs has remarkably changed. We now know that S-promoting and W-promoting sites of the basal forebrain and hypothalamus can modulate in an inhibitory or an excitatory manner, respectively, the activating ascending reticular formation, together with the monoaminergic nuclei (locus coeruleus and raphe n.) involved in both W/arousal and in REMS regulation (see 3.3 and 3.4). However, the ventral forebrain does much more for S–W than being a ‘switch’. The hypothalamus, in particular, is a key structure for coordination: (i) of telencephalic and brainstem S–W influences; (ii) with other bodily functions including thermoregulation, water, autonomic, and endocrine balances (for references, see Steininger et al., 2001); and perhaps, (iii) with the circadian oscillator in the suprachiasmatic n. (e.g. see Colwell and Michel, 2003). As illustrated by our athalamic cats, the thalamus also plays important middle level control functions in both S (Structures which support S–W in the isolated forebrain, a) and W.

For Jackson, the highest level resides in the frontal cortex and ‘posterior part of the cerebrum’. For the S–W system, we propose that the top level is the telencephalon. Removal of the highest level uncovers its most important S–W role, i.e. maintaining the balance between the S- and W-promoting sites of the middle level ‘switch’ process. The main sign of this regulation is inhibitory as shown by the overwhelming dominance of W and motor activity in diencephalic cats with quasi absence of REMS and NREMS. In Jacksonian terminology a ‘release’ of the W/behavioral activity-promoting sites of the ventral brain has taken place and blocking the action of the ‘released’ structure (e.g. by thiopental in our cats), just confirms the functional nature of this phenomenon. The suppression of REMS in diencephalic cats is so strong that we suggest that, even in intact animals, REMS cannot occur unless ‘permitted’ by the forebrain. In addition, it is clear that a permissive, most probably hypothalamic-basal forebrain influence, is required for REMS rebound to occur in the decerebrate animal (see 2.3 and Section 7). In terms of hierarchy, it is also illustrative that injections of carbachol in the basal forebrain suppress REMS generation that would normally occur following injection of this cholinceptive drug in the lower REMS-related sites of the pons (Baghdoyan et al., 1993). In addition, the pattern of POG sequencing is changed after neodecorticization (Gadea-Ciria, 1977).

The REMS-controlling mechanisms of the brainstem are intricate, but investigators have, to a large extent, deciphered their circuitry and neurotransmitters. In contrast, we could hardly make the same statement regarding S–W mechanisms of the forebrain. Thus, in comparison, and as predicted by the Jacksonian hypothesis, the organization of S–W control at brainstem level appears to be much simpler than that at forebrain level. Indeed and, unfortunately, knowledge on the role of the telencephalon in S–W regulation remains limited. How does the telencephalic control operate? As discussed in this essay, a number of forebrain structures influence S–W. These include the neocortex (e.g. our afrontal cats), the caudate n. (our acaudate cats), the amygdala (Charifi et al., 2000), and the limbic system (e.g. Crochet and Sakai, 2003). The cerebellum is also involved (e.g. Cunchillos and de Andrés, 1982). Do all these sites operate by modulating the middle level? Is the entire cortical mantle involved and are there ‘agonist’ and ‘antagonist’ cortical areas? Is there a direct control over hypothalamic sites as our experiments would suggest, and/or does the highest level operate through the thalamus and/or directly through the brainstem (e.g. Lindsley et al., 1972)? Which type of neurons and neurotransmitters/modulators participate? A bright spot within this hiatus is the
body of knowledge, at the cellular electrophysiology level, on the S–W-related thalamic-cortico-thalamic interactions (see 5.2). In terms of hierarchy, there is evidence that the very slow oscillations (0.5–1.0 Hz) of cortical neurons control the synchronization of synaptic and intrinsically generated delta oscillation as well as organize the coherence of spindles in the thalamus (e.g. Steriade, 2001). But how this thalamocortical ‘subsystem’ interacts with the other forebrain ‘subsystem’, i.e. the ventral forebrain ‘switch’, remains little explored, with a few exceptions (e.g. Asanuma and Porter, 1990; Gritti et al., 1998), and this certainly limits our understanding of the integrated role of the thalamus in S–W regulation.

The highest level is certainly responsible for a number of other sophisticated and important S–W functions, including the voluntary maintenance of alertness as required, e.g. in humans, for the performance of nocturnal jobs and social functions; some behaviors preparatory for S, including, in cats, a search for a place to sleep and grooming; and the nictohemeral periodicity of S in some species (e.g. in dogs; Kleitman and Camille, 1932); all of which are absent in decorticate animals. Most importantly, and in compliance with the concept of hierarchy, the highest level provides the functional flexibility which is absent in the lower levels. For example, our collective transection/ablation and chemical site-inactivation experiments provide the notion of a rigid cascading inhibition of W-arousal-motor activation processes (see also McGinty and Szymusiak, 2004). And yet, by virtue of a putative brain site(s), which most probably resides in the telencephalon, an animal or human can override it all and stay awake, e.g. to opportunistically catch a prey or to read a great novel, respectively. Our ignorance of how the telencephalon regulates the middle and lower functional levels underlines the dire need for research to understand the highest level of the S–W system. We believe that the time is overdue to turn more of our attention to these forebrain processes because identifying them would lead to a better understanding of normal as well as abnormal S–W conditions.

Finally, there is an important organizational feature of the S–W system which sets it apart from other complex CNS systems. It possesses two ‘modules’, the forebrain and the brainstem, that can sleep and wake independently from each other (albeit differently). We argued that the IF, or upper level ‘module’, can sustain not only W but also awareness (see 4.3). We believe that we can now move one step further. In an excellent presentation of facts and arguments, it was proposed (Llinás and Paré, 1991) that the substrate for consciousness is in the forebrain and consists of the cortico-thalamo-cortical subsystem, assisted perhaps by the cortico-amygdala-cortical circuitry. The proposal includes the concept that, within this context, W and REM sleep are fundamentally the same type of functional state’, so that the hypothesis applies to both the oneric, restricted consciousness of dreaming during REMS as well as to the integrated full consciousness of W. We fully agree with this hypothesis. It follows, therefore, that the neural basis for consciousness should be present in the IF, and that it does not really matter whether the physiologic state of the IF during behavioral and ECoG arousal is closer to that of W or to that of REMS (see 3.3). Consequently, we propose that the IF should also be capable of supporting consciousness, and this makes our plea for documenting the presence of a putative IF in humans all that more pressing.

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