Markovian Analysis of Phasic Measures of REM Sleep in Normal, Depressed, and Schizophrenic Subjects

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Rapid eye movement (REM) phasic activity refers to brief events that occur in periods of REM sleep, such as individual eye movements (EMs). REM density (RD) is the best-known measure of such activity, although reports of RD differences among normal, depressed, and schizophrenic subjects have been equivocal. RD is a measure with a large variability, and its physiological substrate is not known. We sought a more consistent measure which might also suggest the underlying physiology. Using the time intervals between individual EMs, we calculated empirical probability distributions which showed that EMs fell into two subgroups or states: "burst" and "isolated." Then, a novel Markov chain model of sequential transition between the states was calculated for nine normal, eight schizophrenic, and seven depressed male veterans. A significantly higher probability of remaining in the burst state was observed in both patient groups. The actual number of EMs in the isolated state was nearly identical in the three groups. Possible pontine neurochemical explanations involving cholinergic and serotonergic mechanisms are discussed.

Introduction

Abnormalities of sleep architecture have been noted in several psychiatric syndromes when compared with normal controls. These include decreased slow-wave sleep (Benson and Zarcone 1989; Kupfer et al 1984), shorter REM sleep latency (Kupfer 1976, Keshavan et al 1990), and increased "REM phasic activity," that is, the amount of eye movement activity in REM sleep (Reich et al 1975). Though initial reports showed that many of these abnormalities were found only in patients with major depressive disorder, more recent findings suggest that schizophrenics have similar abnormalities (Zarcone et al 1987).

Of the above abnormalities, REM phasic activity is reported on the least, perhaps because of the need for more complicated data analysis. Simple counts of the number of

EMs are inadequate as the duration of a REM sleep period varies considerably even in a single individual. One solution has been the calculation of a "REM eye movement density" measurement, the number of EMs per unit time in a period of REM sleep. This is often called simply "REM density" (RD), and has been calculated by various methods. Evidence of RD differences between groups of psychiatric patients has been equivocal.

Feinberg et al (1964) found that hallucinating schizophrenics had a RD similar to normals, but that nonhallucinating schizophrenics had a significantly lower RD than either group. Reich et al (1975) observed RD to be significantly higher in schizoaffective patients compared with acute and "latent" schizophrenics. Gillin and Wyatt (1975) found no difference between the RD of schizophrenic and normal controls. Although Foster et al (1976) demonstrated that RD of the first REM sleep period separated primary from secondary depressives, Thase et al (1986) could not replicate the finding. Rather, they found that RD correlated with severity of depressive illness. Reynolds et al (1983) reported RD in narcoleptics to be significantly higher than even the RD of depressed patients. Kempenaers et al (1988) found a nonsignificant trend for RD in schizophrenics to be higher than normals and lower than depressed patients. Benson and Zarcone (1991) found that RD did not differ significantly between depressed and schizophrenic subjects. These authors also showed that in normals, Zung depression scores were positively correlated to RD (Zarcone and Benson 1983).

However, even RD is a rather global measurement. It represents only an average of the underlying pattern of clumping or bursting of the EMs within a REM period. To get at the deeper information, one must measure the time interval between successive EMs, hereafter called the "interrapid EM interval" (IRI).

Unfortunately, conventional statistics are not well suited to IRI data. One older approach was to submit counts of EMs per unit time (*not* IRIs) to time-series analyses such as auto-correlation, Fourier transform, or period analysis (Krynicki 1975; Lavie 1979), but some assumptions of time-series analysis are not met by such data (Ktonas 1974). Two issues are relevant: the statistical frequency distributions of the IRIs, and the "stationarity" of the process that generates IRIs—whether the generating process is constant over time, or varies in ways that would produce a nonrandom trend. The fact that IRIs occur in "bursts" between which there are many "isolated" IRIs violates the stationarity assumption of conventional time-series analysis. Regarding statistical distribution, Boukadoum and Ktonas (1988) fitted different linear functions to burst and isolated IRIs displayed on a semi-log probability distribution, which suggested that the two categories of IRI, burst versus isolated, may be generated by separate physiological processes. A probability distribution can be approximated by a frequency histogram of the IRIs of one REM period from one person, divided by the total number of IRIs in that REM period.

