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Variations in Extracellular Levels of Dopamine, Noradrenaline, Glutamate, and Aspartate Across the Sleep–Wake Cycle in the Medial Prefrontal Cortex and Nucleus Accumbens of Freely Moving Rats

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We used intracerebral microdialysis coupled with electrophysiologic recordings to determine relative changes in the concentrations of several neurotransmitters in the medial prefrontal cortex and nucleus accumbens of freely moving rats during waking, slow-wave sleep, and rapid eye movement (REM) sleep. The concentrations of noradrenaline, dopamine, glutamate, and aspartate in 2-min dialysate samples were analyzed by capillary electrophoresis combined with laser-induced fluorescence detection. Changes in glutamate and aspartate concentrations were found only in the nucleus accumbens, in which a decrease was obtained during both slow-wave sleep and REM sleep compared to waking. A progressive reduction in the release of noradrenaline was observed from waking to REM sleep in both structures. In contrast, dopamine concentrations were higher during waking and REM sleep compared to that during slow-wave sleep. The latter results demonstrate that contrary to the findings of earlier electrophysiologic studies carried out on ventral tegmental area dopaminergic neurons, changes in the release of dopamine in projection areas occur across the sleep–wake cycle. The elevated levels of dopamine during waking and REM sleep in the medial prefrontal cortex and the nucleus accumbens could result from changes during these two states in afferent modulation at the level of cell bodies or at the level of dopaminergic terminals.

Key words: catecholamines; excitatory amino acids; REM sleep; microdialysis; limbic areas

Sleep–wake alternation associated with the generation of characteristic cortical oscillations during waking, slow-wave sleep (SWS), and rapid eye movement (REM) sleep results from complex interactions between different neuronal populations. These interactions involve marked and distinct changes in the firing rate of mesopontine and basal forebrain cholinergic neurons, monoaminergic neurons (i.e., locus coeruleus noradrenergic neurons, dorsal raphe serotoninergic neurons, and tuberomamillary histaminergic neurons) as well as hypothalamic γ-aminobutyric acid (GABA)ergic neurons across the sleep–wake cycle (Gottesmann, 1999; Hobson and Pace-Schott, 2002).

Among the monoaminergic neurons, dopaminergic (DA) neurons from the substantia nigra (SN) and ventral tegmental area (VTA) do not exhibit any significant change in their mean discharge rate across the sleep–wake cycle (Miller et al., 1983; Trulson and Preussler, 1984). Several indirect findings, however, suggest that sleep–wake state-related changes in the activity of the DA system originating from the VTA may occur. The release of acetylcholine, a neurotransmitter known to activate DA VTA neurons (Gronier and Rasmussen, 1998; Forster and Blaha, 2000), is maximal during both waking and REM sleep compared to that in SWS (Williams et al., 1994; Marrosu et al., 1995). The recently identified orexin peptides, which have been implicated in the sleep disorder narcolepsy (Lin et al., 1999), are present within the VTA (Peyron et al., 1998) and display the same pattern of release as acetylcholine does across the cycle (Kiyashchenko et al., 2002). Furthermore, neuroimaging studies in humans have shown that limbic areas innervated by DA VTA neurons such as the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) are activated during waking and REM

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sleep and deactivated during SWS (Braun et al., 1997; Nofzinger et al., 1997). Finally, Maloney et al. (2002) have reported an increase in c-Fos expression in rat DA neurons in the VTA but not SN during REM sleep. Taken together, these data suggest that DA release may be higher in VTA projection areas during waking and REM sleep compared to that during SWS.

In addition to a substantial DA innervation from the VTA, the NAc, and mPFC receive numerous excitatory amino acid projections and reciprocal interactions (direct or indirect) have been described between DA and glutamatergic systems in these structures (Morari et al., 1998; Tzschentke, 2001). Variations across the sleep–wake cycle in glutamate and aspartate release could therefore reciprocally affect DA release. Finally, noradrenergic (NA) inputs are also present in the NAc and mPFC and electrophysiologic data (Hobson et al., 1975; Aston-Jones and Bloom, 1981) suggest that NA release would decrease from waking to REM sleep.

We examined the concentrations of DA, NA, glutamate, and aspartate in dialysates from the NAc and mPFC across the sleep–wake cycle in freely moving rats. Given the short duration of REM sleep in rats, microdialysis was coupled to capillary electrophoresis with laser-induced fluorescence detection making it possible to quantify neurotransmitters concentrations in 2-min dialysate samples (Parrot et al., 2003).

