Neuropsychopharmacology (2007) 32, 1232–1241

www.neuropsychopharmacology.org

Prominent Burst Firing of Dopaminergic Neurons in the Ventral Tegmental Area during Paradoxical Sleep

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Dopamine is involved in motivation, memory, and reward processing. However, it is not clear whether the activity of dopamine neurons is related or not to vigilance states. Using unit recordings in unanesthetized head restrained rats we measured the firing pattern of dopamine neurons of the ventral tegmental area across the sleep–wake cycle. We found these cells were activated during paradoxical sleep (PS) via a clear switch to a prominent bursting pattern, which is known to induce large synaptic dopamine release. This activation during PS was similar to the activity measured during the consumption of palatable food. Thus, as it does during waking in response to novelty and reward, dopamine could modulate brain plasticity and thus participate in memory consolidation during PS. By challenging the traditional view that dopamine is the only aminergic group not involved in sleep physiology, this study provides an alternative perspective that may be crucial for understanding the physiological function of PS and dream mentation.
Neuropsychopharmacology (2007) **32,** 1232–1241. doi:10.1038/sj.npp.1301251; published online 6 December 2006

Keywords: dopamine; ventral tegmental area; bursts; sleep; paradoxical sleep; reward

INTRODUCTION

The dopaminergic mesocorticolimbic system is formed by dopamine neurons located in the ventral tegmental area (VTA), which project to the nucleus accumbens, prefrontal cortex, septum, amygdala, and hippocampus. There is a general agreement that midbrain dopamine neurons play key roles in unpredicted and novel reward processing, although possible activation of dopamine neurons by salient stimuli that are not rewarding is still debated (for review, see Ungless (2004)). Electrophysiological recordings from dopamine neurons in awake animals have demonstrated that these cells fire bursts of spikes in response to novel salient stimuli (Kiyatkin, 1995; Ljungberg *et al*, 1992; Steinfels *et al*, 1983). Electrochemical monitoring of extracellular dopamine concentration showed that this increase in bursting activity is accompanied by a transient increase in dopamine levels (Wightman and Robinson, 2002). When stimuli become familiar or predicted, their presentation fails to activate the dopamine cells (Ljungberg *et al*, 1992; Schultz *et al*, 1993) or to increase the dopamine levels (Kilpatrick *et al*, 2000), indicating that such activations depend on novelty but not on motor components of the behavioral tasks. According to Lisman and Grace (2005), the activation of the dopamine system induced by novel salient stimuli required for memory consolidation is triggered by the hippocampus and the pedunculopontine tegmental nucleus (PPN). This hypothesis is based on the observation that novelty induced increase in extracellular dopamine levels was blocked by intrahippocampal infusion of TTX (Legault and Wise, 2001). The hippocampus is known to activate VTA dopamine cells via a polysynaptic pathway involving the activation of nucleus accumbens which in turn inhibits the ventral pallidum leading to disinhibition of dopamine cells (Floresco *et al*, 2003). The PPN, which sends direct excitatory inputs to the VTA (Omelchenko and Sesack, 2005; Omelchenko and Sesack, 2006), has been shown to be activated by salient stimuli and to participate in dopamine activation induced by salient events (Pan and Hyland, 2005).

In homeothermic animals, sleep is divided into two main distinct stages: slow wave sleep (SWS) and paradoxical sleep (PS). SWS is characterized by high-voltage slow oscillations in the electroencephalogram (EEG) associated with weak electromyographic (EMG) activity. During PS, activity in the forebrain is comparable to waking levels with pronounced and sustained theta rhythm in the hippocampus together with rapid eye movements (REM; PS is also called REM sleep) and a complete loss of muscle tone (Jouvet, 1967). During the past 50 years of sleep research, PS has been associated with various functions, ranging from corneal respiration (Fitt and Gonzalez, 2006) to dreaming (Aserinsky and Kleitman, 1953). It has been proposed

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Received 22 March 2006; revised and accepted 27 September 2006

recently that one possible function of PS is to participate in reconsolidation of newly acquired memories during sleep via off-line replay of previously experienced events (Ribeiro and Nicolelis, 2004; Stickgold *et al*, 2001; Stickgold and Walker, 2005). Indeed, in humans, imaging studies showed that cerebral regions activated during learning are reactivated again during PS following the task (Maquet *et al*, 2000; Peigneux *et al*, 2003). It has also been shown in rats that hippocampal ensemble activity recorded during spatial task is replayed during PS (Louie and Wilson, 2001). Furthermore, the amount of PS increased after spatial learning in the Morris water maze (Smith and Rose, 1997) and specific PS deprivation following such a task impaired following performances (Smith and Rose, 1996).

