Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer’s dementia

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Summary
Disturbed sleep cycles are the principal cause of institutionalization in dementia, and therefore represent a major clinical problem. They may arise from dysfunction within the circadian clock of the hypothalamus that times and consolidates wakefulness, or from neuropathology in output pathways and/or target sites of the clock specifically controlling sleep and wakefulness. To determine the relationship of disturbed activity cycles to other circadian clock-controlled rhythms, cross-sectional and longitudinal actigraphy and serial sampling of saliva were used to compare the impact of early Alzheimer’s dementia on activity/rest and cortisol rhythms in home-dwelling subjects. Mildly demented subjects had daily activity rhythms comparable to those of healthy age-matched subjects. Moderately demented subjects exhibited a range of disturbances of the activity/rest cycle, with reduced stability, increased fragmentation and loss of amplitude. Within the moderately demented group, however, the degree of circadian disruption was not correlated with the individual severity of dementia. All groups of subjects, mild, moderate with normal activity cycles and moderate with abnormal activity cycles, exhibited robust daily profiles of salivary cortisol, with highest levels in the morning (08:00 h) and an evening nadir (20:00–24:00 h). Salivary cortisol levels tended to be higher in the moderately demented subjects in the afternoon, but this effect was not specific to those with abnormal activity/rest patterns. The actimetric data confirm that deterioration of activity/rest cycles is a common and progressive feature in home-dwelling Alzheimer’s patients, occurring early in the disease but after the measurable onset of dementia. The maintenance of highly rhythmic daily cortisol profiles in association with disturbed activity profiles, both on an individual and on a group basis, demonstrates that loss of circadian control to activity/rest cycles is not a consequence of global circadian disruption in early dementia. Rather, pathology may develop in discrete elements of the circadian clockwork and/or its output systems that control activity/rest, sleep and wakefulness. Further characterization of this pathology will facilitate more effective management of sleep patterns in home-dwelling demented patients.

Keywords: actimetry; body clock; circadian; cortisol; sleep

Abbreviations: ANOVA = analysis of variance; HPA = hypothalamo-pituitary-adrenal; IS = inter-daily stability; IV = intra-daily variability; MMSE = Mini-Mental State Examination; NPCRA = non-parametric circadian rhythm analysis; PSG = polysomnographic; RA = rhythm analysis; SCN = suprachiasmatic nuclei of the hypothalamus


Introduction
Depending on the criteria applied, up to half of the elderly suffer from chronic sleep disturbances, and in demented elderly, one-quarter to a half suffer from severe nocturnal restlessness at some stage of the disease (Van Someren, 2000). The clinical and socio-economical relevance of the latter are illustrated by the facts that nocturnal restlessness predicts faster cognitive and functional decline (Moe et al., 1995), and is the principal cause of institutionalization,
having a major negative impact on the well-being of patient and carer in the home setting and consequently increasing the odds ratio for institutionalization by a factor of 10 (Sanford, 1975; Pollack and Perlick, 1991; Bianchetti et al., 1995). Identification of the origin and evolution of such disturbances relative to the overall progression of disease should facilitate the development of specific therapies and effective management strategies designed to defer dependence on institutional care.

Objective evidence of activity/rest disturbance comes mainly from actigraphic studies of moderately to severely demented, institutionalized subjects (Witting et al., 1990; Ghali et al., 1995; Satlin et al., 1995; Van Someren et al., 1996, 1997; Ancoli-Israel et al., 1997). These have revealed more fragmented, less stable activity/rest patterns, with lower daytime and higher nighttime activity relative to cognitively intact controls. Furthermore, the severity of dementia, assessed using the global deterioration scale (Reisberg et al., 1982), has been positively correlated with increased nocturnal activity, and negatively correlated with day-to-day stability of activity patterns (Witting et al., 1990). Polysomnographic (PSG) studies have also shown that severely demented patients typically have lower sleep efficiency, less slow-wave sleep (SWS) and more frequent arousals than age-matched controls (Allen et al., 1987; Benca et al., 1992).

There have been relatively few studies of the sleep/wake patterns of subjects in their home settings, and at an early stage in the development of dementia. Using sleep diaries (Bliwise et al., 1992) and questionnaires (Ancoli-Israel et al., 1994), home-dwelling subjects with Alzheimer’s disease with mild to moderate dementia reported earlier bedtimes and longer sleep times than controls, and both measures were positively correlated with severity of dementia. In a series of 72-h laboratory-based PSG studies (Prinz and Vitiello, 1993; Moe et al., 1995), sleep efficiency and the amount of SWS were reduced compared with controls, even in mildly demented Alzheimer’s disease subjects usually living at home. This declined further in parallel with the severity of dementia, whilst levels of daytime sleep were increased, leading to fragmentation of the sleep/wake rhythm. In contrast, other actigraphic studies reported that moderately demented subjects dwelling at home had activity/rest cycles comparable to those of age-matched controls (Van Someren et al., 1996).