MATERIALS AND METHODS

Animals and Surgery

All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Male Wistar rats (Charles River, Lyon, France) weighing 280–320 g were anesthetized with pentobarbital (60 mg/kg, intraperitoneally) and mounted on a stereotaxic frame. Guide cannulae (CMA Microdialysis, Stockholm, Sweden) were implanted bilaterally, one in the left mPFC (AP from bregma, +3.0 mm; L, 1.6 mm angled 10 degrees toward midline; V, −2.8 mm) and the other in the right NAc (AP, +1.6 mm; L, 1.3 mm; V, −6.2 mm) according to the atlas of Paxinos and Watson (1986). Silver ball recording electrodes were also implanted: two were placed bilaterally in contact with the frontal cortex and two on the occipital cortex. Two additional electrodes made of twisted stainless steel wires were inserted bilaterally in the dorsal neck muscles to record the electromyograms. A ground electrode was attached via a screw to the skull and all electrodes were soldered to a common connector (Samtec, Torcy, France). The two guide cannulae, the connector and the screws were secured to the skull using dental cement. Rats were given two to three weeks to recover after surgical implantation and were housed individually with ad lib access to food and water with a 12/12-hr light/dark cycle (lights on from 8:00 AM to 8:00 PM). Two days before electrophysiologic recordings coupled to microdialysis the rats were moved to the recording room to habituate them to the new environment.

Electrophysiologic Recordings

Animals were connected to the polygraph via a rotating electrical connector. Three states of the sleep–wake cycle were classified according to a standardized procedure (Portas et al., 1998; Gervasoni et al., 2000; Arnaud et al., 2001): (1) waking (active and nonactive) characterized by fast low-voltage activity in the frontal area and by high muscular tone; (2) SWS identified by higher amplitude slow waves associated with spindles and reduced muscular tone; and (3) REM sleep associated with fast low-voltage activity in the frontal cortex, theta rhythm (5–10 cycles/sec synchronized waves) in the occipital cortex, and the absence of muscular tone except for brief contractions. Sleep-waking states were analyzed second-by-second and the percentage of time scored as waking, SWS, or REM sleep during consecutive 2-min periods was calculated to enable direct comparison with dialysate samples, which were collected over the same 2 min.

In Vivo Microdialysis

On the day of the microdialysis experiment, two concentric microdialysis probes (CMA/12, 500-μm diameter, and 20 kDa cut-off) with a membrane length of 3 mm for mPFC or 2 mm for NAc were inserted into the guide cannulae and perfused with Ringer's solution (NaCl, 149 mM; KCl, 2.7 mM; $MgCl_2$, 1 mM; $CaCl_2$, 1.2 mM; Na_2HPO_4, 2.33 mM; and NaH_2PO_4, 0.45 mM) at a flow rate of 1 μl/min. After a 4-hr stabilization period, dialysates were collected at 2-min intervals with a fraction collector maintained at 4°C and stored at −80°C before analysis by capillary electrophoresis. Dialysates collection coupled to electrophysiologic recordings lasted approximately 6 hr. To synchronize electrophysiologic recordings with sample collection, we took into account the time delay due to the dead volume (13 μl) of the microdialysis system (probe and output tubing).

Catecholamine and Amino Acid Analysis by Capillary Electrophoresis

The dialysate samples (2 μl) were sorted according to the percentage of time scored as waking (around 50/50% of active and nonactive periods), SWS, or REM sleep during 2-min intervals. The samples chosen to be analyzed by capillary electrophoresis corresponded to a minimum of 80% of one state during the entire 2-min period.

The concentrations of catecholamines (DA and NA) and amino acids (glutamate and aspartate) in each dialysate sample were measured using an automatic P/ACE MDQ system (Beckman Coulter, Fullerton, CA) equipped with an external laser-induced fluorescence ZETALIF detector (Picometrics, Ramonville, France). The excitation was carried out using a He-Cd laser (Liconix, Santa Clara, CA) at a wavelength of 442 nm. Separations were carried out with a 52 cm × 50 μm internal diameter (ID) fused-silica capillary having an effective length of 39 cm (defined as the length between the injection point and the on-column detection point).

The method used was adapted from that described previously by Bert et al. (1996). On the day of the analysis, 2 μl of dialysates or standard solutions were derivatized by adding 0.8 μl of a mixture (1:2:1 vol/vol/vol) containing the internal

standard (3.10×10^{-7} M dihydroxybenzylamine [DHBA] or 10^{-5} M aminoadipic acid [AAD] for catecholamine or amino acid determination, respectively), a borate/sodium cyanide (NaCN) solution (100:20 vol/vol; borate buffer, 500 mM, pH 8.7; NaCN, 87 mM), and naphthalene-2,3-dicarboxaldehyde (NDA; 2.9 mM in acetonitrile/water, 50:50 vol/vol) as the fluorogene agent. Catecholamine or amino acid analysis was carried out using 110 mM phosphate buffer (pH 7.05) or 75 mM borate buffer (pH 9.2), respectively. The applied voltage was 25 kV. The hydrodynamic injection of the dialysates (20 sec at 2 psi for catecholamines or 10 sec at 0.2 psi for amino acids) was followed by an injection of orthophosphoric acid (sample stacking procedure).

