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NEUROSYSTEMS

Increased neuronal firing in resting and sleep in areas of the macaque medial prefrontal cortex

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Abstract

The medial prefrontal cortex (mPFC) of humans and macaques is an integral part of the default mode network and is a brain region that shows increased activation in the resting state. A previous paper from our laboratory reported significantly increased firing rates of neurons in the macaque subgenual cingulate cortex, Brodmann area (BA) 25, during disengagement from a task and also during slow wave sleep [E.T. Rolls *et al.* (2003) *J. Neurophysiology,* 90, 134–142]. Here we report the finding that there are neurons in other areas of mPFC that also increase their firing rates during disengagement from a task, drowsiness and eye-closure. During the neurophysiological recording of single mPFC cells ($n = 249$) in BAs 9, 10, 13 m, 14c, 24b and especially pregenual area 32, populations of neurons were identified whose firing rates altered significantly with eye-closure compared with eye-opening. Three types of neuron were identified: Type 1 cells (28.1% of the total population) significantly increased (mean + 329%; $P \ll 0.01$) their average firing rate with eye-closure, from 3.1 spikes/s when awake to 10.2 spikes/s when asleep; Type 2 cells (6.0%) significantly decreased (mean −68%; $P < 0.05$) their firing rate on eye-closure; and Type 3 cells (65.9%) were unaffected. Thus, in many areas of mPFC, implicated in the anterior default mode network, there is a substantial population of neurons that significantly increase their firing rates during periods of eye-closure. Such neurons may be part of an interconnected network of distributed brain regions that are more active during periods of relaxed wakefulness than during attention-demanding tasks.

Introduction

Sleep is not a quiescent state (Maquet, 2000; Steriade, 2000; Steriade *et al.*, 2001; Datta & Maclean, 2007). It is actively induced and involves a highly orchestrated series of integrated brain states (Fuster, 2008; Amting *et al.*, 2010). Functional brain imaging (functional magnetic resonance imaging, fMRI) studies have begun to unravel the neural mechanisms that generate the defined stages of sleep which are behaviourally complex and result from distinct physiological mechanisms (Van Someren *et al.*, 2011). Activity in the medial prefrontal cortex (mPFC) is directly involved in the induction and maintenance of the various sleep stages (Steriade, 1996a,b; Maquet, 2000) (see Fig. 3 in Muzur *et al.*, 2002).

In humans, slow wave sleep (SWS) involves oscillatory activity in corticocortical and hippocampal–PFC pathways (Rauchs *et al.*, 2011; Schwindel & McNaughton, 2011). Additionally, the thalamocortical–thalamic pathways actively regulate the passage of sensory information to the cortex (Contreras & Steriade, 1996; Steriade & Pare, 2007). The generation and maintenance of slow waves during SWS are associated with activity in defined cortical areas, including areas of the mPFC and subcortical nuclei, especially the thalamus (Maquet, 2000; Steriade & Pare, 2007). Rapid eye movement (REM) sleep is associated with activation of the pons, thalamus, hippocampus, amygdala, temporal and occipital cortices, and a concurrent alteration in the activity of the dorsolateral PFC (Kubota *et al.*, 2011).

During sleep, the relative activity in different brain regions can thus be increased in a region-specific manner. Such activation may be transient due to waves of activity generated in mediofrontal regions rippling posteriorly through the cortex (Samann *et al.*, 2011). Furthermore, fMRI studies exploring the relationship between sleep and memory have demonstrated a post-learning reactivation during REM sleep (Rauchs *et al.*, 2011; Schwindel & McNaughton, 2011).

The electrophysiological study of Rolls *et al.* (2003) demonstrated that neurons in Brodmann Area (BA) 25 (subgenual cingulate cortex) of macaques significantly increased their firing rates when the subjects disengaged from a task and fell asleep compared with the awake state. On average, the firing rates of these neurons in BA25 when the macaques were asleep or when they were disengaged from a task were increased by + 435% of those when the monkeys were awake.

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It is currently unknown whether the significant increase in the firing rates of some BA25 neurons with the onset of sleep is localized solely to the subgenual cingulate cortex or is a common feature across all mPFC areas. The aim of this study was therefore to establish whether single neurons in other areas of monkey mPFC (BAs 9, 10, 13 m, 14c, dorsal anterior cingulate 24b and especially pregenual cingulate 32) had similar changes of firing rate related to the onset of sleep and eye-closure. Such data would be extremely relevant to understanding the basic neurophysiological mechanisms underlying the involvement of the mPFC in human sleep (Maquet, 2000), both in normal and in abnormal states (Vogt, 2009; Price & Drevets, 2012). It would also be relevant to the interpretation of the increased activation measured in the default mode network in the resting state in neuroimaging studies (Buckner *et al.*, 2008; Mantini *et al.*, 2011), in which the measures relate to increased blood flow or metabolism, and not directly to firing rate.

The data presented in this paper relating to 'sleep active/sleep inactive' neurons were obtained during a series of experiments investigating the response properties of single neurons in monkey mPFC to a variety of gustatory, olfactory, visual, somatosensory and auditory stimuli (as reported previously by Rolls, 2008). The data presented here are the first single-neuron recordings we know reporting how the firing rates of single neurons are related to the onset of sleep in areas of primate mPFC other than BA25, and are a valuable complement to functional neuroimaging studies in humans of the default mode network (Buckner *et al.*, 2008) as well as in monkeys in which a similar default mode network has been identified in the resting state (Mantini *et al.*, 2011; Hutchison *et al.*, 2012). By studying the firing rates of single neurons, we are able for the first time to provide evidence on the proportion of neurons in these regions that change their firing rates in the states of waking vs. resting/sleep, and on their firing rates when in these different states.

Materials and methods

Subjects

Neurophysiological recordings were made of the activities of single neurons in the medial wall areas of the prefrontal cortex (mPFC) in awake behaving unanaesthetized monkeys. The subjects were two young adult male rhesus macaques (*Macaca mulatta*), weighing 3.5–4.5 kg (coded BM and BQ). All procedures were licensed to be carried out at the University of Oxford under the UK Animals (Scientific Procedures) Act 1986. All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were carried out in accord with the 'Policy on the use of animals in neuroscience research' of the Society for Neuroscience (USA), and have been described previously (Rolls *et al.*, 2003). During the experiments, BM and BQ were seated in comfortable restrained positions in primate chairs located in a specially designed hexagonal recording chamber approximately 2.5 m wide. On return to their home cages the animals were kept on healthy calorie-controlled diets with *ad libitum* access to water. The animals were not sleep deprived.

Recording methods

The electrophysiological recording methods have been described previously in companion articles (Rolls *et al.*, 2003; Rolls, 2008). Briefly, recordings of the extracellular electrical activity of single, well-isolated, neurons in the mPFC of both hemispheres, in both subjects (BM and BQ), were made using either glass- or epoxylite-insulated tungsten microelectrodes, with known impedances of 5–10 MΩ [Frederick Haer & Co., Bowdoinham, ME, USA, Catalog UEWLFFSMNNNE - unzapped; see Verhagen *et al.* (2003)].