The novel Markovian statistical approach of Boukadoum and Ktonas (1988) was designed to remedy the shortcomings of conventional time-series analysis of IRIs. The method as yet has only been reported for normal subjects, but it shows considerable promise as a research tool in psychopathology, which the present article explores. It could be described as a categorical time-series approach, the categories being burst and isolated IRIs. The probabilities of transition from one category to the other are calculated from the sequential appearance of IRIs throughout a REM period. Though burst density or isolated density might be calculated in the manner of RD, neither demonstrates the sequence in which an IRI in burst succeeds an isolated IRI in the way that the Markovian analysis does.

The development of this Markovian method began in the 1970s, and is not to be

confused with Markovian methods of predicting the transition from REM sleep to other sleep stages (Kemp and Kamphuisen 1986). Ktonas and Smith (1976) were the first to suggest that a Markov model might be applied to the transition between burst and isolated IRIs within a single REM period. Ktonas and Bonilla (1979) stated the mathematical assumptions underlying a Markovian analysis of IRIs. Briefly, they employed a first-order Markovian model with two dichotomized (quantitized) states: IRIs less than 5 sec (burst IRIs) and IRIs greater than 5 sec (isolated IRIs). A numerical example in Ktonas and Boukadoum (1987) shows how this approach can find differences between two REM periods having identical REM densities. These authors also estimated the theoretical sampling distribution of a parameter "C" derived from the Markovian state transition probability matrix (STPM) using a Monte Carlo simulation (Ktonas et al 1981). This simulation led the author to suggest the minimum number of IRIs in a REM period required for a valid Markovian calculation (e.g., 70 IRI for a 5 × 5 transition matrix, see below). Further elaboration by Jansen and Cheng (1988) showed that one requires, for an accurate calculation, $5n^2$-$8n^2$ IRI, where n is the number of rows in the square transition matrix. For example, a 2 × 2 matrix would require 20–32 IRIs.

Boukadoum (1983), Boukadoum and Ktonas (1988) showed that the "stationarity assumption" for Markovian analysis of REM sleep was met if the REM period was at least 8 min long. They also found that the STPMs have a stationarity (do not differ significantly) between the several REM periods of a single night. This is in contrast to the well-known increase in RD and REM period duration towards morning.

The above techniques have never been used to analyze the REM sleep of psychiatric patients. We applied IRI statistical frequency histograms and Markovian analysis of IRI burst-isolated transitions to the REM sleep of schizophrenic, depressed, and normal subjects. Because conventional REM phasic activity measures such as RD seem to show inconsistent differences between depressed and schizophrenic subjects, we wondered if this novel statistical analysis would show a difference. We thus sought to disprove the null hypothesis: The Markovian patterns of IRIs in schizophrenic and depressed patients versus normals do not differ significantly.

Methods

Patient Selection

We studied 24 subjects, all male veterans; 15 were patients from the Palo Alto VA Medical Center's Psychiatry Service. All were inpatients in the Clinical Research Center, and were diagnosed using Research Diagnostic Criteria (RDC) (Spitzer et al 1978). Seven had major depressive disorder (DEPR) and eight were schizophrenic (SCHZ). In addition, nine normal male veteran paid volunteers (NORM) were recruited from the community to control for socioeconomic and educational factors. All were in good health and had no problems sleeping. None abused alcohol or illicit drugs. They were screened for psychopathology using the Structured Clinical Interview (SCI) of Burdock and Hardesty, which provides 10 indices of psychiatric disorder. None of the controls exceeded the threshold on any of the 10 subscales.

There was a significant difference in the mean age of subjects, likely explained by the differing peak age of onset for depression and schizophrenia (mean ± SD: NORM = 27.3 ± 7.3, DEPR = 40.0 ± 13.9, SCHZ = 28.3 ± 2.5, analysis of variance (ANOVA) $F = 5.01, p = 0.015$).

With the exception of chloral hydrate PRN, all patients were free of psychotropic medications for a minimum of 2 weeks before the all-night polysomnographic recordings. In all cases, no chloral hydrate was given in the 72 hr prior to the study. Following two adaptation nights, data were obtained from two consecutive recording nights to assess reliability of the measured variables.

Procedures

Using a Grass Polygraph, electroencephalogram (EEG), chin electromyogram (EMG), and electrooculogram (EOG) were recorded on both nights. Sleep stages were scored according to the conventions of Rechtschaffen and Kales (1968). The EOG technique was a differential recording between pairs of shunted electrodes attached to the inner and outer canthi of opposite eyes (Hord 1975). Grass Polygraph filter settings for EOG were 30 Hz high-filter, 0.3 Hz low-filter, 50 μV/cm gain. Data were simultaneously recorded on an AMPEX FM tape recorder for off-line computer analysis via the Grass J6 output. Digitization of the raw data and computerized waveform analysis were performed using the REMDTEK software (Schreier et al 1977) with parameters set to accept a waveform as an EM if its onset exceeded 25 μV within 200 msec. Any IRI of less than 60 msec was assumed to be artifactual and was discarded.