Histology

After the termination of each experiment, animals were sacrificed and their brains were rapidly removed. Serial sections of the brains were prepared for verification of probe placement.

Chemicals

NDA and NaCN were purchased from Fluka (Buchs, Switzerland). DA, NA, DHBA, DL-glutamate, L-aspartate, AAD, boric acid, and sodium tetraborate were obtained from Sigma (St. Louis, MO), and mono- and di-basic sodium phosphate were obtained from Carlo Erba (Rodano, Italia).

Statistical Analysis

For each rat, catecholamine or amino acid levels in the NAc and mPFC were obtained from mean of three to five 2-min dialysates collected during the three states of the same sleep–wake cycle. Results are expressed as means ± standard error of the mean (SEM) of concentrations (not corrected for in vitro recovery) of n rats. Separate groups of animals were used to determine the concentrations of catecholamines ($n = 6$) and amino acids ($n = 7$). Comparison of the concentrations of catecholamines and amino acids between waking, SWS, and REM sleep were carried out using one-way analysis of variance (ANOVA) for repeated measures. Paired t-tests were used for posthoc comparisons.

RESULTS

Histologic Verification

Figure 1 shows rat coronal sections and schematic drawings illustrating the placement of the microdialysis probes in the NAc and the mPFC.

Electrophysiologic Recordings and Capillary Electrophoresis Analysis

Figure 2 illustrates the characteristic electrophysiologic patterns of waking, SWS, or REM sleep recorded during 5 sec. These states did not necessarily last the entire 2-min interval corresponding to the dialysate sample. This was especially evident during REM sleep episodes, which are short in rats (2–4 min at most). The mean duration of each state, expressed as a percentage, during the 2-min dialysates chosen for analysis by capillary electrophoresis were 98.26 ± 0.9% for waking, 95.15 ± 0.6% for SWS, and 88.40 ± 0.8% for REM sleep.

Figure 3 shows typical electrophoregrams observed with dialysates obtained from the NAc during waking, SWS, or REM sleep in catecholamines or amino acids separation conditions.

Noradrenaline Levels in the NAc and mPFC Across the Sleep–Wake Cycle

The mean concentrations of NA obtained in dialysates from the NAc and mPFC during the three states are shown in Figure 4A. In the NAc, the levels of NA decreased during SWS ($4.34 \times 10^{-10} \pm 0.6$ M) compared to that during waking ($6.8 \times 10^{-10} \pm 1.35$ M) and decreased further during REM sleep ($3.7 \times 10^{-10} \pm 0.41$ M). One-way ANOVA for repeated measures indicated that the NA levels were significantly different between the three states ($P = 0.009$). Posthoc analysis of the NA levels showed a significant difference between waking and both SWS and REM sleep ($P < 0.05$).

In the mPFC, the extracellular levels of NA displayed a pattern of change similar to that found in the NAc, with the highest concentrations occurring during waking and the lowest during REM sleep. The mean concentrations of NA in dialysates were $20.9 \times 10^{-10} \pm 3.82$ M during waking, $12.67 \times 10^{-10} \pm 2.78$ M during SWS, and $9.98 \times 10^{-10} \pm 2.12$ M during REM sleep. The difference in NA concentrations was significant between waking and both SWS and REM sleep ($P < 0.01$).

Dopamine Levels in the NAc and mPFC Across the Sleep–Wake Cycle

In the NAc (Fig. 4B), the DA levels were significantly different between the three states ($P = 0.02$). The concentrations of DA in dialysates were higher in both waking ($7.72 \times 10^{-10} \pm 1.72$ M) and REM sleep ($8.56 \times 10^{-10} \pm 1.74$ M) compared to that in SWS ($6.21 \times 10^{-10} \pm 1.36$ M). The difference in DA levels between waking and SWS was significant ($P < 0.05$) as was that between SWS and REM sleep ($P < 0.05$).

In the mPFC, the concentrations of DA were maximal during waking ($3.14 \times 10^{-10} \pm 0.35$ M), minimal during SWS ($1.47 \times 10^{-10} \pm 0.16$ M), and intermediate during REM sleep ($2.3 \times 10^{-10} \pm 0.41$ M). The DA levels were significantly different between waking and both SWS and REM sleep ($P < 0.01$). The difference in DA levels between SWS and REM sleep was not significant ($P = 0.07$).