The sleep/wake cycle is timed by the circadian pacemaker of the suprachiasmatic nuclei of the hypothalamus (SCN), which acts via subcortical networks to regulate cortical function (Pace-Schott and Hobson, 2002). The impairments to daily activity/rest patterns in later dementia may therefore arise from dysfunction outside the SCN in subcortical and/or cortical neural pathways cueing and consolidating sleep and wakefulness. Alternatively, they may reflect a global dysfunction of the SCN clockwork, in which case they would be accompanied by corresponding deficits in other circadian functions. Several studies have therefore attempted to assess whether various daily rhythms are disturbed in dementia, but have yielded inconsistent findings. Oral and rectal temperature rhythms in mildly and moderately demented Alzheimer’s disease subjects are reported to be of increased amplitude (Touitou et al., 1986), unchanged (Prinz et al., 1984, 1992), phase delayed (Satlin et al., 1995; Harper et al., 2001) or disorganized (Okawa et al., 1991) relative to elderly controls. Melatonin secretion patterns are reported to be unchanged (Touitou et al., 1981) or of reduced amplitude (Mishima et al., 1999) in moderately demented Alzheimer’s disease subjects.

Cortisol secretion is a well characterized daily rhythm intimately dependent on the SCN clock (Hastings et al., 2003). Assessment of the impact of dementia on cortisol patterns is, however, complicated by a tendency for nocturnal levels to be elevated in healthy ageing (Ferrari et al., 2001a; Vgontzas et al., 2003). This rise, observed especially around midnight until 4 am, may be a consequence of age-related changes in sleep patterning, because it is also seen in young insomniacs (Vgontzas et al., 2001). Subjects with Alzheimer’s disease share this nocturnal elevation (Ferrari et al., 2001a), but although the overall level of cortisol secretion increases in moderate dementia (Swannick et al., 1998; Umegaki et al., 2000; Ferrari et al., 2001b), there are no reports of altered daily patterning to its secretion that could be indicative of a more widespread circadian dysfunction attributable specifically to dementia.

Notwithstanding the difficulty in linking sleep/wake disorders to a more global disturbance of timing, post mortem studies have revealed neuropathology in the SCN of severely demented patients, implicating degeneration in the core clock mechanism as a cause of sleep disorders, at least in the final stages of Alzheimer’s disease (Swaab et al., 1985; Stopa et al., 1999). Clarification of when, how and what type of circadian disorders develop is clearly important to a full understanding of the behavioural symptoms of dementia. This study therefore aimed to use actimetric recordings to assess the impact of probable Alzheimer’s dementia on activity/rest cycles in home-dwelling subjects at early stages of disease progression, and to compare this with neuroendocrine rhythmicity exemplified by cortisol secretion. Both cross-sectional and longitudinal comparisons were made to monitor changes in circadian competence as dementia progressed.

**Subjects and methods**

**Subjects**

Ethical approval was granted by Cambridge and Huntingdon Health Authority, Local Research Ethics Committee. Written informed consent was obtained from subjects and/or their next of kin. Healthy elderly subjects (10 male and nine female; age 66–84 years, mean 71.8) were recruited from the MRC Cognition and Brain Sciences Unit subject panel. Fifteen male and 12 female subjects (age 60–82 years, mean 68.5) who met the Diagnostic and Statistical Manual (DSM)-IV definition of dementia and the National Institute of Neurological and Communicative Diseases and Stroke, Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA)
criteria for probable Alzheimer’s disease (McKhann et al., 1984) were recruited from Addenbrooke’s Hospital Memory Clinic. All Alzheimer’s disease subjects were living at home with a carer. Mini-Mental State Examination (MMSE) was used to classify them as having either mild (MMSE ≥20/30, n = 13), or moderate (MMSE ≤19/30, n = 14) dementia (Folstein et al., 1975). These groupings correspond to Clinical Dementia Rating (CDR) levels of 1.0 (mild) and 2.0 (moderate) (Hughes et al., 1982; Morris et al., 1997). Carers completed a comprehensive behavioural questionnaire, the Cambridge Behavioural Inventory (CBI) (Bozeat et al., 2000) relating to the subjects’ cognitive and behavioural symptoms, and levels of activities of daily living skills over the previous month. None of the subjects met the criteria for a diagnosis of depression using the Hamilton depression rating scale (Hamilton, 1960). Ten mild and 10 moderate Alzheimer’s disease subjects and 18 elderly control subjects recruited during the first year of the 2-year study were potentially available for follow-up after 1 year. Seventeen of the elderly control subjects, eight of the mild Alzheimer’s disease subjects and six of the moderate Alzheimer’s disease subjects participated in the follow-up study. Eight of the healthy elderly subjects took cardiovascular medication including diuretics, beta-blockers and calcium channel antagonists, eight took non-steroidal anti-inflammatory drugs (NSAIDS), two cholesterol-lowering agents, two proton pump inhibitors, one used steroid and bronchodilator inhalers for asthma, one allopurinol (xanthine oxidase inhibitor), one oxybutin (anti-muscarinic), and one a bisphosphonate for osteoporosis. Of the Alzheimer’s disease subjects, 15 were taking cholinesterase inhibitors, 10 NSAIDs, seven cardiovascular medication, four cholesterol-lowering agents, one a proton pump inhibitor, one tobutamide (oral hypoglycaemic), one allopurinol, two serotonin-specific reuptake inhibitors (SSRIs), four antipsychotics and one a benzodiazepine.