A computer with real-time digital and analog data acquisition collected spike arrival times and displayed online summary statistics as well as peristimulus time-histograms and rastergrams. To ensure that the recordings were made from single cells, the interspike interval was repeatedly monitored to make sure that intervals of < 2 ms were not present. The waveform of the action potentials was also continually monitored.

Screening of neurons and classification of behavioural states

During the course of 31 electrode penetrations, a total population of 249 neurons throughout identified mPFC areas were electrophysiologically tested with a comprehensive battery of visual, auditory, gustatory, somatosensory and olfactory stimuli, and were recorded from during states of waking and sleep (Fig. 1A). The aim was to investigate the involvement of mPFC neurons in taste and food-related processing mechanisms and thus in appetite control with potential relevance to understanding obesity (Rolls, 2008, 2011; Grabenhorst & Rolls, 2011). Stimulus parameters are detailed in the companion paper (Rolls *et al.*, 2003). The results of these experiments have been reported previously by Rolls (2008) and are not considered further here.

However, during the experimental sessions described above, it was noticed that the two animals, when not engaged in specific behavioural tasks, became drowsy and would frequently close their eyes. Concomitant with the onset of eye-closure was the finding that some mPFC neurons either markedly increased or decreased their spontaneous firing rates, whereas the activity of other neurons was unaffected. The studies described here were undertaken to systematically investigate these observations.

During the 'peri-task' periods referred to above, the monkeys would wax and wane in and out of three readily identified behavioural states: *wakefulness* [eyes fully open – designated here as Behavioural State (BS) 3]; *drowsiness* (eyes partially closed for > 3 s; BS2); and *sleep* (eyes fully closed – BS1). Classification of BS1, BS2 and BS3 was made by the experimenter from live video images of the monkey displayed on a video monitor placed outside the recording chamber. Electrocorticogram (ECG) recordings in both animals were used to validate the classification procedure (see below). The method is similar to the procedures described by Balzamo *et al.* (1998) and Rolls *et al.* (2003), which also used ECG data to define 'awake' vs. 'sleep' states. Such an approach is a reliable and standard method of observing animal behaviour that has been in use since the early days of ethology (Balzamo *et al.*, 1998).

The experimental procedure was that every 10 s a mean firing rate (together with a standard error estimate calculated in 1-s portions of the 10-s period) was calculated and automatically saved by the computer. For each of these 10-s periods the experimenter recorded on a data spreadsheet the mean rate, and the experimenter's assessment of the behavioural state (BS1, 2 or 3) in that period, using the categories just described.

Recordings from 85 of the cells in the above populations revealed responsive neurons in BAs 9, 10, 13 m, 14c, 24b and 32 that significantly altered their firing rates on eye-closure. The recording sites of these cells are shown in Fig. 1C–E. During the recording sessions the animals had access to water *ad libitum* and some food (nuts, fruit) given by the experimenter. After the recording sessions the animals were returned to their home cages.

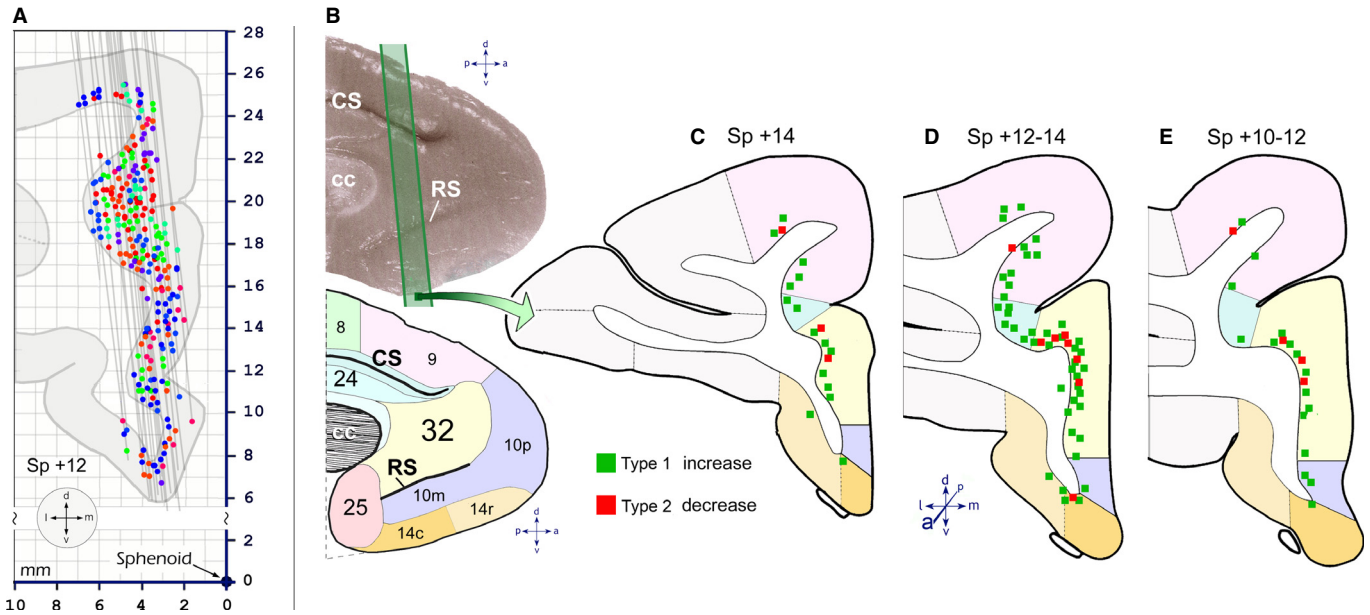

FIG. 1. (A). Representative distribution of a sample of the 833 neurons that were studied electrophysiologically shown on coronal sections. The electrode tracks were angled medially between 5° and 9° (commonly 7°), and anteriorly between 10° and 24° (commonly 17°) with respect to the vertical meridian. The colour coding of neurons represents specific physiological properties of units as defined in a separate investigation reported elsewhere (Rolls, 2008). The neurons described in this study comprised a subset that it was possible to test during wakefulness and sleep of this overall population. (B). Representative medial view of the left anterior hemisphere showing the position and orientation of the antero-posterior slab of tissue (green) in which the recordings were made. Three coronal sections within this slab from sphenoid (Sp) + 10 to + 14 are shown in C–E. Cingulate sulcus, CS; rostral sulcus, RS; corpus callosum, cc. The lower diagram in B shows the cytoarchitectural division of the monkey medial prefrontal cortex. The 'pregenual' position of area 32 and its location around the lower bank of the anterior aspect of the cingulate sulcus is shown. Area 32 occupies a large swathe of the anterior medial wall cortex and directly abuts onto area 25 posteriorly (see B). (C–E). Three coronal sections taken from positions anterior (+ 10 mm to + 14 mm) to the sphenoid bone promontory (Sp). The distribution of Type 1 and 2 cells reported in this study is indicated by squares on the three sections. Regional territories of the medial and ventromedial prefrontal cortex (BAs 9, 10, 13 m, 14c, 14r, 24b and 32) are also indicated (cross-referenced by colour to B). The positions of neurons were identified histologically using the X-ray coordinates of each neuron as described by Feigenbaum & Rolls (1991). The reconstruction indicated that many of the recorded neurons were in the deep-lying cortical layers. Whilst all medial wall areas in this anteroposterior sector have cells that were recorded from, the majority (40.6%) of such neurons were in area 32 (which is larger than the other areas).