Excitatory Amino Acid Levels in the NAc and mPFC Across the Sleep–Wake Cycle

A significant decrease in the concentrations of glutamate in dialysates obtained from the NAc (Fig. 5A) was observed during both SWS ($P \leq 0.05$) and REM sleep ($P \leq 0.05$) compared to that during waking. The

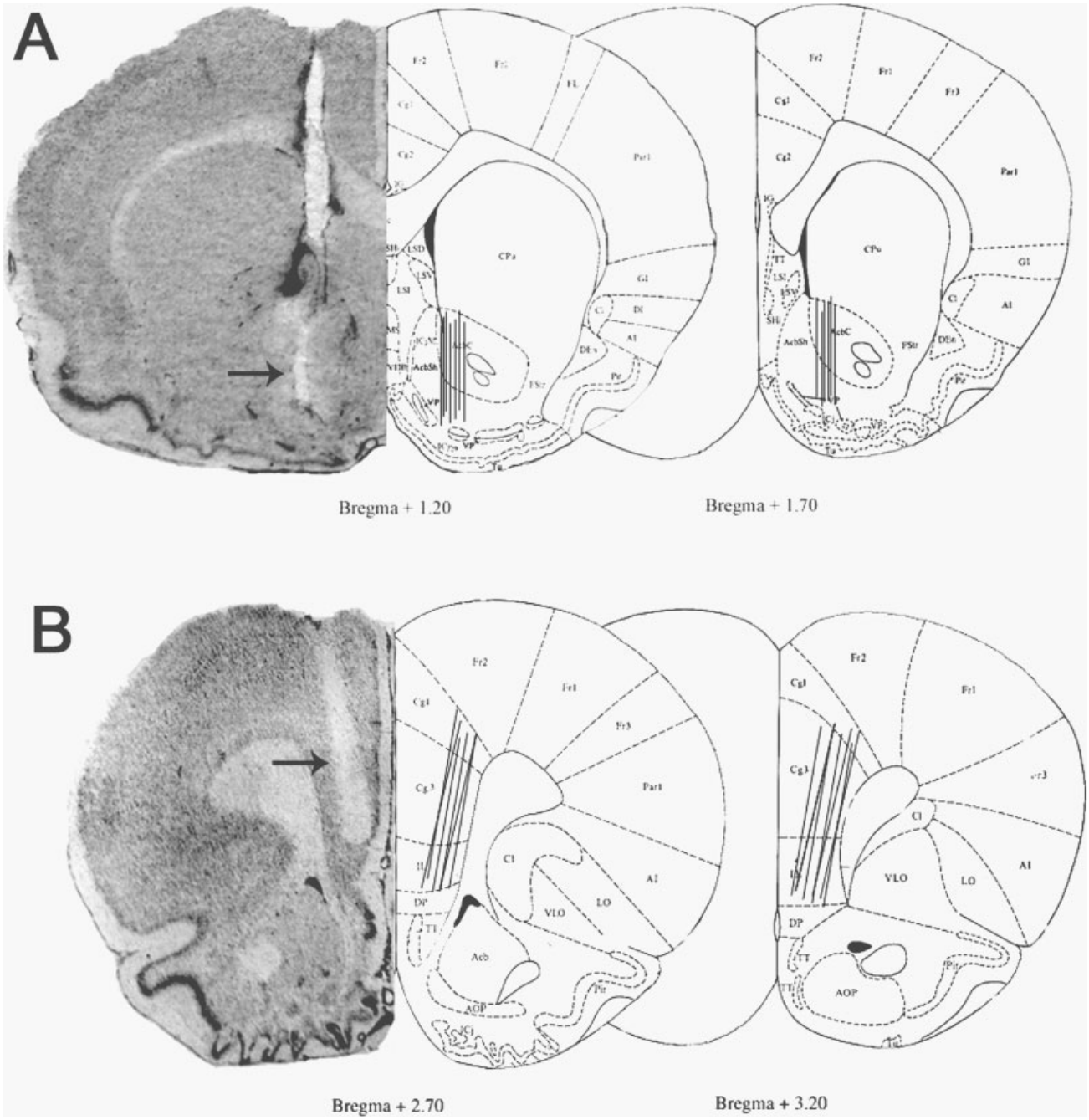

Fig. 1. Location of the microdialysis probes in the nucleus accumbens (NAc; **A**) and the medial prefrontal cortex (mPFC; **B**). The histologic sections (left) show the tract of a microdialysis probe and the arrow indicates the location of the dialysis membrane. The schematic drawing (right) adapted from Paxinos and Watson (1986) represents the tracts of the dialysis membranes (2 mm for NAc and 3 mm for mPFC) in all experiments ($n = 13$). AcbC, core of the NAc; AcbSh, shell of the NAc; VP, ventral pallidum; Tu, olfactory tubercules.

mean concentrations of glutamate were $9.28 \times 10^{-7} \pm 2.89$ M during waking, $5.69 \times 10^{-7} \pm 1.53$ M during SWS, and $5.57 \times 10^{-7} \pm 1.47$ M during REM sleep. In contrast, glutamate dialysate levels in the mPFC did not change significantly during the three states of the cycle (mean concentrations: $16.2 \times 10^{-7} \pm 2.9$ M during waking, $16.1 \times 10^{-7} \pm 2.5$ M during SWS, and $15.9 \times 10^{-7} \pm 2.6$ M during REM sleep).