**Activity/rest assessment**

Subjects wore a wrist-mounted activity monitor (Actiwatch; Cambridge Neurotechnology, Cambridge, UK) for 28 days and completed a daily sleep diary (Lockley et al., 1999). Data were initially plotted and analysed with Clocklab software (Actmetrix, Evanston, IL, USA), and then subjected to non-parametric circadian rhythm analysis (NPCRA). This is more suitable than cosinor or other parametric analyses for the quantitative analysis of non-sinusoidal data (Van Someren et al., 1999). Inter-daily stability (IS) is a measure of stability across days, while intra-daily variability (IV) reflects the relative consolidation/fractionation within days based on how many transitions occur between activity and rest. The third variable, rhythm amplitude (RA), reflects the difference in activity level between the 10 most active and five least active hours in the day (LS). It therefore provides a more comprehensive representation of rhythm amplitude than the simple difference between single measures of peak and nadir. One moderate Alzheimer’s disease subject completed only 10 days of activity monitoring because of a failure to tolerate the activity monitor. Fourteen days of activity monitoring from another moderate Alzheimer’s disease subject who became bed-ridden after a fall were discarded.

**Endocrine measures**

Subjects provided samples of saliva (0.5–2.0 ml) by free collection every 4 h from 08:00 to 24:00 h on two consecutive days during the second week and a further two consecutive days during the final week of actimetry. These times were selected to include the high morning levels of cortisol and progressive decline through the day (Krieger et al., 1971; Goodyer et al., 1996; Van Someren et al., 1996; Ferrari et al., 2001a, b; Vgontzas et al., 2001, 2003). No samples were taken at 04:00 h. Although this inevitably prevented the complete description of the endocrine profile, it was necessary to avoid disturbing the spontaneous activity/rest cycle. No artificial aids were used to promote salivation, and subjects were instructed not to clean their teeth for at least 1 h beforehand, and to rinse their mouths out with clean water just before collection. Cortisol concentrations were determined from the saliva samples by ELISA (Goodyer et al., 1996). To increase compliance in the follow-up study, samples were obtained using Salivette swabs (Sarstedt Ltd) (Shirtcliff et al., 2001). Two of the six moderate Alzheimer’s disease subjects taking part in the follow-up study were unable to provide saliva samples. All samples from the follow-up study were assayed together, but independently of the year 1 samples from the same subjects.

**Statistics**

Main effects and their interactions were analysed by repeated measures and/or factorial one-and two-way analysis of variance (ANOVA) using SPSS for PC. Where ANOVA revealed significant main effects and/or interactions, differences between group means were determined by Scheffe’s post hoc test. Unpaired or paired t-tests were used as appropriate where only two groups were to be compared, e.g. in the follow-up studies. One moderately demented subject failed to provide 08:00 h saliva samples. To incorporate the data from the other samples provided by this subject into the repeated measure analysis, a nominal value was assigned equal to the mean value obtained from other subjects in that group at 08:00 h. The outcome of the statistical analyses was the same whether or not this nominal data point was included, or the entire dataset of this subject was excluded. Figures are plotted without the 08:00 h data for this subject, but do include the remaining data. Hormone data were log-transformed to normalize variance (Carroll et al., 1981; Goodyer et al., 1996).

**Results**

**Actograms**

Healthy elderly subjects (Fig. 1A) exhibited robust, high amplitude activity bouts, clearly consolidated to the daytime hours. The night hours were characterized by very low levels of activity. Morning activity onsets and late evening offsets were well defined and consistent from day to day. Activity/rest cycles in subjects with mild dementia were not appreciably different from those of controls (Fig. 1B). In contrast, there was a broad range of activity patterns in subjects suffering from moderate dementia, ranging from consolidated, well defined patterns comparable to those of mildly demented subjects and controls (Fig. 1C), to severely disrupted patterns (Fig. 1D). In this latter category, onsets and offsets to activity were not well defined, they were variable from day to day, and the subjects exhibited fragmented patterns of rest and activity.
Activity profiles
Activity of the group of control subjects peaked in the morning hours after waking and declined gradually through the afternoon and into the evening (Fig. 2A). The mildly demented group had an activity profile comparable to that of controls, albeit with a slightly lower amplitude. As a group, the moderately demented subjects showed appreciably less activity throughout the day relative to that of controls and mildly demented subjects. Repeated measures ANOVA revealed significant effects of clock time $[F(47,2021) = 68.68, P < 0.001]$, group $[F(2,2021) = 4.02, P = 0.025]$ and a clock time $\times$ group interaction $[F(94,2021) = 3.09, P = 0.001]$. Activity in moderately demented subjects was lower than in controls between 07:30 and 12:00 h, and between 14:00 and 16:00 h (post hoc Scheffe’s test).

Non-parametric analysis of activity patterns
NPCRA revealed that the stability, consolidation and peak/trough changes of activity in the mild subjects were indistinguishable from the control, non-demented, subjects.