Electrocorticogram recordings

Electrocorticograms were recorded on two occasions (once in each animal) to confirm that the behavioural states, BS1 and BS3, defined periods when the monkeys were respectively either 'asleep' or 'awake' – these ECG recordings were obtained using the procedure described by Rolls *et al.* (2003). The ECGs were made between an electrode contacting the dorsal parietal cortex and a reference ground wire attached to the skull. The ECGs were measured for a cumulative total of 40 s of recording in 1-s samples. Half of the 40 data segments were when the monkeys were 'asleep' and half whilst they were 'awake'. The recorded potentials were sampled at 100 Hz and low-pass filtered to include the frequency range 0–50 Hz. The power spectra of the ECG were then calculated separately for awake (BS3) and sleep states (BS1) using the spectral calculation performed by fast Fourier transform (FFT) methods, utilizing the procedures and C code described by Press *et al.* (1992). The use of multiple independent data segments to compute an average of the power spectra for each state ensured that the resulting power spectra for each state were statistically reliable, as described elsewhere (Press *et al.*, 1992; Bendat & Piersol, 2010).

The ECGs demonstrated that when the subjects were rated by the experimenter as being in BS3 (eyes-open/awake) the ECG showed low-voltage fast activity, and this was reflected in the power spectra (range 2–20 Hz) which had a peak in the frequency range 23–28 Hz, as shown in Fig. 2. Increased power at low frequencies is a sign of SWS (Finelli *et al.*, 2001). When the subjects were rated by the experimenter as being in BS1 (eyes-closed/asleep), high-voltage

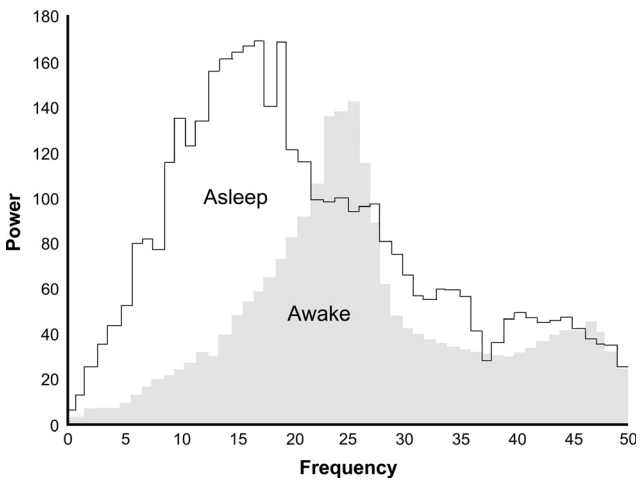

FIG. 2. Power spectra (linear scale) of the electrocorticogram (ECG) when the monkey was 'awake' (eyes-open = BS3) vs. 'asleep' (eyes-closed = BS1). The increased power at low frequencies (< 15 Hz) provides evidence that in the eyes-closed state the subject was asleep. This record was obtained by averaging 40 separate 1-s power spectra to give a reliable average spectrum. The ECG helps to validate the assessment of BS1. The y-axis is in relative units of power. The data were obtained during epochs when the monkey was either only awake or only asleep.

slow waves appeared in the ECG, and this was reflected in the power spectra with relatively more power than when awake in the lower frequencies between 5 and 18 Hz (which include the alpha

and theta bands), as illustrated in Fig. 2. The power spectra shown in Fig. 2, taken together with similar data obtained in other macaques (Rolls *et al.*, 2003), confirm the experimenter's assessment of the behavioural states as BS3 or 'awake' (i.e. periods when the monkeys had their 'eyes-open'), and as BS1 or 'asleep' (i.e. when the animals had their 'eyes-closed').

Classification of cell types

Cells in mPFC showing responses to eye-closure or eye-opening could be classified on the basis of their firing rate changes during transitions between behavioural states (see Figs 3–7). Type 1 cells significantly increased their firing rate when the subjects closed their eyes and went to sleep, and returned to their previous levels on reopening of the eyes. Type 2 cells significantly decreased their firing rate on eye-closure, and returned to their former level of activity with eye-reopening. Type 3 cells were unaffected by both eye-opening and eye-closure.

Data acquisition and analysis

Neuron firing rates were recorded every 10 s as described above for periods of many minutes that could include several (up to nine) discrete periods of eye-closure/eye-opening (Fig. 4). Mean firing rates were calculated separately for each BS3, BS2 and BS1 epoch. Mean epoch values were then used to obtain the overall mean BS3, BS2 and BS1 firing rates for each neuron. 'Grand mean' firing rate estimates (together with standard error values) for each behavioural state (BS1, 2 and 3) were subsequently generated for each of the three cell types 1–3 (Table 1).

Significant differences between 'grand mean' BS3 and BS1 firing rate values for each cell type (1–3) were identified using Student *t*-tests with the formal value for statistical significance being set at $P < 0.05$.

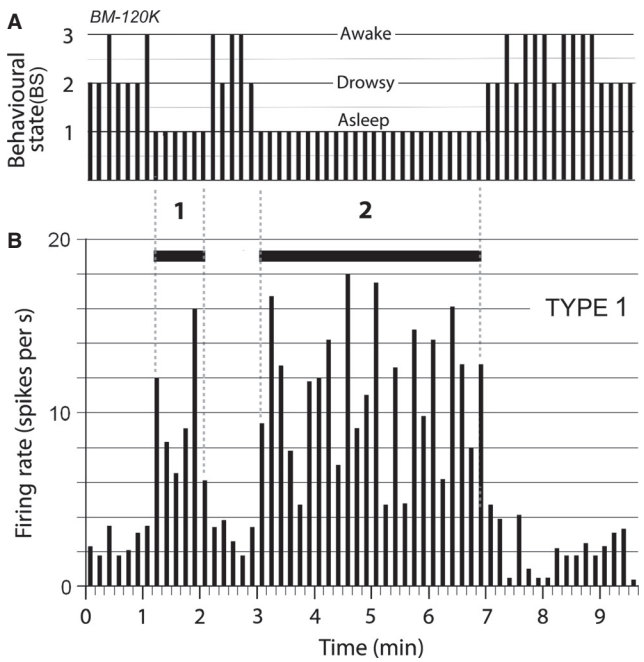

FIG. 3. Firing rates of a Type 1 neuron situated in the deep layers of area 24b during a 9 min 40 s recording period. The mean firing rates (spikes/s) in each successive 10-s time periods were recorded and the 'Behavioural State (BS)' noted [BS3 = eyes-open (awake); BS2 = eyes partially closed (drowsy); BS1 = eyes-closed (asleep)]. Two distinct epochs (1, 2) of eye-closure (BS1) are associated directly with periods of significantly higher (+ 423% $P \ll 0.01$; $P = 4.1 \times 10^{-9}$) firing rates than the flanking epochs (mean ± SD; BS1 = 9.3 ± 3.8 spikes/s; BS2/3 = 2.2 ± 1.1 spikes/s).