The concentrations of aspartate (Fig. 5B) in dialysates obtained from the NAc exhibited a pattern of change comparable to glutamate with a decrease during both SWS ($2.24 \times 10^{-7} \pm 1.3$ M) and REM sleep

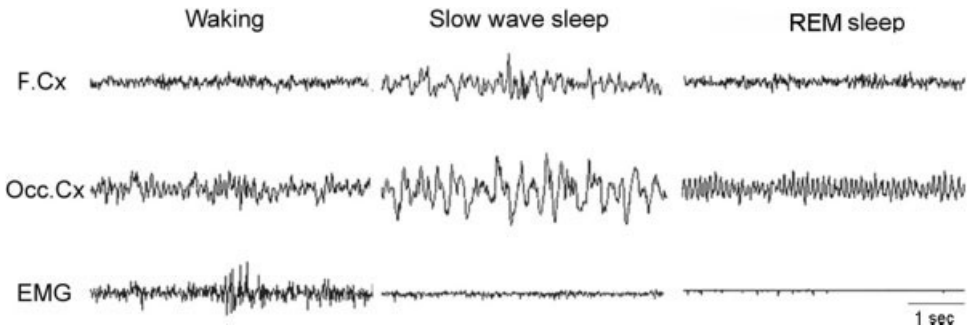

Fig. 2. Electrophysiologic activity obtained during waking, slow-wave sleep, or REM sleep. FcX, frontal cortex; Occ.Cx, occipital cortex; EMG, electromyogram.

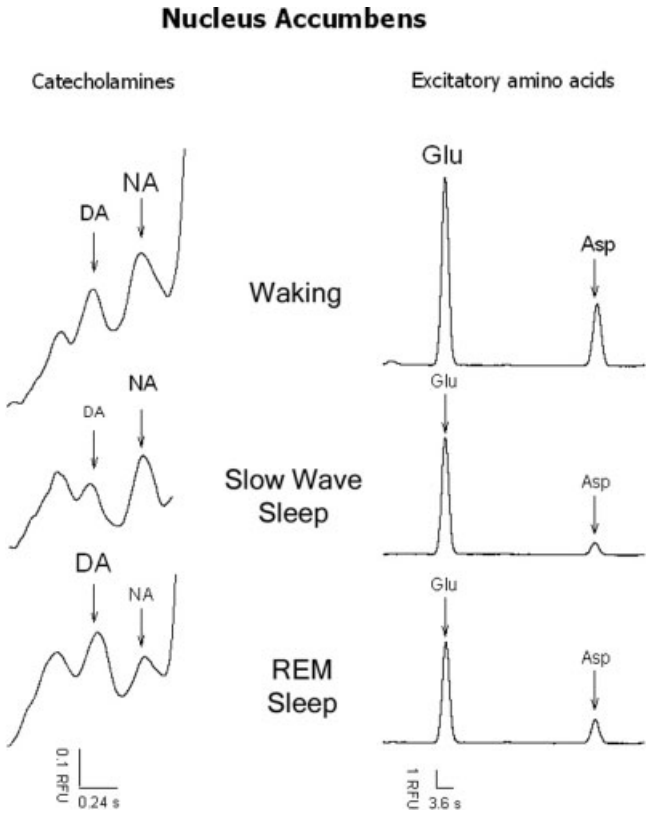

Fig. 3. Typical electropherograms obtained during analysis of catecholamines (left) or excitatory amino acids (right) in 2-min microdialysates obtained from the nucleus accumbens across the sleep–wake cycle. Dopamine (DA) peak areas are the highest during REM sleep whereas noradrenaline (NA) peak areas significantly decrease during both slow-wave sleep and REM sleep. Glutamate (Glu) and aspartate (Asp) peak areas decrease during both slow-wave sleep and REM sleep.

($2.25 \times 10^{-7} \pm 1.21$ M) compared to that during waking ($5.1 \times 10^{-7} \pm 2.3$ M). One-way ANOVA for repeated measures indicated that the aspartate levels were significantly different between the three states ($P = 0.03$) but posthoc analysis showed no significant difference. Similarly to glutamate, no change in aspartate dialysate levels were observed in the mPFC (Fig. 5B) across the sleep–wake cycle (mean concentrations: $2.51 \times 10^{-7} \pm 0.18$ M during waking, $2.79 \times 10^{-7} \pm 0.58$ M during SWS, and $2.33 \times 10^{-7} \pm 0.28$ M during REM sleep).