Fig. 1 Representative actograms from healthy control (A, E), mildly demented (B, F) and moderately demented (C, G, D, H) subjects recorded first in year 1 (A–D), and for the same subjects at follow-up in year 2 (E–H). Data from 28 consecutive days are double-plotted on a 48-h time base for clarity.
The moderately demented group, however, showed marked perturbations, with significantly lower stability \([F(2,43) = 5.77, P = 0.006]\), consolidation \([F(2,43) = 5.67, P = 0.007]\) and peak/trough differences \([F(2,43) = 3.42, P = 0.042]\). In addition, the activity level for highest 10 h (M10) was significantly lower in this group \([F(2,43) = 3.73, P = 0.032]\), although the phase of the profiles, as represented by timing of the onset of M10, was not significantly different between groups (mean ± SEM, controls 08:34 ± 16 min, mild 08:47 ± 10 min, moderates 08:50 ± 11 min).

Fig. 2 Group daily activity profiles (plotted as mean ± SEM) for (A) control, mildly demented and moderately demented subjects, and (B) control subjects and moderately demented subjects grouped as normal or abnormal according to non-parametric rhythm analysis of the activity/rest profile (data from mildly demented subjects omitted for clarity). (C) As in B, but data plotted relative to each individual’s mean activity level and time of activity onset, as defined by sleep diary. *Indicates significantly different from aged control by Scheffe’s post hoc test \((P < 0.05)\).
Increased variability and reduced RA of the profiles were not correlated with the severity of dementia, as defined by MMSE score (Spearman correlations: IV, $r = -0.314$, $P = 0.11$; RA, $r = 0.253$, $P = 0.203$). There was, however, a significant positive correlation between MMSE score and inter-day stability ($r = 0.45$, $P = 0.018$), indicating that a greater degree of dementia was associated with less regular activity/rest profiles.

Notwithstanding the clear group differences reported above, it was obvious that the activity profiles within the group of moderately demented patients were heterogeneous. NPCRA was used therefore to further categorize this group. Specifically, a subpopulation of disrupted, ‘abnormal’ patients ($n = 7$) was defined on the basis of having one or more non-parametric measures $>2$ SD from the mean value of the control group. The remaining moderately demented subjects ($n = 7$) with all three non-parametric measures within $2$ SD of the control group mean were classified as ‘normal’ in their activity/rest cycles.

Comparison of activity/rest profiles in moderately demented patients

The activity/rest profiles of the moderately demented subjects categorized according to their non-parametric analyses were markedly different. Repeated measures ANOVA of the raw activity data for controls, mild and the two groups of moderately demented subjects revealed significant effects of clock time [$F(47,1974) = 51.7$, $P < 0.001$] and group [$F(3,1974) = 3.61$, $P = 0.025$], and a significant clock time $\times$ group interaction [$F(141,1974) = 2.87$, $P = 0.001$], confirming that the daily profile was different between the groups (Fig. 2B). The moderately demented subjects with ‘normal’ behaviour had clearly defined profiles comparable to age-matched controls, but with a markedly reduced peak level of activity. In fact the activity profiles of this group were indistinguishable from those of mildly demented subjects (Fig. 2A). The average profile of moderately demented subjects with ‘abnormal’ behaviour, however, showed pronounced disorganization. The daytime peak was poorly defined and of very low amplitude and the activity levels in this group were significantly lower than in the controls (mean within the range 07:30 and 17:00 h. This loss of definition was not an artefact of altered phasing or desynchronization between individuals. There was no significant difference between groups in activity acrophase times [mean $\pm$ SEM, controls 13:46 $\pm$ 12 min, mild 14:18 $\pm$ 15 min, moderates normal 15:05 $\pm$ 21 min, moderates abnormal 14:45 $\pm$ 4.9 min; $F(3,42) = 2.31$ not significant (n.s.)]. Equally, there were no significant differences in the time to 50% of maximum activity [$F(3,42) = 1.56$ n.s.], actigraphically defined activity onset [$F(3,42) = 1.82$ n.s.] and M10 onset [$F(3,42) = 2.56$, $P = 0.067$ n.s.]. Moreover, the difference in pattern remained when the data were plotted relative to individual wake-up times rather than solar time (Fig. 2C). The altered daily pattern was also independent of the change in overall activity level, because when the activity counts were expressed relative to each individual’s mean, the significant interaction between clock time and group remained [$F(141,1974) = 2.037$, $P = 0.001$], indicating that the ‘abnormal’ group had a significantly different waveform to their 24-h activity/rest cycles, with less daytime activity and more nocturnal activity (Fig. 2C). The moderately demented subjects with abnormal behaviour patterns were therefore able to maintain a 24-h life in phase with solar time, but the definition and consolidation of the daytime activity phase and nighttime rest phase were severely impaired.