Areal location of neurons

At the beginning and end of each electrode tract, two X-radiographs (coronal and sagittal planes) were taken to identify the initial and final positions of the microelectrode tip in the brain. From these X-radiographs, the spatial locations of the electrode tip at the beginning and end of each electrode penetration could be accurately defined with respect to the posterior lip of the sphenoid bone – a bony promontorial landmark in the skull clearly visible in X-radiographs (Aggleton & Passingham, 1981). As a result, the location of the electrode tip with reference to the known defined laminar cytoarchitecture of mPFC could, to a first approximation, be assessed from a stereotaxic X-radiographic atlas of the macaque brain (Feigenbaum & Rolls, 1991) in conjunction with the standard laboratory atlas for macaques of Paxinos *et al.* (2000). (The positions of electrode tracts were subsequently confirmed histologically in serial Nissl-stained sections through mPFC – see Fig. 1A.)

Using the posterior lip of the sphenoid bone as reference, the positions of each recorded cell along the path of each electrode tract could be accurately mapped in the coronal (mediolateral) and sagittal (anteroposterior) planes. By consulting monkey brain atlases (Aggleton & Passingham, 1981; Feigenbaum & Rolls, 1991; Paxinos *et al.*, 2000) the areal locations of each recorded neuron could be defined reliably.

Histology

At the end of all experimental work, electrolytic microlesions were made through the tip of a recording electrode to mark the locations of typical neurons in the mPFC of each hemisphere for both BM and BN. The animals were deeply anaesthetized with sodium pentobarbitone (Sagatal) and transcardially perfused, initially with physiological saline (0.9%) and subsequently with 0.1 M phosphate-buffered (PB) 4% paraformaldehyde (pH 7.4 at room temperature). The brains remained in the skulls overnight before being carefully dissected from the cranium. Following infiltration with graded sucrose solutions (10, 20 and 30%), complete sets of serial 1-in-2 sections (50 μm thick) from the entire rostrocaudal extent of each brain were then prepared in the coronal plane using a freezing microtome. Sections were collected into 0.1 M PB and subsequently mounted in order onto glass slides and air-dried. Finally, the sections were stained with cresyl violet to reveal areal and laminar cytoarchitectures then passed through an ascending series of alcohols before being embedded in DePeX mountant and coverslipped.

The microlesions together with the associated X-radiographs and stereotaxic atlases enabled the areal positions of all cells to be reconstructed from the Nissl-stained sections using the method of Feigenbaum & Rolls (1991).

Cortical areal and laminar definitions

The definitions of cortical areas and laminar patterns in macaque mPFC were made from the studies of Carmichael *et al.* (1994), Carmichael & Price (1995), Freedman *et al.* (2000) and Paxinos *et al.* (2000).

Preparation of figures and illustrations

Digital image files were imported into Adobe Photoshop 7 or CS3 and were processed routinely for grey/colour levels, brightness and