As the hippocampus is highly active during PS and since pharmacological activation of hippocampus increases the activity of dopamine neurons (Floresco *et al*, 2003; Lodge and Grace, 2006b), one would expect dopamine neurons to be activated during PS sleep. Such a hypothesis is further supported by the fact that PPN, which regulates dopamine cell bursting activity (Lodge and Grace, 2006a), is also strongly activated during PS (Datta and Siwek, 2002; El Mansari *et al*, 1989; Maloney *et al*, 1999; Verret *et al*, 2005). Until now, there have been few reports concerning the activity of dopamine cells during PS. On the one hand, c-fos expression is increased in dopamine cells during a rebound of PS (Maloney *et al*, 2002) and a recent microdialysis study demonstrated an increase in extracellular levels of dopamine in nucleus accumbens and prefrontal cortex during PS (Lena *et al*, 2005). On the other hand, early electrophysiological studies in rats and cats concluded that the firing rate of these neurons was unrelated to the sleep–wake cycle (Miller *et al*, 1983; Trulson and Preussler, 1984). As a result of these latter reports, dopamine neurons are generally viewed as the only aminergic neurons not affected by vigilance states (for review see Pace-Schott and Hobson (2002)). However, among the early electrophysiological studies, there is the suggestion of a possible modification of the firing pattern during PS since an increased variance of interspike intervals during PS was reported (Miller *et al*, 1983). Other studies only considered changes in the mean firing rate of dopamine neurons and overlooked any possible state-dependent shifts in the discharge pattern.

Dopamine neurons, *in vivo*, exhibit tonic irregular single spike firing interrupted by bursts of spikes often with decreasing spike amplitude followed by brief silences (Grace and Bunney, 1983) and it has been shown that the mean firing rate and the bursting pattern of dopamine neurons can be modulated independently (Floresco *et al*, 2003). A state-dependent shift in discharge pattern might be crucial since burst firing of dopamine neurons was shown to result in a much larger synaptic dopamine accumulation than single spike firing (Floresco *et al*, 2003; Gonon, 1988; Venton *et al*, 2003).

To test the hypothesis that mesolimbic dopamine neurons are activated during PS, we examined their activity across vigilance states with special attention to possible changes in the pattern of firing. In order to compare bursting activity of dopamine cells across sleep–wake cycle and bursting activity during a well-documented activation of dopamine system, we performed recordings during the consumption of palatable food. This condition is known to increase extracellular levels of dopamine (Bassareo and Di Chiara, 1997; Westerink *et al*, 1997) with kinetics suggesting sustained burst firing of VTA dopamine neurons (Roitman *et al*, 2004).

MATERIALS AND METHODS

All procedures were approved by the Claude Bernard University ethical committee in accordance with the corresponding European Communities Council Directives (86/609/EEC). They also met international standards and were previously described in detail (Souliere *et al*, 2000; Urbain *et al*, 2000). Experiments were performed during the light part of the 12/12 h light/dark cycle. Twelve male Sprague–Dawley rats (Charles River, l'Arbresle, France) weighing 280–350 g were deeply anesthetized (chloral hydrate 400 mg/10 ml freshly diluted in NaCl, priming injection: 400 mg/kg i.p., supplemented by perfusion: 120 mg/kg/h i.p.). They were mounted in a classical stereotaxic frame with conventional ear and incisors bars. Temperature was maintained at 37°C with a regulated electric heating pad (Harvard Apparatus). Three small stainless-steel screws were turned into small holes bored into parietal and frontal or occipital parts of the skull in order to monitor bipolar EEG. Three steel wires were inserted into the neck muscles in order to record the electromyogram (EMG). A liquid bonding resin (Superbond, Sun Medical, Japan) was then applied on the skull surface to enhance binding of the dental cement, except above the reference point (lambda) needed for positioning further stereotaxic tracks. A U-shaped aluminum piece, fixed to a flexible carriage (GFG Co., Pierre-Benite, France) fastened to the stereotaxic apparatus, was positioned above the VTA and lambda suture. This U-piece was then embedded in dental cement with the EEG screws and EMG wires and their six-pin connector, as already described (Souliere *et al*, 2000; Urbain *et al*, 2000, 2002, 2004, 2006), leaving a well inside the U-piece. At the end of the surgery, the well was closed with bone wax and wound-healing cream (Madecassol, Serdex, France) was poured out on all the borders of the implant to facilitate healing. The rat was then removed from the stereotaxic apparatus and placed in his individual home cage. The U-shaped piece (about 5 g weight) was well tolerated by the rats, which were able to move, sleep, feed, and drink normally in their home cage.

After 2 days of recovery, animals were progressively habituated to the head fixation procedure (8–12 days). Their head was painlessly secured to the stereotaxic frame by screwing the U-shaped piece, cemented to the rat's head, with its associated carriage; their body lying comfortably in a hammock. This procedure allowed forelimb movements thereby enabling the animals to groom and grasp food when offered by the experimenter. At the end of the training period, the rats stayed calm for a period of 5–6 h during which quiet wakefulness, SWS, as well as PS episodes were typically observed. After training, rats were again anesthetized (chloral hydrate 400 mg/10 ml freshly diluted in NaCl, 320 mg/kg i.p., supplemented as needed. Lidocaine was applied locally on the dura mater just before its dissection) and the skull and dura matter were removed. To prevent any infection, an antibiotic solution (neomycin trisulfate

hydrate, Fluka) was laid on the skull inside the well, which was then closed with bone wax. This wound dressing was replaced every day.