Although there were significant differences between the two moderately demented groups and the mildly demented subjects in several sections of the CBI carer report questionnaire [ANOVA memory, $F(2,23) = 4.64$, $P = 0.02$; attention, $F(2,23) = 4.82$, $P = 0.02$; practical everyday skills, $F(2,23) = 5.86$, $P = 0.01$] (Table 2), the contrasting daily activity profiles of the two groups of moderately demented subjects

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>MMSE $\pm$ SEM</th>
<th>CBI memory $\pm$ SEM</th>
<th>CBI everyday skills $\pm$ SEM</th>
<th>CBI self care $\pm$ SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild AD (13)</td>
<td>24.08 $\pm$ 0.65</td>
<td>12.5* $\pm$ 1.0</td>
<td>27.2 $\pm$ 2.5</td>
<td>11.2 $\pm$ 2.4</td>
</tr>
<tr>
<td>Moderate AD, normal (7)</td>
<td>13.71 $\pm$ 1.02</td>
<td>18.4 $\pm$ 1.5</td>
<td>24.1 $\pm$ 3.9</td>
<td>13.3 $\pm$ 3.6</td>
</tr>
<tr>
<td>Moderate AD, abnormal (7)</td>
<td>12.57 $\pm$ 1.96</td>
<td>17.9 $\pm$ 2.3</td>
<td>24.1 $\pm$ 3.9</td>
<td>13.3 $\pm$ 3.6</td>
</tr>
</tbody>
</table>

Increased variability and reduced RA of the profiles were not correlated with the severity of dementia, as defined by MMSE score (Spearman correlations: IV, $r = -0.314$, $P = 0.11$; RA, $r = 0.253$, $P = 0.203$). There was, however, a significant positive correlation between MMSE score and inter-day stability ($r = 0.45$, $P = 0.018$), indicating that a greater degree of dementia was associated with less regular activity/rest profiles.
were not accompanied by differences in any cognitive and
behavioural indices, including their MMSE scores (t-test =
\(-0.52, P = 0.62\)). Disturbance of the activity/rest cycle
therefore occurred independently of other behavioural con-
sequences of the progression of dementia.

**Endocrine function**
The control subjects showed their highest levels of cortisol at
08:00 h, with a progressive decline to the low levels observed
in the evening. Repeated determinations showed this daily
pattern was highly consistent for all individuals (Fig. 3).
Although it is not possible to assert that the 08:00 h value was
the peak, it is clear that cortisol secretion in controls followed
the rhythmic daily profile that would be expected for normal
healthy subjects. The pattern of cortisol secretion in the
mildly demented group was comparable to that of controls,
and equally reproducible (Fig. 3B). Normal cortisol rhythms,
with high levels at 08:00 h declining to basal around 20:00 or
24:00 h, were also observed in 12 of the 14 moderately

![Fig. 3 Representative individual daily salivary cortisol profiles for (A) control, (B) mildly demented, and (C) moderately demented subjects with normal and (D) abnormal activity patterns. Samples were taken over two separate 48-h intervals, one during the second week (days 1–2) and one during the fourth week (days 3–4) of actigraphic recording. For each individual the mean daily profile is presented on the right (mean ± SEM).](http://brain.oxfordjournals.org/)
demented subjects, with both normal and abnormal activity profiles (Fig. 3C and D). One subject in each of the moderately demented subgroups failed to exhibit a rhythmic profile of cortisol secretion in the first year of assessment (but see ‘Follow-up study’ below). One-way repeated measures ANOVA therefore confirmed that all groups exhibited strong and highly significant \((P < 0.01)\) daily rhythms in salivary cortisol: controls \([F(3,54) = 92.9]\), mildly demented \([F(3,36) = 53.5]\), all moderately demented subjects \([F(3,36) = 27.9]\), moderate with normal activity/rest patterns \([F(3,18) = 14.1]\) and moderate subjects with abnormal activity patterns \([F(3,15) = 13.3]\).

Repeated-measures ANOVA of cortisol in control subjects compared with all of the demented subjects combined into a single group again revealed a significant effect of clock time \([F(3,129) = 166, P < 0.0001]\), but no group effect nor any interaction between clock time and group. Comparison of controls with mildly and moderately demented subjects grouped separately also produced a significant clock time effect \([F(3,126) = 163, P < 0.0001]\), and a marginally significant group effect \([F(2,126) = 3.33, P = 0.045]\) arising from higher levels of cortisol in the moderately demented subjects (Fig. 4A). This effect was most obvious in the afternoon samples (16:00 h). The highest levels at 08:00 h and the evening low points were, however, comparable between the three groups. Moreover, there was no significant interaction \([F(6,126) = 1.31, P = 0.26]\), indicating that, overall, the daily profile of cortisol secretion in moderately demented subjects was not significantly different from that of control subjects. With further categorization of the moderately demented subjects according to their activity profile, the trend for higher afternoon cortisol levels was apparent in both groups (Fig. 4B). The association between moderate dementia and higher cortisol levels was not, therefore, a specific feature of subjects with disturbed daily activity/rest cycles. Indeed, direct comparison of the daily cortisol profiles in the two groups of moderately demented subjects by two-way repeated measures ANOVA revealed a clock time effect \([F(3,33) = 26.5, P < 0.001]\), but no group effect \((P = 0.49)\) or group \(\times\) clock time interaction \((P = 0.76)\).