DISCUSSION

The present study was designed to evaluate the neurochemical changes that occur in the NAc and mPFC during waking, SWS, and REM in freely moving rats. Changes in the extracellular levels of DA and NA were observed in both structures. DA levels were higher during waking and REM sleep compared to SWS whereas NA levels decreased progressively from waking to REM sleep. In contrast, state-related changes in the levels of glutamate and aspartate were found only in the NAc.

The most important finding of the present study is the existence of changes in the extracellular levels of DA in the terminal regions of VTA DA neurons over the course of the sleep–wake cycle. These changes could result from variations during the cycle in the activity of afferents either at the level of DA cell bodies in the VTA or at the level of DA terminals in limbic areas.

In the VTA, DA neurons are known to discharge in vivo either in a single spike or bursting mode. It has been established that burst stimulations, although having the same mean frequency as regularly spaced spikes, are twice as potent at inducing release of DA (Gonon, 1988; Chergui et al., 1994). Previous electrophysiologic studies have demonstrated a relatively unchanged mean discharge rate of VTA DA neurons across the sleep–wake cycle; however, changes in the discharge pattern of these neurons were not examined (Miller et al., 1983; Trulson and Preussler, 1984). The increased release of DA during waking and REM sleep compared to that during SWS may therefore be explained by an increased burst activity of VTA DA neurons, which would not necessarily alter the mean discharge rate during these two states. It has been reported that burst activity of VTA DA neurons can be induced by glutamatergic or cholinergic afferents. Glutamatergic afferents from functionally and anatomically distinct regions have been implicated in the regulation of burst activity. These areas include the pedunculopontine nucleus, which is associated with the control of the sleep–wake cycle, and the mPFC (Kitai et al., 1999). With respect to the mPFC, the relative stability in the concentrations of glutamate and aspartate over the sleep–wake cycle cannot corroborate or exclude this assumption, because these levels most likely represent

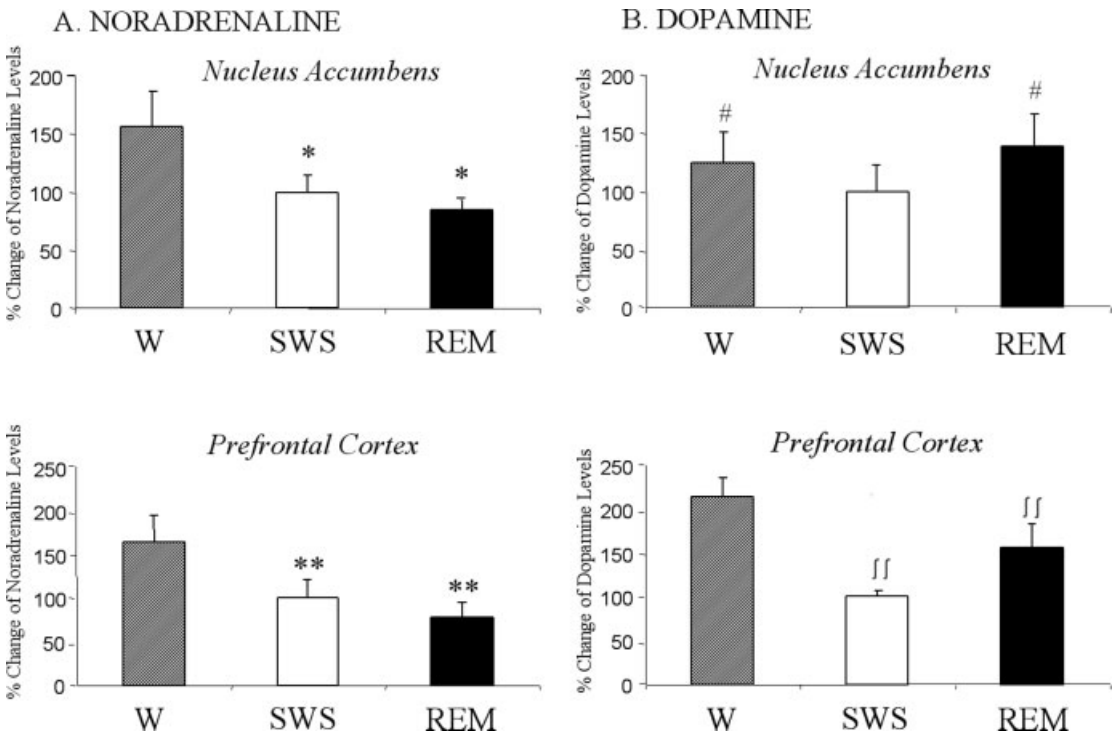

Fig. 4. Noradrenaline (**A**) or dopamine (**B**) levels in the nucleus accumbens and medial prefrontal cortex across the sleep–wake cycle. Results are expressed as mean ± SEM of the percentage change as a function of slow-wave sleep (SWS) ($n = 6$ rats). $^{*}P < 0.05$, $^{**}P < 0.01$ compared to waking (W); $\#P < 0.05$ compared to SWS; $\int\int P < 0.01$ compared to waking (paired t-test).