After habituation, daily recording sessions were typically performed over a maximum of 7–10 days, each session lasting about 4–6 h. Extracellular recordings of VTA neurons (−5.3 to −6.0 mm posterior, 0.5–0.9 mm lateral and 7.4–8.6 mm ventral to bregma) were performed using glass micropipettes (broken back to an external tip diameter of 2–3 μm, impedance of 6–12 MΩ at 10 Hz) filled with 2% Pontamine Sky Blue in 0.5 M sodium acetate (pH 7.5). The electrode signal was filtered and amplified (0.2–10 kHz; P16, Grass Instruments, West Warvick, USA), fed to a 50 Hz noise eliminator (Hum-Bug, Quest Scientific, Vancouver, Canada) and filtered again (1–3 kHz; 5A22N differential amplifier, Tektronix Co). Single-unit activity (signal-to-noise ratio of at least 3:1) was isolated with an amplitude spike discriminator (CEMI, Lyon, France). Samplings of electrode signal (sampling rate 10 kHz), signal from the spike discriminator and polygraphic signals (EEG and EMG amplified using a P55 from Grass Instruments, sampling rate 256 Hz) were collected on a personal computer via a CED interface (Cambridge Electronic Design, Cambridge, UK) using the Spike 2 software. After each recording session, the well was filled with saline solution (NaCl 0.9%) and covered with wax. At the end of the last recording session, the animals were killed and subsequent histological localization of the recording sites, labeled by iontophoretic deposit of Pontamine Sky Blue during the last three recording sessions, were made on cresyl violet-stained coronal sections.

The activity of some dopamine neurons was monitored during the entire period of consumption of a highly palatable food. Pieces of cake (Galettes Saint-Michel®, France, 0.5–1 g) were given by hand after at least 40 s of basal recording during quiet waking (no predictive cue was intentionally used). Rats were habituated to eat such pieces of cake in their home cages and in the restraining frame during the week preceding the recording sessions. Palatable food was always present in the experimental room in an open box so that the animals were habituated to the smell.

All spontaneously active neurons encountered within the VTA were recorded. To identify the different cell types, we computed average action potentials (at least 30 traces) for each cell and analyzed the shape of the waveform and the duration of action potentials (from the onset to the end). Dopamine neurons were identified by their long-duration triphasic action potentials, the distinction between short and long durations being determined *a posteriori* by the analysis of the repartition of action potential durations of all recorded VTA neurons (see Results).

Further analyses were conducted only on putative dopamine cells. Single unit discharge of dopamine neurons was analyzed off-line using Spike 2 analysis software. Burst detection was performed using a custom Spike 2 script based on the criteria of Grace and Bunney (1984), a burst beginning with the occurrence of two spikes with an interspike interval <80 ms, and ending with the occurrence of an interspike interval >160 ms. Mean firing, percentage of spikes fired in bursts, mean number of bursts per second, mean number of spikes per burst and intraburst firing rate of individual cells were calculated in each vigilance state recorded during at least 20 s. Correlations to EEG rhythm were assessed by computing spike triggered averaging of the EEG signal. The same method was used to analyze the firing and burst activity before and during the palatable food consumption. ANOVA, followed by Tukey–Kramer *post hoc* pair-wise comparisons, were employed to determine if discharge rate and bursting parameters varied as a function of vigilance stages. Student's *t*-test for paired values was used to compare activity before and during eating palatable food and the activity at different times relatively to PS onset and offset. The significance level was set at $p<0.05$ for all statistical analyses. All data are expressed as mean ± SEM.

RESULTS

Characterization of Dopamine Neurons

One hundred twenty-two neurons were recorded within the VTA of 12 rats (see recording sites location Figure 1c). An analysis of the total length of action potentials of all recorded neurons (Figure 1b) divided the population into 3 groups. Twelve neurons exhibited short biphasic action potentials, the others had short ($n=16$) or long ($n=94$)

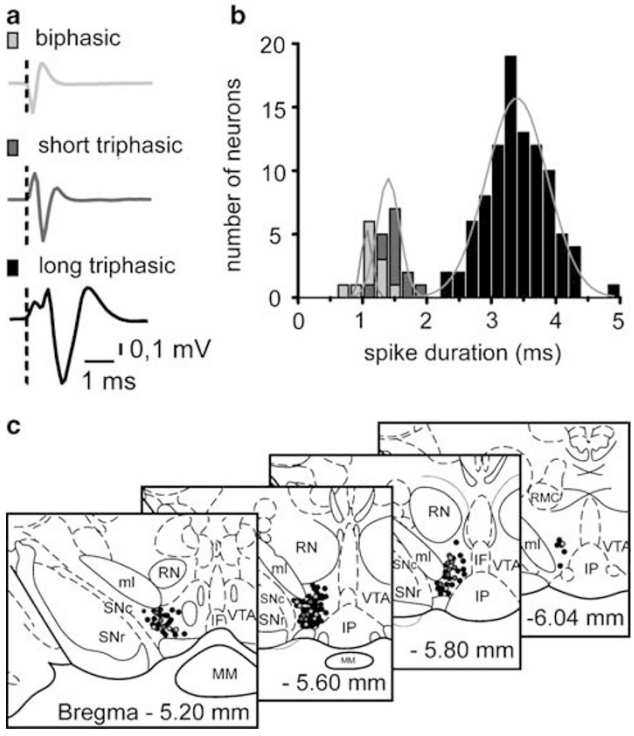

Figure 1 Electrophysiological characterization of three neuronal populations within the VTA. (a) Typical examples of short bisphasic, short triphasic, and long triphasic average extracellular waveforms. (b) Distribution of spike duration of all recorded neurons. Note clear separation of the group of long spikes on the trimodal histogram. A triple-Gaussian fit ($r^2>0.9$) confirms the >2 ms criterion for broad triphasic neurons. (c) Mapping of recording sites within the VTA. Neurons with biphasic, short triphasics, and long triphasic waveforms are represented by light gray, dark gray, and black bars and dots, respectively. IF: interfascicular nucleus, IP: interpeduncular nucleus, ml: medial lemniscus, MM: medial mammillary nucleus, RN: red nucleus, SNc/SNr: substantia nigra pars compacta and recticulata, VTA: ventral tegmental area.