Regardless of their behavioural profile, therefore, all classes of demented subject continued to express a robust daily cortisol rhythm comparable to that of healthy subjects, and the only potential effect of dementia was a trend for higher afternoon levels. To examine further the relationship between dementia and cortisol secretion, the mean level of cortisol between 08:00 and 20:00 h was compared. Although higher in moderately demented subjects (ng/ml, mean \(\pm\) SEM: controls 1.63 \(\pm\) 0.12, mild 1.51 \(\pm\) 0.11, moderates 2.08 \(\pm\) 0.26), this effect escaped statistical significance \([F(2,42) = 2.74, P = 0.10]\). Moreover, there was no significant difference in overall levels of cortisol secretion between moderately demented subjects with disturbed activity/rest patterns and other groups (moderate dementia normal activity/rest = 1.95 \(\pm\) 0.31, moderate dementia abnormal activity/rest = 2.22 \(\pm\) 0.43). There were, nevertheless, differences in the detail of the cortisol profile associated with dementia. In the demented groups as a whole, mean salivary cortisol levels (08:00 to 20:00 h) were negatively correlated with MMSE \((r = 0.47, P = 0.02)\), i.e. levels tended to be higher with increasing dementia (Fig. 4C). Consequently, elevated levels of saliva cortisol, especially at 16:00 h and, to a lesser extent, at 12:00 h, were correlated with actigraphic disturbance. In particular, IS was negatively correlated with an increased cortisol level.
at 16:00 h (r = -0.36, P < 0.03), whilst IV was positively correlated with cortisol level at 16:00 h (r = 0.57, P < 0.0001) and at 12:00 h (r = 0.42, P < 0.004). Finally, lower relative amplitude was associated with higher cortisol at 16:00 h (r = ±0.57, P < 0.0001), 12:00 h (r = ±0.34, P < 0.02) and 20:00 h (r = ±0.34, P < 0.02). Of note, none of the correlations between the actigraphic variables and the average cortisol level (8:00-20:00 h) reached significance. The data are therefore indicative of a selective relation between afternoon cortisol secretion, dementia and actigraphic competence, in the absence of any compromise to overall daily control of cortisol release in Alzheimer’s disease.

**Follow-up analysis of actimetric and endocrine rhythms**

**Cognitive state**

Control subjects showed no decline in cognitive function, as assessed by MMSE scores, over the year (mean ± SEM: year 1 = 29.3 ± 0.2, year 2 = 29.5 ± 0.2; n = 17; paired t = -0.525, P = 0.72). In contrast, cognitive function declined significantly in the mild Alzheimer’s disease subjects between assessments (year 1 = 24.6 ± 0.8, year 2 = 22.4 ± 1.1; n = 8; t = 4.03, P = 0.005), as it did in those moderate Alzheimer’s disease subjects available for follow-up (year 1 = 13.0 ± 1.2, year 2 = 7.5±2.5; n = 6; t = 4.20, P = 0.009).

**Actimetry**

For all categories of subject, the actograms recorded after 1 year were very similar to the original traces (Fig. 1E-H), with the exception of two moderately demented subjects with abnormal activity on year 1 who showed a further decline (Fig. 1H). No subjects showed improved daily patterning to activity, and none of those with normal patterns at the start of the study deteriorated. The group activity profiles reflected this stability (Fig. 5), and for all groups there remained highly significant time of day effects, but no year-to-year effect, nor any interaction of year with time of day. Non-parametric measures of activity cycles were also highly consistent over the 2 years (Table 3), and none of the groups showed year-on-year differences in stability or fragmentation. In the moderately demented group alone the RA measure of cycle amplitude declined further (paired t = 2.72, P = 0.042). The consistency across 1 year emphasizes the
matched controls. Daily pro®les of cortisol secretion comparable to those of age-
therefore, all moderately demented subjects had exhibited abnormal behaviour) displayed robust daily rhythms of
in the ®rst year (one with normal behaviour and one with
moderately demented subjects with disturbed cortisol pro®les
were also consistent between the ®rst and second years in the
subjects with moderate dementia provided saliva samples in
ANOVA therefore re¯ected a highly signi®cant effect of clock time
levels were again highest at 08:00 h in year 2,
being somewhat higher than in year 1, falling to an evening
ANOVA therefore re¯ected a highly signi®cant effect of clock time
thus con®rming the stability of the pro®le. Only four
subjects with moderate dementia provided saliva samples in the follow-up study, precluding extensive analysis of group
effects. However, an important observation is that the two
moderately demented subjects with disturbed cortisol pro®les
in the ®rst year (one with normal behaviour and one with
abnormal behaviour) displayed robust daily rhythms of
salivary cortisol at follow-up, with highest levels at 08:00 h
and lowest in the evening. Over the course of the study,
therefore, all moderately demented subjects had exhibited
daily pro®les of cortisol secretion comparable to those of age-
matched controls.

Outcome
Of the 10 moderately demented subjects recruited during the
®rst year of the study, ®ve were still living at home 1 year
later (good outcome) and ®ve were admitted to an institution
or died. Although the MMSE scores were not signi®cantly
different between these two groups (mean ± SEM: good
outcome 13.8 ±1.16 versus poor outcome 10.6 ±2.34), the
subjects with a poor outcome had, on the ®rst year,
significantly lower parametric measures of stability (good
outcome IS = 0.49 ± 0.04, poor outcome = 0.31 ±0.04; t =
3.22, P = 0.012) and amplitude for the activity/rest cycle (RA
= 0.87 ±0.02 versus 0.66 ±0.07; t = 2.85, P = 0.022).