Statistical Analysis

Boukadoum and Ktonas (1986) suggested using a 200 msec refractory period after the detection of each EM, to avoid mis-identifying amplifier artifacts as EMs. The 200 msec rule is not universally used as it depends on the filter settings used on the polygraph amplifier (our own system used a 60 msec rejection rule). Yet, we wished to compare our findings with those of Boukadoum and Ktonas (1988). We therefore wrote a program that summed any IRIs in our raw data within 200 msec after a given IRI, and added the time to the next one. This procedure was repeated until the minimum IRI was 200 msec. This approximation provided the fairest comparison of our data with theirs, and is shown in Table 2 (middle section). Our raw data were used for all other calculations, including Figure 1, the CATMOD procedure, and the results in Tables 3–5.

Ten conventional measures of activity were calculated for each REM sleep period: (1) number of IRIs (#IRI); (2) number of minutes of REM sleep (#MIN); (3) REM density (RD = #IRIs/#MIN); (4) number of IRIs in bursts where burst IRIs were less than 2.0 sec apart, expressed per hr of REM sleep (BURIRI/HR) (Note that this definition of a burst differs from the "less than 5 sec" definition in the Markovian analysis section. The reason for reporting 2-sec bursts is to allow comparison with previous research reports where this definition was commonly used); (5) percent of #MIN spent in bursts (BUR%TM); (6) number of isolated IRIs per hr of REM sleep (ISOLIRI/HR); (7) percent of REM sleep time spent as isolated IRIs (ISOL%TM); (8) number of IRIs per 2-sec burst (IRI/BUR); (9) the number of bursting episodes detected per hr of REM sleep (BURCNT/HR); and (10) a measure of the amount of fragmentation (FRAG) of REM sleep by other sleep stages, where 100% refers to unbroken REM sleep. As these measures were likely to be intercorrelated, a nonparametric intercorrelation matrix (Spearman's Rho) was calculated. Those found to be least correlated to others (variables 2 and 10), and three relating to burst-, or isolated-RD (variables 1, 4, 6) were submitted to ANOVA: group × night × REM period, with Bonferroni comparisons of means post hoc. Three-way ANOVAs were

initially performed, but the NIGHT variable was never significant. Accordingly, 2-way ANOVAS were done, pooling both nights together. Statistical Analysis System (SAS) version 6.03 General Linear Model (GLM) procedure was used.

The frequency and probability distributions of the IRIs measured in these three groups have already been published (Douglass et al 1985). The data were replotted in the manner of Boukadoum and Ktonas (1988) using semi-log axes. Linear regressions were done to estimate the burst and isolated IRI probability density functions (PDFs).

The Markovian analysis proceeded as follows: the transition between burst and isolated IRIs required a two-state model (2 × 2 raw transition matrix, RTM) where state 1 represented IRIs quantitized as less than 5 sec and state 2 represented IRIs greater than 5 sec. The matrix was filled using frequency counts (n) of transitions between the two quantitized states. A numerical example is shown in Table 1. This RTM was then converted into a maximum-likelihood estimate of the STPM for each REM sleep period (from Boukadoum 1983):

$$\hat{P}_{ij} = \frac{n_{ij}}{n_i} \qquad \text{[equation 1]}$$

where

$\hat{P}$ is the estimated STPM

n_{ij} is the frequency count of the cell of the RTM, row i column j

The sum of probabilities in each row of this matrix is 1.00, by definition. Also generated was the "state probability" for each of the two states, which is the likelihood of each state occurring in that REM sleep period, irrespective of order of appearance.

A statistical test was suggested by Boukadoum (1983) to determine significant differences between pairs of STPMs. A χ^2 value is obtained with dfs defined by the dimensions of matrices involved:

$$\chi^2 = \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{n_i \, (\hat{P}_{ij} - \dot{P}_{ij})^2}{\dot{P}_{ij}} \qquad \text{[equation 2]}$$

where

N = number of rows in STPM matrix

df = degrees of freedom = $N(N - 1)$

$n1_i$ = row frequency count, row i of $\wedge$ STPM

$n2_i$ = row frequency count, row i of $\cdot$ STPM

n_i = SQRT ($n1_i \times n2_i$)

$\hat{P}$ = STPM predicted from observed data

$\dot{P}$ = any other STPM to which $\hat{P}$ is compared.