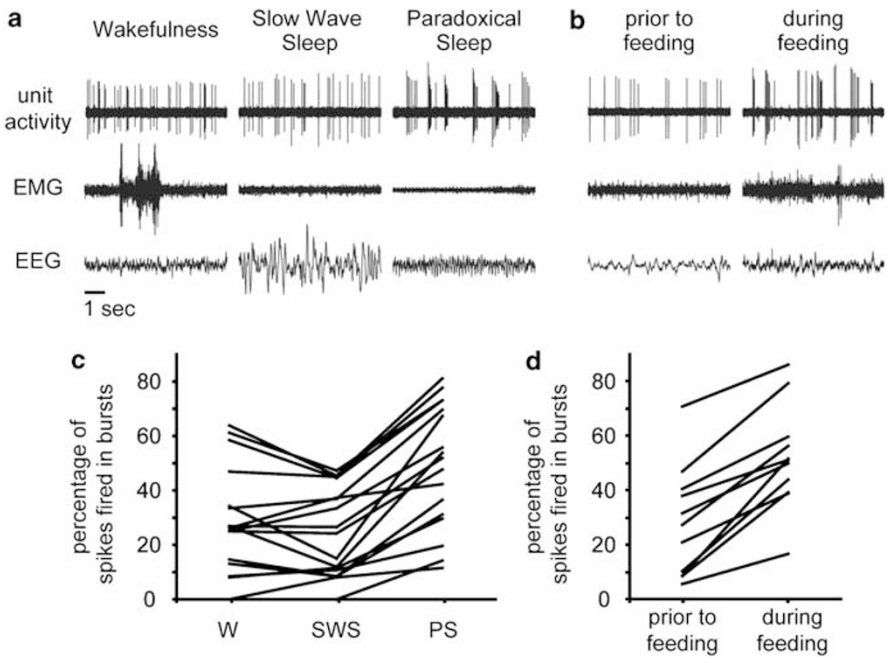

Figure 2 Dopamine neurons switch to a bursting mode during PS and palatable food consumption. (a, b) Shows examples of spike firing pattern (top traces) of two dopamine neurons, one (a) recorded during the three vigilance states and the other (b) recorded before and during palatable food consumption. Sleep wake cycle was monitored by EMG (middle traces) and electroencephalographic (EEG; bottom traces) recordings. Wakefulness (W) is characterized by low amplitude, desynchronized EEG and sustained EMG activity, SWS by high-voltage slow oscillations in the EEG associated with weak EMG activity, and PS is characterized by pronounced theta rhythm and a complete loss of muscle tone. Dopamine neurons firing switched from irregular spiking with few doublets during wakefulness and SWS to a pronounced bursting pattern with many spikes of decreasing amplitude during PS and feeding. Note the lack of modification of firing during the phasic EMG activation during wakefulness. (c, d) Represent the bursting activity (expressed as percentage of spikes fired in bursts) of 17 neurons recorded across all the three vigilance states (c) and 11 neurons recorded before and during the consumption of palatable food (d). Note that bursting increased during PS and feeding across the entire population of dopamine neurons. No statistical difference was observed between W and SWS using *t*-test for paired values.

triphasic spikes (Figure 1a). Both raw data and a triple Gaussian fit showed a clear separation of short and long action potential durations at 2 ms. Therefore, cells with spike duration >2 ms were considered as dopamine cells.

VTA Dopamine Neuronal Activity during the Sleep–Wake Cycle

Among 94 dopamine neurons recorded, 84 were recorded together with EEG and EMG recordings allowing a good sleep–wake cycle monitoring. Among these, 68 were recorded during wake (W), 52 during SWS, and 25 during PS. The activity of one of the 17 cells recorded across the three vigilances states is illustrated in Figure 2a. Irregular activity was observed during quiet W and SWS, with rare bursts composed of a few spikes, mostly doublets. In contrast, the activity during PS switched to a pronounced bursting pattern characterized by numerous bursts composed of several spikes of decreasing amplitude.