the activity of glutamatergic afferents rather than efferents. Concerning mesopontine cholinergic afferents, activation of nicotinic and muscarinic receptors on VTA DA neurons has been shown to increase bursting activity and DA release in projection areas (Grenhoff et al., 1986; Nisell et al., 1994; Gronier and Rasmussen, 1998; Forster and Blaha, 2000; Gronier et al., 2000). Interestingly, VTA DA neurons are innervated by the collaterals of mesopontine cholinergic neurons sending projections to the thalamus (Oakman et al., 1999), a key structure in the control of cortical activation during the different states, and in which the release of acetylcholine is higher during waking and REM sleep compared to that during SWS (Williams et al., 1994), as we observed for DA release. Finally, the recently described orexin peptides may contribute to the changes in DA levels we observed during the sleep–wake cycle. Nevertheless, although only an excitatory effect has been demonstrated to date in other neuronal populations (Kilduff and Peyron, 2000), evidence for such an action on VTA DA neurons is lacking.

In support of the notion that the activity of afferents to VTA DA neurons may change over the sleep–wake cycle, at least during REM sleep, Maloney et al.(2002) have reported an increase in c-Fos expression, i.e., in neuronal activation, in rat VTA DA neurons, but not SN DA neurons, in association with naturally enhanced REM sleep during rebound from REM sleep deprivation.

An alternative explanation for changes in the release of DA over the sleep–wake cycle may be a modification in the activity of afferents at the level of DA terminals in the NAc and mPFC, rather than at the level of DA cell bodies in the VTA. This could explain, in part, the relatively unchanged mean discharge rate of VTA DA neurons described in the two earlier electrophysiologic studies. It has been thus postulated that an increase of DA release during REM sleep might occur in limbic areas and result from a presynaptic mechanism of disinhibition consecutive to the silence of noradrenergic and serotoninergic neurons during this state (Gottesmann, 2002, 2004). Another possibility could be a presynaptic interaction between glutamatergic afferents and DA terminals (Morari et al., 1998).

Finally, it is possible that changes in the activity of afferents across the sleep–wake cycle could occur at the level of both VTA DA cell bodies and DA terminal fields and could concomitantly contribute to the changes in DA release.

The present results may seem inconsistent with two previous microdialysis studies examining the release of DA across the sleep–wake cycle but may be explained by different experimental procedures. On the one hand, Shouse et al. (2000) did not detect any difference in the DA concentrations across the sleep–wake cycle in the amygdala and the locus coeruleus of the cat. DA concentrations in these two areas, however, seem to be close to