Table 1. Example of 2 × 2 State Transition Probability Matrix (STPM) Calculation

50 Sequential interrapid-eye-movement intervals (IRI, sec)

23.73	0.33	8.13	113.73	3.53	10.13	9.13	2.33	0.47	0.27	0.80	0.60	0.53	2.60	2.40
2.67	0.20	10.13	1.87	0.73	0.40	0.87	1.87	2.60	0.33	0.73	2.40	4.33	0.47	0.47
3.87	0.53	1.60	5.67	0.53	1.20	3.07	30.87	0.33	0.33	4.87	1.73	0.93	0.33	1.47
3.67	6.87	0.27	0.40	0.80										

Two-state quantitization at <5, >5 sec

2 1 2 2 1 2 2 2 1 1 1 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 2 1 1 1 1 1 1 1 1 2 1 1 1

2 × 2 raw transition matrix			2 × 2 STPM			State prob	Renewal prob matrix			Renewal test
	<5	>5		<5	>5			<5	>5	$\chi^2 = 20.12$,
<5 sec:	34	6	<5 sec:	0.850	0 150	0.816		0.5	0.5	df = 2, $p < 0.001$
>5 sec:	7	2	>5 sec:	0.777	0.222	0.184		0.5	0.5	

Calculation example, using data from one REM sleep period of a normal subject. An interrapid eye movement interval (IRI) is the number of sec between two successive rapid eye movements in REM sleep IRIs are categorized here into two states, those under 5 sec duration (burst), and those over 5 sec (isolated). State probability is the probability of occurrence of each state, irrespective of sequence The raw transition matrix (RTM) is a frequency count of transitions from the row-state to the column-state. The Markovian STPM is calculated from the RTM (see text, equation 1). Each cell in an STPM is defined as the probability of state transition from the row-state to the column-state The renewal test (see text, equation 2) compares the observed STPM to a theoretical matrix (renewal probability matrix) representing random transition between the states. As the χ^2 differs significantly here, it may be concluded that there is a pattern to the state transitions in these 50 IRIs, and the random (renewal) state transition model can be rejected with $p < 0.001$.

A special case is the comparison of an experimental STPM against a "renewal process" STPM representing random transition. If significant, this comparison indicates that the experimental STPM shows patterned transition between states and does not correspond to a renewal (purely random) process.

2×2 STPMs were calculated for each REM period that was over 8 min long and which had over 75 IRIs (see CATMOD below). Each STPM was then compared to the theoretical "renewal matrix" using equation [2], and a tally was made of how many REM periods differed from a renewal model at a $\chi^2 \geq 9.21, p < 0.01,$ df $= 2$.

As the RTM frequencies underlying the STPMs of each REM sleep period are essentially 2×2 contingency tables, the question arises of what statistical test to use on them in a factorial experimental design such as the present one. The solution of Boukadoum and Ktonas (1988), reporting on a single group, was to perform a nonparametric test on the STPMs from the REM periods of one night for each subject in order to demonstrate stationarity. But, due to the small number of REM periods in one subject night, true differences between REM periods across subjects could be missed due to type II error. Though this method is both conservative and valid, it does not provide a statistical test for differences between experimental groups.

For a group summary, they reported the mean and SD of the 4-cell STPMs across all subjects for each REM period. We have generated a similar table from our data for the purposes of comparison (Table 2), but there is a problem with this approach: the mean is not the best measure of central tendency for samples from nonnormally distributed populations like proportions, as is the case with the STPMs. A maximum-likelihood estimate applied to pooled frequencies of all 2×2 tables would be a better estimate of the true group cell probabilities, or a logit transformation could be employed to normalize such a distribution (Snedecor and Cochran 1980). These methods would produce tables that better reflected the true group probabilities. This criticism applies only to the tabular display; the statistical tests employed by Boukadoum and Ktonas (1988) were appropriate.