Discussion
Increasing severity of dementia was associated with progres-
sive disorganization and decreasing amplitude of the daily
pattern of activity and rest within home-dwelling Alzheimer’s
disease subjects. Whereas mildly demented subjects showed
no signi®cant deterioration, moderately demented subjects
exhibited a spectrum of daily patterns, ranging on an
individual basis from intact to severely disrupted.
Longitudinal analysis con®rmed these observations, and
also revealed that disruption to activity/rest patterns is a
slow process, the cycles remaining unaltered despite signi®-
cant decline in cognitive function between yearly assess-
ments. The salivary cortisol levels analysed in the same
individuals, even those with disturbed activity rhythms,
showed a robust high amplitude daily pro®le, comparable to
that seen in control and mildly demented subjects. In all
cases, highest levels were observed at 08:00 h and lowest
levels in the evening, and the amplitude of the pro®le was not
signi®cantly different from control subjects in the initial or
follow-up study. There was a tendency, however, for
moderately demented subjects to have higher levels of
cortisol in the afternoon, although this occurred both in
subjects with and without marked activity/rest disturbance.
These results indicate that the disturbance of activity/rest
pro®les in early dementia occurs independently of any
changes in daily cortisol secretory pro®les, and so it is
unlikely to arise from global disorder within the circadian
pacemaker of the SCN. Rather, it probably involves
dysfunction in pathways from the SCN that speci®cally
control sleep and wakefulness, or of distal targets of those
output pathways.
The mildly demented subjects investigated in the current
study are at the earliest stage of dementia to be analysed by
actigraphy to date. Laboratory-based PSG analysis of sleep
patterns in comparably demented subjects (Prinz and Vitiello,
1993) detected slightly more fragmented nocturnal sleep and
greater daytime naps than in control subjects, but coherent
24-h patterning to the sleep/wake cycle remained. In com-
bination with our ®ndings, this suggests that in the very early
stages of dementia, circadian disturbances are subtle if
present at all. In contrast, our group of moderately demented
subjects had signi®cantly disrupted activity/rest patterns,
although the patterns varied widely from individual to individual. The degree of (dis)organization within a subject was, however, stable through time. Consistent with our actimetric data, PSG-defined sleep measures in moderately demented subjects living at home also reported marked deterioration, including increased fragmentation and napping (Prinz and Vitiello, 1993). The effect is not, however, an inevitable consequence of moderate dementia, because some moderately demented subjects in the current study had regular activity/rest cycles, and Van Someren et al. (1996) also reported no lack of organization to daily patterns in actimetric measures of home-dwelling, moderately demented subjects. Moderate dementia appears, therefore, to be the first stage where marked disturbances to the pattern of sleep and wakefulness can be identified. Although consistent and sustained within an individual, these disturbances occur sporadically between subjects. These findings in home-dwelling subjects examined relatively early in the progression of disease contrast with the consistently reported major disturbances to the daily activity patterns of severely demented, institutionalized patients (Witting et al., 1990; Ghali et al., 1995; Satlin et al., 1995; Van Someren et al., 1996; Ancoli-Israel et al., 1997). Together, these observations indicate that the activity/rest disturbances are a slow and progressive, but nevertheless common, feature of dementia. Moreover, although they develop in parallel with cognitive deterioration, and indeed this and other studies (Witting et al., 1990; Moe et al., 1995) have shown that the degree of disturbance can be inversely correlated with cognitive score, disturbances to activity rhythms and cognitive decline do not necessarily occur together, nor progress in an individual at the same rate: subjects with comparable degrees of moderate cognitive loss may, or may not, show circadian disturbances. This implies a differential breakdown of the neuroanatomical substrates leading to cognitive decline and impaired circadian control of activity. Nevertheless, our demonstration of abnormal activity/rest patterns in home-dwelling subjects confirms that circadian dysfunction is a true component of the behavioural symptomatology of dementia, and not simply an artefact of institutionalization (Bliwise, 1993; Harper et al., 2001). Moreover, in our own limited follow-up population and in more extensive analyses (Moe et al., 1995), it appears that disturbances of the activity rhythm in subjects with dementia of comparable severity, as assessed by MMSE, can be differentially predictive of further functional decline.

The current study is the first reported use of salivary cortisol measurement in home-dwelling, demented subjects. The salivary levels observed (<1–5 ng/ml) are consistent with previous reports (Goodyer et al., 1996), and in combination with actimetry, the protocol sustained high compliance and provided an independent measure of a circadian clock-dependent function. The absence of a sample at 04:00 h inevitably precluded any analysis of the impact of dementia on the elevation of nocturnal cortisol secretion reported in older subjects (Ferrari et al., 2001a; Vgontzas et al., 2003). To take such a sample, however, would have confounded the recording of spontaneous activity/rest cycles, and so was not possible within the constraints of sampling subjects in their home setting. In addition, with our every 4-hourly sampling regime and lacking the 04:00 h sample it would be unwise to rule out small changes in the phasing of these cycles during dementia. Although it is possible to identify phase-shifts smaller than the sampling interval (Klerman et al., 2002), if the cortisol profiles of our subjects delay shifted as reported for the circadian temperature rhythm (Satlin et al., 1995; Harper et al., 2001), we would not necessarily detect them. Should such delays have occurred in our subjects; however, they would have been independent of the activity/rest cycle, which did not show any significant phase differences between groups. Moreover, the temporal profiles with equivalent peaks at 08:00 h and evening nadirs at 20:00 to 24:00 h in both control and demented subjects match very well the patterns of cortisol secretion reported from plasma samples (Krieger et al., 1971; Van Cauter et al., 1996; Ferrari et al., 2001a, b; Vgontzas et al., 2001, 2003), suggesting that appreciable phase-shifts of cortisol secretion did not occur in our demented subjects.