The mean firing rate increased slightly but not significantly in PS compared to W and SWS (Figure 3a, W: 3.93 ± 0.16 spikes/s, $n=68$, SWS: 3.95 ± 0.16 spikes/s, $n=52$, PS: 4.63 ± 0.26 spikes/s, $n=25$, ANOVA, $F_{2,142}=3.038$, $p>0.05$). There was no significant difference in the percentage of spikes fired in bursts between W and SWS. However, there was a highly significant increase in this parameter during PS compared to W and SWS (Figure 3b,

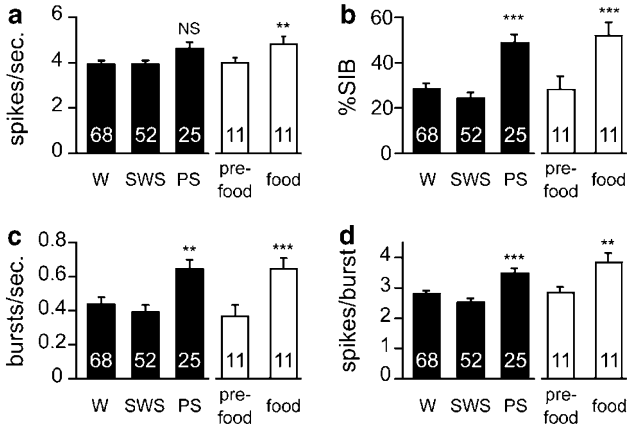

Figure 3 Discharge properties of dopamine neurons during the sleep wake cycle (filled columns) and palatable food consumption (open columns). We measured four complementary parameters: (a) firing rate, (b) the percentage of spikes fired in bursts (%SIB), (c) the number of bursts per second, and (d) the number of spikes per burst. Note the slight non significant increase in the mean firing rate and the large and highly significant increase in all bursting parameters during PS compared to quiet wakefulness (W) and SWS. This increase was very similar to the activation observed during palatable food consumption (food) as compared to quiet wakefulness preceding food presentation (pre-food). Numbers inside each column indicate the number of analyzed cells. NS $p>0.05$; $^{**}p<0.01$; $^{***}p<0.001$ using appropriate tests (ANOVA, followed by Tukey–Kramer *post hoc* pair-wise comparisons for vigilance states and Student's *t*-test for paired values for palatable food consumption).

W: 28.4 ± 2.6, $n = 68$, SWS: 24.3 ± 2.4, $n = 52$, PS: 48.8 ± 3.8, $n = 25$, ANOVA, $F_{2,142} = 13.6$, $p < 0.001$, *post hoc* analysis, $p < 0.05$ for PS compared to W and SWS, NS between W and SWS). Importantly, this increase in the percentage of spikes fired in bursts, which was observed in all 17 neurons recorded across the three vigilance states (Figure 2c), gradually appeared 10–20 s before the onset of PS (Figure 4a), was sustained all along the duration of the PS episode (20–200 s—see example in Figure 5) and suddenly stopped with the offset of PS (Figure 4b).

Burst characteristics were further analyzed in the three vigilance stages by calculating the burst occurrence, the number of spikes per burst and the intraburst frequency. Both burst occurrence (Figure 3c) and the number of spikes per burst (Figure 3d) significantly increased in PS as compared to W and SWS (W: 0.44 ± 0.04 bursts/s, 2.81 ± 0.09 spike/burst, $n = 68$; SWS: 0.39 ± 0.04 burst/s, 2.54 ± 0.07 spike/burst, $n = 52$; PS: 0.63 ± 0.06 bursts/s, 3.49 ± 0.15 spike/burst, $n = 25$, ANOVA, $F_{2,142} = 5.558$, $p < 0.01$ and $F_{2,142} = 19.089$, $p < 0.001$, respectively, Tukey's *post hoc* analysis, $p < 0.05$ for PS compared to W and SWS for both parameters, NS between W and SWS). On the other hand, the mean frequency within bursts remained stable across the sleep–wake cycle (W: 22.1 ± 0.9, SWS: 25.1 ± 1.0, PS: 22.0 ± 1.1 spikes/s, ANOVA, $F_{2,142} = 2.98$, $p > 0.05$). Four neurons never fired a burst during W and one of them never fired a burst during SWS, either.

Averaging of EEG triggered by unit activity did not reveal any correlation between EEG oscillations and neuronal activity in any vigilance state (data not shown).

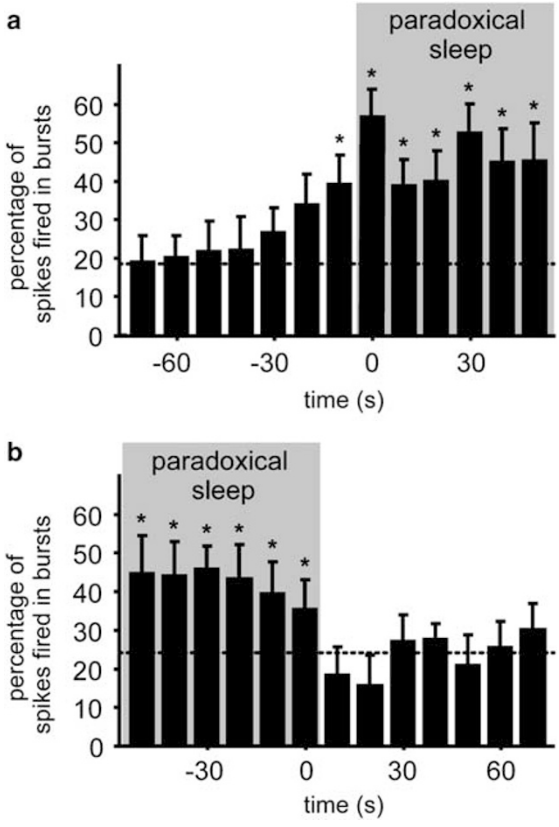

Figure 4 Increase in bursting activity of dopamine neurons anticipates the onset of PS and stops as soon as the animal wakes up from PS. PS onset was defined by the observation of both pronounced theta rythm sychronization on the EEG together with atonia on the EMG, the offset corresponded to awakening characterized by a decrease of theta rhythm and the dispartition of atonia. In all 10 dopamine neurons were recorded during the transitions from SWS to PS (a) and 12 during the transition from PS to W (b). Time zero corresponds to the onset (a) and the offset (b) of PS. Each bar represents the mean percentage of spike in burst measured over 10 s. Dotted lines represent the mean baseline values recorded for this sample of cells during SWS (a) and W (b). Comparison to baseline using *t*-test for paired values shows significant increase in bursting starting before the onset of PS. *$p < 0.05$.