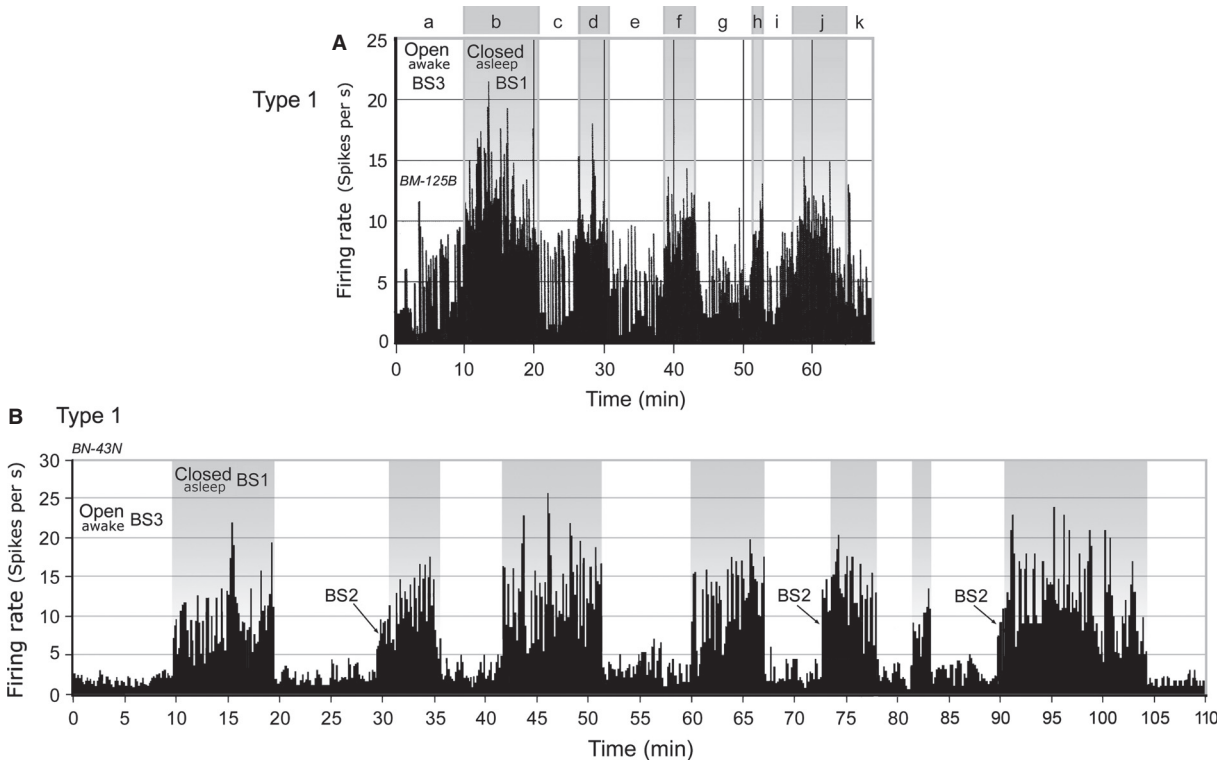

FIG. 4. (A). Firing rates of a unit in deep area 32 (layer 6) during a 65 + min recording period. Alternating 'eyes-open' (awake/BS3) and 'eyes-closed' (asleep/BS1 – shaded grey) epochs are clearly evident and were associated directly with distinct variations in the firing rate (spikes/s) of the recorded unit. Mean firing rates ± SD (spikes/s) during eyes-open epochs (a, c, e, g, i and k) and eyes-closed epochs (b, d, f, h and j). Eyes-open epochs: a = 6.6 ± 2.5; c = 7.8 ± 2.0; e = 6.2 ± 1.7; g = 5.5 ± 1.7; i = 7.0 ± 1.1; k = 5.1 ± 1.3 (overall mean of eyes-open epochs = 6.3 ± 1.0). Eyes-closed epochs: b = 12.9 ± 3.2; d = 10.4 ± 1.9; f = 10.2 ± 1.9; h = 9.23 ± 1.3; j = 9.0 ± 2.7 (overall mean of eye-closed epochs = 10.4 ± 1.6). Analyses of variance and multiple paired *t*-tests identified that the firing rates during eye-closed epochs were significantly higher ($P \ll 0.01$) from each of the firing rates of the associated flanking eyes-open epochs. Note in particular the high variation of mean values for eyes-closed epochs, and that the mean firing rate of the unit gradually declines across each successive eye-close epoch. (B). Neuron in area 32 in subject BN. The firing rate response of a Type 1 cell. Statistical comparisons identified that the mean firing rates of eye-closed periods (shaded grey) were significantly increased ($P \ll 0.01$) compared with the mean firing rates of the flanking eyes-open periods. Note that firing activities during the eyes-open and eyes-closed epochs are relatively constant throughout the extended period of monitoring (110 min).

contrast before being composed into figure illustrations for publication.

Results

The data were obtained in two behaving unanaesthetized young adult macaque monkeys (BM, BQ). A total of 249 neurons were screened in both animals [172 (69%) in BM and 77 (31%) in BQ] using a selection of visual, auditory, gustatory, somatosensory and olfactory stimuli (Rolls, 2008). In addition, the firing rates of each cell were assessed to see if they were influenced by eye-closure during periods when the animals were not being actively tested. Figure 1A illustrates the wide areal distribution of the 249 electrophysiologically sampled cells in the PFC. The single neuron recordings were made from mPFC areas – BAs 9, 10, 13 m, 14c, 24b (dorsal anterior cingulate cortex) and 32 (pregenual area; Fig. 1B). The anterior–posterior extent of the recordings ranged from + 10 mm to + 14 mm anterior to the posterior lip of the sphenoid bone (Fig. 1C–E).

Behavioural data

After a period without behavioural testing and interaction with the experimenter, the subjects would adopt a relaxed position in their chairs in which the arms and legs became motionless, and the eyelids would gradually droop and eventually close. When closed, the eyes showed a slow drift typical of drowsiness prior to entry into SWS. These behavioural criteria for the animals being 'awake' (BS3 – eyes-open), 'drowsy' (BS2 – partial eye-closure) or 'asleep' (BS1 – eyes-closed) were made from live images of the monkeys displayed on a video monitor placed outside the hexagonal recording chamber (Balzamo *et al.*, 1998). ECG evidence obtained during the initial recording sessions in both animals confirmed that when the animals were in BS1 they were most probably in a state of SWS (Fig. 2).

Electrophysiological classification of cell types 1–3 in PFC

Several distinct types of neuronal responses were observed as the animals passed between BS1, 2 and 3 (see Table 1 and Figs 5 and 6). As a result, a preliminary cell classification based on significant changes in firing rates associated with BS1, 2 and 3 was defined (see Figs 3–7 and Tables 1 and 2):

Type 1 cells (28.1% of the screened population) significantly *increased* (+ 329 ± 26%; mean ± SEM, $n = 70$; $P \ll 0.01$) their firing rate from the spontaneous rate when the subjects closed their eyes and went to sleep (mean ± SEM, $n = 70$; Awake = 3.1 ± 0.4 spikes/s; Asleep = 10.2 ± 0.8 spikes/s; $P \ll 0.01$; $P = 3.4 \times 10^{-15}$).

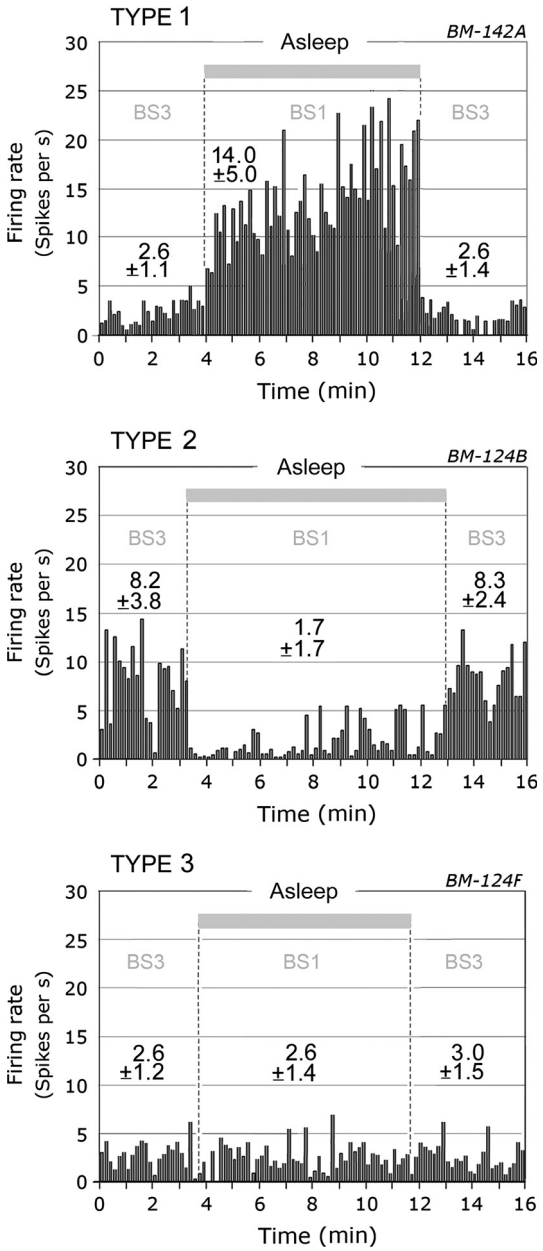

FIG. 5. Examples of Type 1, 2 and 3 neurons showing their activities during eyes-open (BS3 awake) or eyes-closed (BS1 asleep) periods**.** The neurons were from BA32 (subject BM). The mean firing rates (± SD) in spikes/s are given for each 'eye-open/eye-closed' epoch. The Type 2 neuron had a relatively high eyes-open firing rate which reduced significantly during eye-closure. Type 3 cells were not affected during either the eyes-open or eyes-closed periods.