Cortisol secretion was nevertheless related to cognitive state, insofar as the moderately demented group had higher afternoon levels, and daily mean levels within the demented subjects were correlated with the severity of dementia, as reported previously (Raskind et al., 1982; Hartmann et al., 1997). The maintained rhythmicity to the daily profiles, however, even in the group with disturbed activity/rest patterns, suggests that circadian control to the hypothalamo-pituitary-adrenal (HPA) axis is retained in patients with moderate dementia. This conclusion is supported by our observation that salivary levels of dehydroepiandrosterone, a corticosteroid implicated in physiological ageing and neuroprotection (Kimonides et al., 1998; Karishma and Herbert, 2002), were also rhythmic in control, mild and moderately demented subjects, being higher at 08:00 than at 20:00 h (Hatfield et al., unpublished data). Our conclusion that circadian regulation of the HPA axis is effective in the demented home-dwelling subjects is consistent with reports of intact temperature rhythms (Prinz et al., 1984; Touitou et al., 1986; Prinz et al., 1992; Mishima et al., 1997), plasma cortisol rhythms (Hartmann et al., 1997; Ferrari et al., 2001a) and melatonin secretion (Touitou et al., 1981) in institutionalized subjects. Daily cortisol profiles in demented subjects do not, therefore, share the loss of control shown by the activity/rest cycle.

Only one other study, to our knowledge, has employed follow-up actigraphic measures in Alzheimer’s disease, and based on cosinor analysis, reported no significant change in individuals tested between 6 and 36 months after initial assessment (Ysavage et al., 1998). Of our own moderately demented subjects, those with the most impaired activity/rest profiles were unavailable for follow-up, either because of death (n = 3) or institutionalization (n = 2). No subjects with well-defined activity/rest cycles were lost during the intervening year. More extensive analysis would be necessary to
confirm the extent to which impaired activity/rest measures might have prognostic value, although there are reports in this regard (Bianchetti et al., 1995; Moe et al., 1995). Our observation of stability in both actigraphic measures and cortisol profiles in individuals sampled after 12 months, increases confidence in the reliability of the measures. It also extends the conclusion from cross-sectional analysis that the declines of circadian and cognitive function during early dementia exhibit different rates, and presumably have distinct causal mechanisms.

These contrasting rates of cognitive and circadian decline may reflect differential neuropathology, consistent with the conclusion that whilst sensitive to the pathological changes seen in Alzheimer’s disease, the hypothalamus is less severely affected than other brain areas (Stopa et al., 1999). It is likely, therefore, that circadian disorders involving the daily cortisol profile would become more prevalent as neuropathology progresses from the cortex to subcortical/hypothalamic areas in severe Alzheimer’s disease. The higher afternoon levels of cortisol secretion in the moderately severely affected than other brain areas (Stopa et al., 1999). Alternatively, neuropathology of the hippocampus has been linked to altered circadian feedback dynamics with the HPA axis (Ferrari et al., 2001a), and the glucocorticoid cascade hypothesis (Sapolsky et al., 1986) proposes that increased activation of the glucocorticoid receptor contributes to neuronal death, potentiating cognitive decline. Equally, loss of glucocorticoid-sensitive hippocampal neurons may disinhibit the HPA axis, leading to further corticosteroid-dependent neurotoxicity. Higher afternoon levels of cortisol in our more demented subjects are consistent with this hypothesis, although causality cannot be inferred (Muller et al., 2001).

Neuropathological examination, so effective in other studies of dementia (Harper et al., 2001), would clearly add to our understanding of the origin of circadian disorders. Based on the current data, we would not anticipate global disruption to the SCN in the early stages of dementia. Rather, pathological changes restricted to subregions of the SCN specifically implicated in circadian regulation to activity/rest cycles, and not HPA output (Buijs and Kalsbeek, 2001). Alternatively, the SCN may be unaffected, and the pathology located in pathways linking a functional biological clock to centres timing sleep and the consolidation of wakefulness. It might be anticipated that with further progression, pathology involving elements of the SCN may arise, leading to severe circadian dysfunction. The correlations between elevated afternoon levels of cortisol, dementia and actigraphic measures may be a forerunner of such changes. Nevertheless, animal models of age-related disturbance to circadian timing have revealed a fully competent circadian oscillation in the SCN in vitro, despite loss of precision to circadian function in the periphery (Yamazaki et al., 2002). Further characterization of SCN function in animal models of dementia should therefore complement human studies, where pacemaker properties of the SCN cannot currently be determined directly, but only inferred by measurement of SCN-dependent outputs. Identification of the site of breakdown of circadian control of the activity/rest cycle will have important implications for therapy and management of the patient. Even if the course of dementia cannot be slowed, successful management of dementia-related circadian disorder holds great potential for improving the well-being of home-dwelling patients and carers alike, and for delaying the need for institutional care.

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References


Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB, et al.

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