VTA Dopamine Neuronal Activity during Palatable Food Consumption

Eleven dopamine neurons were recorded during the consumption of a highly palatable food. As illustrated in Figure 2b, dopamine neurons switched, during food consumption, to a bursting pattern similar to that observed during PS.

There was a slight increase in mean firing rate (4.82 ± 0.32 *vs* 4.01 ± 0.21 spike/s, $p < 0.01$) and a large and highly significant increase in the percentage of spikes fired in bursts (51.91 ± 5.75 *vs* 28.06 ± 6.08, $p < 0.001$), burst occurrence (0.64 ± 0.06 *vs* 0.37 ± 0.07 bursts/s, $p < 0.001$), and number of spikes per burst (3.84 ± 0.31 *vs* 2.84 ± 0.20 spike/burst, $p < 0.01$) during food consumption as compared to preceding quiet W (Figure 3). The increase in the

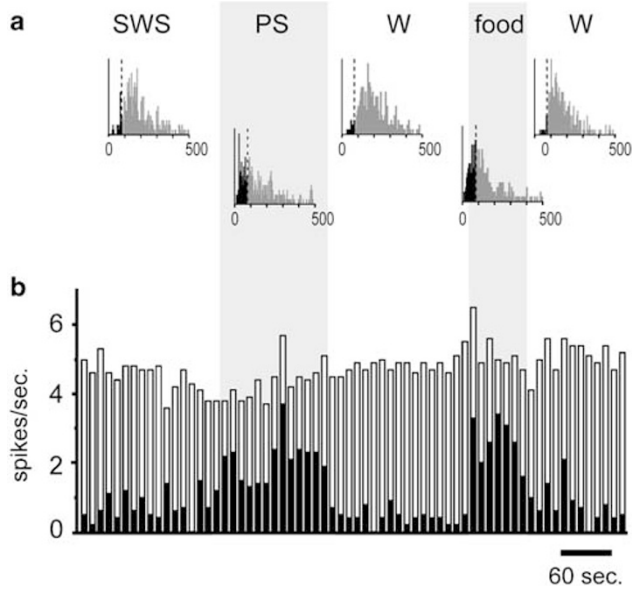

Figure 5 Activity of a dopamine neuron recorded across the sleep–wake cycle and during the consumption of palatable food. (a) Inter-spike interval histograms (ISIH) for each vigilance state. The switch to a prominent bursting pattern during PS is characterized by an increase of the proportion of short intervals (as an indication, bins corresponding to values <80 ms are filled in black on the ISIH). (b) Rate-meter representing the tonic and the bursting activity. Each bar represents the mean firing rate recorded over 10 s. The proportion of spikes fired in burst is shown by the filled proportion of the bar. A bar would be fully black if all spikes were fired in burst during the corresponding 10 s and it would be completely white if no spike spikes were fired in bursts. Note similar increase in burst firing during the entire PS and palatable food consumption (food) periods without clear variation in the mean firing rate, as compared with waking (W) and SWS.

percentage of spikes fired in bursts was confirmed by the increased proportion of short intervals on comparative interspike interval histograms (Figure 5). It was observed in all recorded neurons (Figure 2d) and lasted throughout the entire period of food consumption (20–120 s), as illustrated in Figure 5. There was no significant variation in the mean frequency within bursts (21.4 ± 1.1 *vs* 22.7 ± 1.5 Hz, $p > 0.34$).

There was no significant difference between mean firing rate, or any of the burst parameters between palatable food consumption and PS conditions (Student's *t*-test for unpaired values, firing rate $p > 0.67$, percentage of spikes fired in bursts: $p > 0.70$, spikes/burst: $p > 0.31$, bursts/s: $p > 0.98$, intraburst mean frequency: $p > 0.48$).

DISCUSSION

Here, we provide the first evidence of a robust increase in bursting activity of VTA dopamine neurons during PS. Dopamine cells were not only more likely to burst during PS, but the bursts also contained more spikes. This increase was similar to the bursting induced by the consumption of palatable food.

A possible difficulty of electrophysiological identification of dopamine neurons has recently been indicated by juxtacellular labeling of VTA non-dopaminergic cells, which did not appear easily distinguishable from dopamine cells by their electrophysiological parameters (Ungless *et al*, 2004). These authors stressed the necessity for a careful separation of two types of neurons exhibiting short and long triphasic action potentials. However, the shape of action potentials depends on the filtering parameters (Marinelli *et al*, 2006), thus the criterion used to distinguish different cell types may vary with the use of different filters. By analyzing the action potential duration of each cell recorded in the VTA (Figure 1), we provide evidence that, when using our filtering parameters, the classical and widely used criterion (spike length > 2 ms) proposed by Grace and Bunney (1983) allows good discrimination of each cell group, and therefore a good identification of VTA dopamine cells.