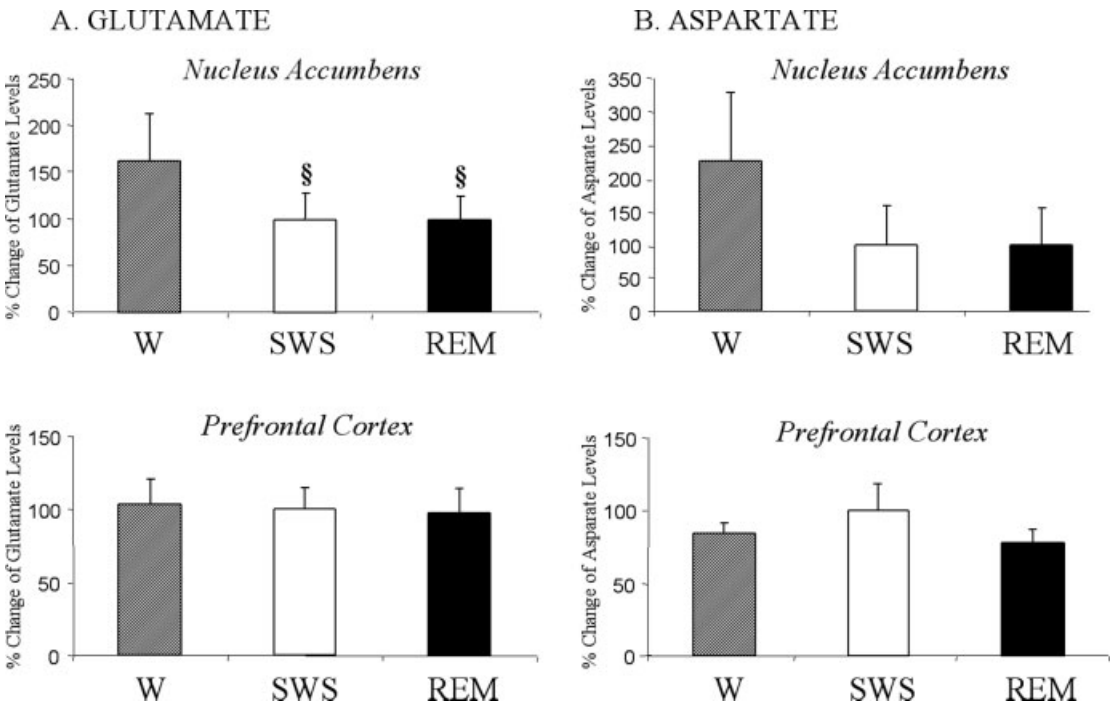

Fig. 5. Glutamate (**A**) or aspartate (**B**) levels in the nucleus accumbens and medial prefrontal cortex across the sleep–wake cycle. Results are expressed as mean ± SEM of the percentage change as a function of slow-wave sleep (SWS) ($n = 7$ rats). §$P \leq 0.05$, compared to waking (W; paired t-test).

the limit of detection of the analytical technique used, i.e., high performance liquid chromatography (HPLC) combined with electrochemical detection. On the other hand, de Saint-Hilaire et al. (2000) reported in the rat PFC an unexpected decrease of DA release during waking compared to that during SWS as well as bidirectional changes of DA levels during REM sleep, depending on whether this state was followed by SWS or waking. The areas of the PFC (prelimbic and orbital) in which the DA levels were measured by de Saint-Hilaire (2000) were not the same than those we studied (anterior cingulate, prelimbic, and infralimbic i.e., mPFC) and the DA levels obtained by this group were far higher (10–20-fold) than levels we observed and currently reported in the mPFC (Yonezawa et al., 1998; Lorrain et al., 2003). Secondly, the samples chosen for REM sleep in the de Saint-Hilaire study could represent only 66% of this state, suggesting a possible dilution of REM sleep-specific DA changes.

The functional significance of changes in DA levels in the terminal fields of VTA DA projections across the sleep–wake cycle remains to be determined. It has been proposed that activation of the mesocorticolimbic DA system during REM sleep may participate in the generation of dreams (Solms, 2000; Gottesmann, 2002, 2004). This assumption was based on clinical observations showing that lesions of presumed DA pathways suppressed dreaming without affecting REM sleep (Solms, 1997).

In contrast to the marked changes in the release of DA obtained in the mPFC, the concentrations of glutamate and aspartate in this structure remained relatively constant across the sleep–wake cycle. The mPFC receives numerous excitatory amino acid afferents originating from the dorsomedial thalamus, hippocampus, amygdala, and other cortical areas (Conde et al., 1995). Glutamate and aspartate concentrations measured in the dialysates reflect therefore the combined activity of these inputs, to which it must be added amino acid release from collaterals of pyramidal neurons and from glial cells. It is therefore difficult to determine if the absence of change in glutamate and aspartate concentrations represents a lack of alteration in the activity of excitatory neurons or changes in the opposite direction in these different neuronal and glial populations. In contrast, in the NAc, which receives glutamate and aspartate-containing projections from the mPFC, hippocampus, and amygdala (Zahm and Brog, 1992), glutamate levels decreased significantly during both SWS and REM sleep compared to that during waking. Similarly, a parallel reduction in the concentrations of aspartate was observed in this structure. The decrease in glutamate and aspartate levels during SWS may be related to the decrease in cerebral energy metabolism, which has been described in humans in the NAc during this state (Braun et al., 1997). The decrease in the concentrations of glutamate and aspartate we observed during REM sleep could either reflect decreased activity in one or more structures innervating the NAc or result from a presynaptic inhibition of amino acid release (Nicola et al., 2000) triggered by the increase

in DA release. Because the mPFC, hippocampus. and amygdala are highly activated during REM sleep in humans (Maquet et al., 1996; Braun et al., 1997; Nofzinger et al., 1997), the latter explanation seems the most plausible.

We also found that the release of NA in the NAc and mPFC was higher during waking than during SWS and even lower during REM sleep. This finding is consistent with the progressively reduced firing rate of locus coeruleus noradrenergic neurons that occurs from waking to REM sleep and is required for the generation of REM sleep (Hobson et al., 1975; Aston-Jones and Bloom, 1981). The present results are also in agreement with the only microdialysis study (Shouse et al., 2000) carried out to date examining the release of NA over the sleep–wake cycle, in which a decrease was observed in the locus coeruleus and amygdala of the cat. Our results extend these data to other projection areas of the noradrenergic system, i.e., the NAc and mPFC.

In conclusion, the present study has demonstrated the existence of changes in DA release in both mPFC and NAc as a function of sleep–wake state in rats. During REM sleep, the elevated levels of DA observed in the mPFC and NAc could contribute to the specific cognitive processes taking place during this state. The changes in glutamate levels obtained in the NAc during REM sleep compared to waking could also participate to the typical information processing of that state. Finally, NA levels, as expected from electrophysiologic data, decreased from waking to REM sleep.

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