Type 2 cells (6.0% of the screened population) significantly *decreased* (−68 ± 7.2%; mean ± SEM, $n = 15$; $P < 0.01$) their firing rate on eye-closure, returning to their former level of activity with eye-reopening (mean ± SEM, $n = 15$: Awake = 7.7 ± 1.7 spikes/s; Asleep 2.5 ± 0.9 spikes/s; $P < 0.05$; $P = 1.1 \times 10^{-2}$).

Type 3 cells (65.9% of the screened population) were unaffected by either eye-opening or eye-closure (mean ± SEM, $n = 164$: Awake = 3.2 ± 0.5 spikes/s; Asleep 3.3 ± 0.3 spikes/s; $P > 0.05$).

Examples of the activity of these three cell types during individual eyes-closed (BS3) epochs are illustrated in Fig. 5.

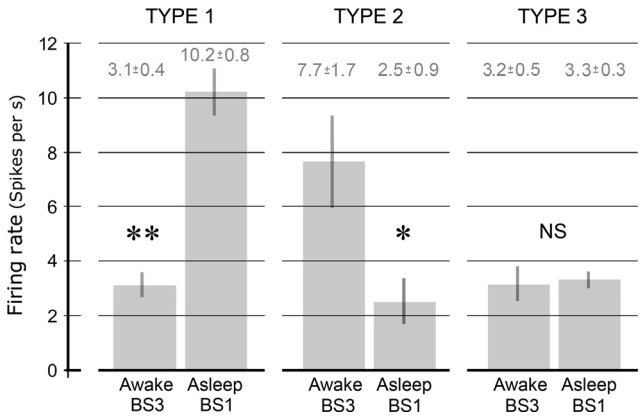

FIG. 6. Histograms showing the overall mean firing rates and error estimates for cell Types 1, 2 and 3 during Behavioural States (BS) 3 (eyes-open/awake) and 1 (eyes-closed/asleep). Mean values ± SEM (Type 1 cells $n = 70$; Type 2 cells $n = 15$; Type 3 cells $n = 50$). Statistical significance: $^{**}P \ll 0.01$; $^{*}P < 0.05$; ns = not significant (see also Table 1).

Summary population data for the firing rates of cell Types 1, 2 and 3 are given in Table 1 and illustrated graphically in Fig. 6. None of the Type 1 and Type 2 cells had significant responses to any of the taste, olfactory and visual stimuli being tested (Rolls, 2008). Of note is that only three of the Type 3 cells displayed significant responses to sensory stimuli (see Rolls, 2008); the lack of eye-close responses of these three cells was similar to the other Type 3 cells.

The population responses for a large sample of epochs ($n = 100$) from Type 1 cells during the transitions from being 'awake to asleep' (BS3 to BS1) and from being 'asleep to awake' (BS1 to BS3) are shown in Fig. 7A and B. These data plots allow an assessment of the overall variability in firing rate changes for Type 1 cells across behavioural states. The data have been plotted so that each transition point occurs at $t = 0$ s (Fig. 7) with a 1-min period 'before' and a 4-min period 'after' each transition being included for comparison. Figure 7A and B clearly indicate for a large number of Type 1 epochs the general robust and consistent physiological responses of these neurons to periods of 'eye-closure' (Fig. 7A) or 'eye-opening' (Fig. 7B).

Some neurons, however, had epochs that did not display such a marked and consistent change in firing rate between behavioural states. For example, there were some Type 1 neurons that had BS3 to BS1 transitions which showed gradual increases in firing rate some 5–40 s prior to eye-closure. The monkey's eyelids would seemingly become heavy and start to droop before finally closing tightly. These neurons can be described as responding to a period of inattention, drowsiness and rest prior to the onset of sleep. Conversely, there were a small number of Type 1 cells that had BS3 to BS1 transitions where there was an increase in cell firing rate several seconds (3–6 s) after the monkey's eyelids closed. In contrast to the period prior to eye-closure/sleep (BS1), where monkeys would sometimes display a state of drowsiness with their eyes partially closed (BS2), they would in general wake up from sleep by opening their eyes fully, producing a sharp BS1 to BS3 transition (Fig. 7B).

Recordings of mean firing rates over longer time periods (up to tens of minutes, which was continuously monitored by the experimenter) revealed the longer term firing rate architecture of 'awake/asleep' epochs and their periodicity, with repeating BS1, BS2 (where present) and BS3 periods, and the reliable changes in firing

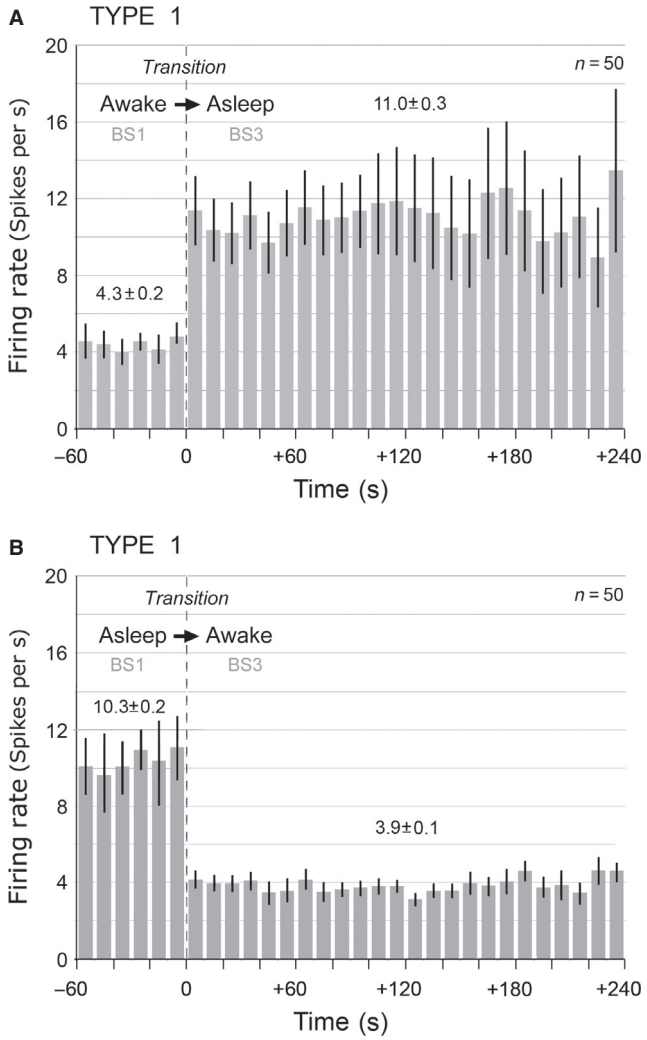

FIG. 7. Histograms showing the changes in firing rate during the transitions from (A) 'awake (BS3) → asleep (BS1)' and (B) 'asleep (BS1) → awake (BS3)' for 100 epochs sampled from different Type 1 cells in BA32. The histograms have been co-aligned so that the transition points occur at $t = 0$ s and include a 1-min period before and a 4-min period after each transition. The data have been averaged within each 10-s sampling bin (mean values ± SEM, $n = 50$). The overall mean firing rates (mean values ± SEM) are given before and after the transitions.