The mean firing rate and bursting parameters of VTA dopamine cells recorded in head restrained rat during quiet waking are consistent with those reported previously in freely moving rats during this state (Freeman and Bunney, 1987; Hyland *et al*, 2002; Kiyatkin and Rebec, 1998; Miller *et al*, 1983). These values contrast with those reported in a recent study which showed that acute stressful restraint increased dopamine neuron firing rate and bursting activity (Anstrom and Woodward, 2005). This provides evidence that our head restraint procedures, after appropriate habituation, were not associated with undue stress as we have previously shown (Souliere *et al*, 2000).

We showed for the first time that VTA dopamine neurons switch to a bursting mode of activity for the entire duration of PS episodes. This activation started 10–20 s before the onset of PS. This period corresponds to a transition period characterized by an increase in theta rhythm on the EEG, which is not yet accompanied by the muscular atonia (Gottesmann, 1992). The increase in burst firing during PS is consistent with the pioneering observation by Miller *et al* (1983) of an increased variance of interspike intervals during PS as compared with SWS. We also confirmed a lack of significant variation in the mean firing rate across vigilance states reported previously in freely moving rats (Miller *et al*, 1983; Trulson and Preussler, 1984), although there was a tendency to an increase during PS. Such a discrepancy between mean firing rate and bursting activity of VTA dopamine neurons is not surprising since several physiological events known to increase mesocorticolimbic dopamine transmission, such as alerting or rewarding stimuli, induce burst firing but generally fail to alter mean firing rate calculated for relatively long intervals (for review see Kiyatkin, 1995). In addition, an increase in bursting activity without any variation of the mean firing rate has been shown to massively increase extracellular dopamine levels (Floresco *et al*, 2003). Hence, on the basis of our findings, dopamine should be released at synaptic terminals at VTA outputs. This is also supported by a recent microdialysis study which reported an increase in extracellular dopamine levels within the nucleus accumbens and prefrontal cortex during PS as compared to SWS (Lena *et al*, 2005). The rather modest increase reported in this study might rely on the difficulty to measure synaptic release of dopamine elicited by bursting activity by using microdialysis without pretreatment with dopamine uptake blockers (Floresco *et al*, 2003). Thus, although the overall mean firing rate of VTA dopamine neurons does not vary across vigilance states, the large increase in their burst firing most likely underlies a strong activation of the mesocorticolimbic dopamine system during PS episodes, in agreement with the report of an increase in c-fos expression by VTA dopamine cells during PS rebound (Maloney *et al*, 2002).

Burst firing of dopamine neurons largely depends, *in vivo*, on glutamatergic NMDA transmission (Chergui *et al*, 1993; Kuznetsov *et al*, 2006; Tong *et al*, 1996) together with cholinergic $\alpha 7$ and $\beta 2$ transmission (Erhardt *et al*, 2002; Kitai *et al*, 1999; Mameli-Engvall *et al*, 2006). The PPN, which sends both glutamatergic and cholinergic inputs to VTA (Omelchenko and Sesack, 2005, 2006), is essential for burst firing of VTA dopamine neurons (Lodge and Grace, 2006a) and elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the VTA (Forster and Blaha, 2000). PPN has also been shown to be highly active during PS (Datta and Siwek, 2002; El Mansari *et al*, 1989) and it is considered a main PS executive region (Pace-Schott and Hobson, 2002). Thus, it seems likely that the main input responsible for the activation of dopamine cells during PS should arise from the PPN. A recent study has demonstrated that the hippocampus, via its indirect action on dopamine cells, also regulates the intensity of their phasic activation (Lodge and Grace, 2006b). The increase in burst firing during the transitional period, when atonia is not yet triggered but hippocampal theta rhythmic activity is already pronounced, suggests a possible involvement of the reciprocal loop between hippocampus and VTA (Lisman and Grace, 2005) in dopamine cell activation during PS. More experiments will be needed to confirm the possible involvement of both hippocampus and PPN in this phenomenon.

Increase in burst firing before the onset of PS might be compatible with the involvement of dopamine in the generation of this state. However, pharmacological increase

of dopamine transmission triggered by psychostimulants promotes waking and reduces both SWS and PS (Wisor *et al*, 2001) and D1 receptor antagonists increase the amount of PS while D1 agonists suppress it (Trampus *et al*, 1991). Although sleep is fragmented in Parkinson disease and in animals which received local injections of 6-OHDA in the VTA, dopamine depletion does not induce any clear modification in the time spent in PS over 24 h (Lai and Siegel, 2003; Sakata *et al*, 2002). Therefore, it is unlikely that VTA dopamine cells participate in generating or maintaining PS. However, dopamine has been shown to increase theta rhythm in the hippocampus via the activation of D1/D5 receptors in the medial septum (Fitch *et al*, 2006; Miura *et al*, 1987) and injections of 6-OHDA in the VTA decrease the power and frequency of theta rhythm observed during PS (Sei *et al*, 1999). Thus, although there is no evidence of any involvement of dopamine in the generation of PS, it most likely plays a role in modulating theta rhythm in the hippocampus during PS.