TABLE 1. Mean firing rates of the individual cell types (1–3) during specific behavioural states (BS1–3)‡

Behavioural state	Cell type 1 ($n = 70$)	Cell type 2 ($n = 15$)	Cell type 3 ($n = 164$)
BS3 (eyes-open/awake)	3.1 ± 0.4**	7.7 ± 1.7*	3.2 ± 0.5†
BS2 (drowsy)	6.4 ± 0.8	5.8 ± 0.8	3.0 ± 0.4
BS1 (eyes-closed/asleep)	10.2 ± 0.8**	2.5 ± 0.9*	3.3 ± 0.3†

‡Cell type overall mean ± SEM (n = number of cells). Mean firing rates for each behavioural state (BS1–3) were determined for individual neurons in a cell type (1–3). These data were then averaged to provide a 'grand mean firing rate' for each cell type during each of the behavioural states. Statistical analyses (t-tests) for the firing rates of: Type 1 cells – BS3 vs. BS1 $^{**}P \ll 0.01$; Type 2 cells – BS3 vs. BS1 $^{*}P < 0.05$; and Type 3 cells – BS3 vs. BS1 †not significant.

rate associated with each epoch (Fig. 4A and B; Table 2). Epochs of eye-closure (BS1) could last from a brief 10 s up to 15 min or more (Fig. 4B). There was no apparent relationship between the duration of the eyes-closed/asleep (BS1) periods and the length of the intervening eyes-open periods (BS3; Fig. 4). On some occasions the monkeys would have numerous 'eye-closed' periods of short duration or only a few eye-closed epochs of extended duration.

It was notable that the vast majority of the Type 1 neurons described here had regular firing patterns during sleep, as illustrated for a typical single neuron in Fig. 8. The same property was described by Rolls *et al.* (2003) for single neurons in the subgenual cingulate cortex BA25 during periods of eye-closure. However, of note is that a few Type 1 cells showed minor variations in the fine temporal patterning of neuronal firing during some 'eyes-closed' epochs, with some exhibiting 'burst-like' responses.

The quantitative areal distribution of cell Types 1, 2 and 3 neurons in mPFC are given in Table 2 (see also Fig. 1C–E).

Finally, it was not possible to ascertain unequivocally whether the neurons being studied electrophysiologically were excitatory projection pyramidal cells or local circuit inhibitory neurones. However, the likelihood is that most of the recorded cells were pyramidal projection neurons as the spike durations were typically greater than 1.2 ms, which is highly characteristic of cortical pyramids (Rolls *et al.*, 2003).

Discussion

The principal results of this study indicate that there are two populations of neurons throughout the monkey mPFC that significantly altered their firing rates when the subjects 'closed' or 'opened' their eyes. Type 1 cells (8.4% of all cells recorded) significantly increased their firing rate when the monkey became drowsy or closed its eyes, whilst Type 2 cells (1.8%) significantly decreased their firing rate on eye-closure. Together these electrophysiological cell types represent a modest population (10.2%) of all the mPFC neurons screened in this study. Histological reconstructions confirmed that the cells studied electrophysiologically were in BAs 9, 10, 13 m,14c, 24b (dorsal anterior cingulate cortex) and 32 (pregenual cingulate cortex in primates), with many of the recorded cells being located in the deep layers of the cortex (see Fig. 1C–E).

A previous paper from our laboratory reported that neurons in BA25 (subgenual cingulate cortex) of the macaque mPFC also significantly increased their firing rates when monkeys went to sleep (Rolls *et al.*, 2003). Of note is that comparable to the neurons reported here, the cells studied by Rolls *et al.* (2003) did not respond to gustatory, olfactory and most visual stimuli. Rolls and colleagues also presented evidence of four neurons in the orbitofrontal cortex (BA13) responding in a similar manner. The present study thus confirms and extends to further areas of mPFC the observations of the earlier companion paper. Taken together these two studies indicate that there are distributed populations of neurons throughout the mPFC of monkeys that selectively respond to being either 'asleep' or 'awake'. Of importance is that the greatest numbers of sleep 'active' and sleep 'inactive' cells were located in BA32, a cortical area in primates fundamental to the integration of 'higher' cognitive and emotional processing streams and a central component area of the default mode network (Bush *et al.*, 2002; Rolls & Grabenhorst, 2008; Larson-Prior *et al.*, 2009, 2011; Vogt, 2009; Grabenhorst & Rolls, 2011). Although sleep active/inactive cells were found throughout the medial and ventromedial areas of the mPFC, it is in area 32 that the highest numbers of cells were found. This highlights the central 'hub-like' position of area 32 in the functional architecture of monkey mPFC with regard to awake/asleep-related mechanisms (see also Fig. 3 in Muzur *et al.*, 2002).

TABLE 2. Quantitative areal distribution of the individual physiological cell Types 1–3 in mPFC*

	Area							
Cell classification	BA9	BA24b	BA32	BA10 m	BA14c	BA13 m	Total	% Types
Type 1	16	10	34	3	4	3	70	28.1
Type 2	3	0	11	0	1	0	15	6
Type 3	62	18	58	19	1	6	164	65.9
Tot	81	28	103	22	6	9	249	
Areal distribution (%)	32.5	11.3	41.4	8.8	2.4	2.6		$\Sigma = 100\%$

*Type 1 = cells that significantly increased their firing rate during the period of eye-closure; Type 2 = cells that significantly decreased their firing rate during the period of 'eye-closure'; Type 3 = cells that did not change their firing rate during eye-closure/eye opening. (The positions of the 85 Type 1 and 2 cells in mPFC are shown in Fig. 1C– E.) [Correction added on 5 April 2013, after first online publication: The headings to the last two columns are interchanged. '%Types' should be the heading for the last column whereas 'Total' should be the heading for the penultimate column].

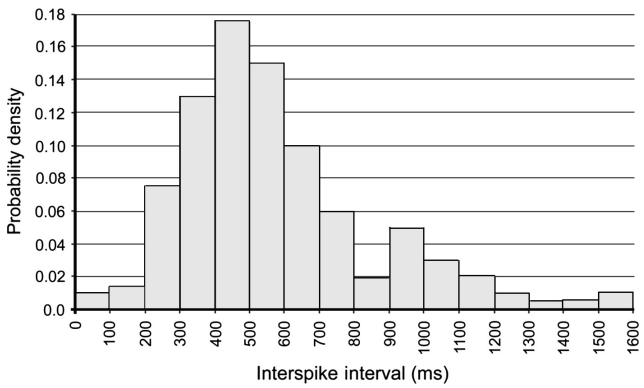

FIG. 8. Interspike interval histogram of a Type 1 neuron in deep area 32 when the monkey was asleep. Note that when the monkey was awake the cell had a very low background firing rate (*c*. 0.1 spikes/s).

Network anatomy of Area 32

Previous tract-tracing studies have identified cortical and subcortical systems projecting to the mPFC as well as inter-areal circuits within the mPFC that are centred on the pregenual cingulate cortex area 32 (Hamani *et al.*, 2011). Subcortical, corticocortical and intracortical (excitatory and inhibitory) afferent input (defining the cortical receptive fields of area 32 neurons) are derived from: (i) lateral area 9, ventral and dorsal area 46; (ii) medial areas 9, 10, 14, 24, subgenual 25 and from regions within area 32; and (iii) orbitofrontal areas 14, medial and lateral area 13, and lateral area 12 (Carmichael *et al.*, 1994; Carmichael & Price, 1996; Öngür & Price, 2000). Input from dorsolateral areas (cognitive executive) and from the orbitofrontal cortex (reward, emotion-related stimuli, etc.) support the idea that area 32 in primates is fundamental to the integration of cognitive and emotional processing streams (Bush *et al.*, 2002; Rolls & Grabenhorst, 2008; Rolls, 2009, 2013; Grabenhorst & Rolls, 2011).

Functional architecture of mPFC and the default mode network

What function do the 'sleep' active/inactive cells recorded here serve? Of importance is that whilst only a single cell was being recorded from at any one time during the awake/asleep periods, it is likely that cell Types 1 and 2 were active in concert.

The network of neurons in macaque mPFC showing increased responses during sleep states described here belong to the same set of areas of the human medial PFC represented in the anterior default mode network, which is active in the resting state (Raichle *et al.*, 2001; Buckner *et al.*, 2008; He *et al.*, 2008; Larson-Prior *et al.*, 2009, 2011). A similar default mode network has been identified in macaques in resting-state fMRI investigations (Mantini *et al.*, 2011). At least some of the neurons described here are relevant to the resting state, as they increased their activity before the eyes were closed prior to the onset of sleep. The undisturbed transition from wakeful rest to sleep represents a period in humans during which attention to the external environment diminishes and the subject becomes free from exteroceptive vigilance. Such transitions show defined but subtle shifts in the functional architecture of mPFC networks with a concomitant increase in internal and self-referential processing. The move into sleep leads to a further change in the connectivity of the mPFC areas of the default mode network (Larson-Prior *et al.*, 2011).

In humans, the default mode network not only consists of mPFC areas but also medial parietal areas (including midline anterior and posterior cingulate cortices; Raichle *et al.*, 2001). Recent investigations in macaques have identified electrophysiological correlates of default mode processing in both mPFC and posterior cingulate cortices (Hayden *et al.*, 2009; Kojima *et al.*, 2009). The positron emission tomography imaging study of Kojima *et al.* (2009) in awake unanaesthetized monkeys clearly demonstrated a default mode of cortical activity with higher rest-related activity in mPFC areas compared with working memory tasks. The activity in macaque mPFC reported here before and during eye-closure may therefore represent in part alterations in the activity of mPFC areas associated with the default mode network in monkeys. It is of interest that Rudolph *et al.* (2007) reported that a significant proportion (~45%) of presumed pyramidal (broad spike/regularly spiking) neurons in parietal association cortex also discharged during SWS and were silent during waking.

In relation to these default mode network studies, the value of the present investigation is that it shows electrophysiologically that the firing rates of a significant number of mPFC neurons (those of cell Type 1 representing about 28% of sampled neurons) in the monkey were low in the awake state (mean 3.1 spikes/s) and increased significantly during sleep (mean 10.2 spikes/s). The firing rates of the neurons involved in default mode network activities, and exactly how they may change, is not directly measured in human neuroimaging studies. Given the increase in the human BOLD (blood oxygen level-dependent) response during operation of the default mode network, it is tempting to speculate that some of the neurons whose firing rates increased during periods of 'eye-closure' may have intracortical axonal arbors instrinsic to the mPFC that innervated nitric oxide (NO)-producing cells (Gabbott and Bacon, 1996). The activity of such cells would lead to local vasodilatation (through NO-mediated mechanisms) and thus increased blood flow in specific mPFC

regions with raised metabolic demands during periods of augmented information processing (Duchemin *et al.*, 2012).

Possible roles in neuropsychiatric disorders

The data from the present study have implications for the generation of sleep activity in humans, both in health and in disease. Many neuropsychiatric and neurodevelopmental disorders, for example depression, schizophrenia and autism, which include functional modifications of the default mode network, have symptoms that include poor sleep architecture (Drevets *et al.*, 1997; Wichniak *et al.*, 2000; Vogt, 2009; Gregory *et al.*, 2011; Vukadinovic, 2011; Price & Drevets, 2012). Patterns of abnormal sleep structure (narcolepsy, sleep inertia, parasomnias, non-REM and REM sleep behaviour disorders, etc.) could be the result of dysfunctional mechanisms in medial and ventromedial prefrontal and other cortical areas and/or abnormal activity in the thalamus and subcortical nuclei projecting to mPFC (Mahowald *et al.*, 2011). We note that defective frontal functioning is also observed after sleep deprivation. This paper and the companion article (Rolls *et al.*, 2003) thus serve to provide preliminary baseline observations and data for more detailed sleep studies of this important PFC region in monkey and humans (Vogt, 2009; Teffer & Semendeferi, 2012). The investigations also provide unique data on the firing rates of mPFC neurons during wakefulness, drowsiness and sleep.

In summary, we have shown that in many areas of the primate mPFC, there is a significant population of neurons (about 28% of the sampled cells) that significantly increase their firing rates during periods of inattention and eye-closure. The firing rates of this set of mPFC neurons (Type 1 cells) averaged 3.1 spikes/s when awake, and 10.2 spikes/s in the eyes-closed and drowsy state. Such neurons may be part of an interconnected network of distributed brain regions that are more active at rest than during tasks requiring attention. In humans and monkeys, these areas are part of the anterior default mode network, defined by increased activation in functional neuroimaging studies during the resting state (Raichle *et al.*, 2001). The novel findings reported here provide direct electrophysiological evidence that many single neurons in these areas of mPFC significantly increase their firing rates during periods of eye-closure and rest.

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Abbreviations

BA, Brodmann area; fMRI, functional magnetic resonance imaging; mPFC, medial prefrontal cortex; REM, rapid eye movement; SWS, slow wave sleep.

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