A possibility concerning the functional role of dopaminergic activation during PS could be that these cells are similarly activated by salient rewarding events as is the case during waking, except that the salient events are internally generated. In most studies reporting burst firing of dopamine neurons in response to reward or salient stimuli, the duration of stimuli is comparatively short, thus activations of dopamine cells are typically composed of only one time-locked burst (Dommett *et al*, 2005; Ungless *et al*, 2004, see also Schultz, 2002 for review). This form of activation is different from the sustained bursting that we observed in PS. Indeed, here we show that the consumption of palatable food that lasted few minutes led to a sustained increase in bursting, similar to what was observed during PS. This latter observation is in agreement with fast scan cyclic voltammetry experiments that show a sustained phasic release of dopamine in nucleus accumbens during sucrose consumption (Roitman *et al,* 2004). This observation constitutes, to our knowledge, the first electrophysiological observation of such a long lasting activation of burst firing which may underlie the increase in dopamine levels reported during various behaviors such as feeding, reward, punishment, or sex (Schultz, 2002). The similarity between dopaminergic activity observed during PS and food consumption suggests that, even if sensory thresholds were high and the animal was not perceiving external stimuli, neuronal reactivations occurring during PS, throughout the brain (Louie and Wilson, 2001; Maquet *et al*, 2000; Peigneux *et al*, 2003; Ribeiro and Nicolelis, 2004), could underlie the perception of internally generated rewarding or at least salient experiences.

Many studies have shown that dopamine is involved in synaptic plasticity in nucleus accumbens, cortex, and hippocampus (Sajikumar and Frey, 2004, for review see Jay, 2003; Lisman and Grace, 2005). Dopamine promotes both LTP in the hippocampus and long-term memory (Frey *et al*, 1991; Li *et al*, 2003; Morris *et al*, 2003; Swanson-Park *et al*, 1999) via the activation of D1/D5 receptors, which activate the cAMP/CREB pathway (Bernabeu *et al*, 1997; Blitzer *et al*, 1998; Otmakhova and Lisman, 1996) and leads to the activation of zif-268 (Bozon *et al*, 2003). Zif-268 is an immediate early gene necessary for both late LTP and long-term memory (Barco *et al*, 2002; Bozon *et al*, 2003; Jones *et al*, 2001), which triggers the synthesis of proteins involved in synaptic function such as synapsins (Rosahl *et al*, 1995; Thiel *et al*, 1994). Zif-268 has been shown to be upregulated during PS following exposure to enriched environment by mechanisms yet to be determined (Ribeiro *et al*, 1999). Others have hypothesized that Zif-268 expression during PS and memory consolidation could be triggered via cholinergic modulation since the ascending cholinergic system (arising from PPN and basal forebrain) was thought to be the only neuromodulatory system activated during PS (Jones, 2005; Pace-Schott and Hobson, 2002). We propose that, as it does during W, dopamine could participate in synaptic plasticity in the hippocampus and long-term memory consolidation occurring during PS (Walker *et al*, 2003, Walker and Stickgold, 2004, Walker and Stickgold, 2006) via its action on D1/D5, cAMP, CREB, and finally zif-268 triggering protein synthesis.

PS has also been associated with the experience of vivid dreaming (Aserinsky and Kleitman, 1953, for review see Hobson and Pace-Schott, 2002). Cessation of dream recall has been documented in Parkinson disease (Sandyk, 1997) while L-DOPA therapy enhances vivid dream recall (Hobson and Pace-Schott, 1999). In addition, patients with lesions of dopamine related cortical areas such as ventromedial prefrontal lobe also have deficits in dream recall (Solms, 2000). Although there is still debate concerning the fact that dopamine could either be required for dreaming or for recall, these observations led Solms to hypothesize that dreaming is not triggered by PS but by activation of the mesolimbic dopamine system that should occur during PS (Solms, 2000). The main objection to such a theory was the lack of any clear evidence of an increase in the activity of the mesolimbic dopamine system. As we now provide such evidence, perhaps it is time to reconsider this theory and speculate that the activation of the dopamine system, we report here could participate in dreaming occurring during PS.

In summary, our results constitute the first electrophysiological evidence of an increased activity of VTA dopamine neurons during PS. This observation challenges the usual thought that dopamine neurons are the only aminergic group not involved in sleep physiology. It seems unlikely that dopamine could be involved in PS generation. However, as during waking, dopamine system could be activated by internally generated rewarding or salient stimuli and participate to the modulation of hippocampal activity and plasticity and, thus, to memory processing. In the light of these results, physiological and cognitive events characteristic of PS should no longer be considered to result from cholinergic neuromodulation alone, but rather from a combined action of high cholinergic and dopaminergic states.

ACKNOWLEDGEMENTS

Lionel Dahan and Nicolas Vautrelle were recipients of fellowships from MILDT (grant MSU, 2001) and 'Région Rhône-Alpes' (Grant 'Emergence' 2001) respectively. This work was also supported by Université Claude Bernard-Lyon1, INSERM, MENRT (ACI, Grant 0220411); Fondation de France (Grant 2002-011947) and NIH (Grant MH62525).

We thank C Peyron, E Pace-Schott, V Coizet and P Redgrave for their comments on the manuscript, E Duboeuf, T Duffau, F Lorent and D Vannereau for their technical assistance.

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