The analysis of Markovian data by log-linear models has been well described by Bishop et al (1975). Our solution employed the categorical log-linear model (CATMOD program, SAS version 6.03, SAS Institute, Cary, NC) which is designed for data of this type. CATMOD required a minimum of 75 data points (IRI transitions) to estimate a 2×2 table. The response profile for CATMOD was defined as the RTM frequency counts underlying the 2×2 STPM of each REM period. The logit method was used to estimate STPM probabilities in a full factorial design (3 groups $\times$ 2 nights $\times$ 4 REM periods $\times$ 2 states—burst and isolated IRI). Individual subjects were not entered into CATMOD. Though a repeated-measures design on subjects would have been desirable, there was sufficient missing data to preclude this approach. It is possible to use raw frequency counts directly in CATMOD, but due again to the small number of subjects and substantial intersubject variation, we chose to estimate the group frequency count by squaring the mean of square-root-transformed individual frequencies (Snedecor and Cochran 1980). This method is more conservative than pooling frequency counts where subjects in a single group differ a great deal from one another.

CATMOD results are reported in a tabular form designed to resemble the familiar ANOVA table, except that χ^2 values replace the F ratios of ANOVA. The χ^2 contribution of the classification variables group, night, and REM period and their saturated crossed effects were calculated. As the night factor was never significant, nor were group $\times$ night $\times$ REM period $\times$ state 3- and 4-way interactions, the final CATMOD model was reduced from a fully saturated factorial model to group $\times$ REM period $\times$ state main

Table 2. Group Comparison of STPMs

	Normals n = 9 (49 REM periods)			Schizophrenics n = 8 (48 REM periods)			Depressed n = 7 (52 REM periods)		
	Destination state			Destination state			Destination state		
	<5 sec	>5 sec	State Prob	<5 sec	>5 sec	State Prob.	<5 sec	>5 sec	State Prob.
From IRI defined as >60 msec									
<5	0 833 ± 0 007	0 167 ± 0 007	0 822 ± 0 007	0 870 ± 0.008	0.129 ± 0.008	0.851 ± 0.009	0.904 ± 0.005	0.095 ± 0.005	0.889 ± 0.007
>5	0 784 ± 0 008	0 216 ± 0 008	0 178 ± 0 007	0.775 ± 0 009	0.225 ± 0.009	0 149 ± 0.009	0.798 ± 0.010	0.202 ± 0.010	0.111 ± 0.007
From IRI defined as >200 msec									
<5	0 806 ± 0 014	0 195 ± 0 014	0 796 ± 0.014	0 858 ± 0 011	0.141 ± 0.011	0.838 ± 0.013	0 118 ± 0 009	0.118 ± 0.009	0.861 ± 0.012
>5	0 753 ± 0 016	0 247 ± 0 016	0 204 ± 0.014	0 759 ± 0 019	0.241 ± 0.019	0.162 ± 0.013	0.764 ± 0.017	0.236 ± 0.017	0.139 ± 0.012
From Logit method of CATMOD, IRI >60 msec									
<5	0.869 ± 0 007[a]	0 131 ± 0.007[a]	0.859 ± 0.007[c]	0.923 ± 0 004[a]	0.077 ± 0.004[a]	0.911 ± 0.004[c]	0.927 ± 0.004[a]	0.073 ± 0.004[a]	0.914 ± 0.004[c]
>5	0 809 ± 0 019[b]	0 191 ± 0.019[b]	0 141 ± 0 007[c]	0 805 ± 0 019[b]	0 195 ± 0.019[b]	0.089 ± 0.004[c]	0.781 ± 0.021[b]	0.219 ± 0.021[b]	0.086 ± 0.004[c]

2 × 2 STPM derived from Markovian analysis of IRIs, followed by the 2 × 1 state probabilities. STPMs are calculated for each REM period for each subject, and are shown as a simple mean ± SE of the first four REM periods from 2 nights (total = 8, less missing data) If more than four REM periods occurred in a night, #5 onwards were not used Labels <5, <5 are cutoff points (sec) defining membership of IRIs in the Markovian states burst and isolated, respectively. Number of subjects is n. Initial state is shown as rows, destination state as columns. Our IRIs were originally defined as being >60 msec apart as shown in the top section of the Table. To enable comparison with the method of Boukadoum and Ktonas (1988), the data were transformed so that a 200 msec refractory period occurred after each EM detection, this reduced the state probability of short-duration IRIs (middle of Table). The bottom third of the table shows STPMs estimated by the CATMOD method (see text), which may be more accurate for these data.

[a]For initial state = burst, burst destination state probability differed significantly between groups (χ^2 = 63.28, def = 1, p = 0.0000, contrast of DEPR ± SCHZ vs. NORM).

[b]For initial state = isolated, isolated destination state probability did not differ between groups (χ^2 = 0 82, df = 1, p = 0.36, contrast cf DEPR + SCHZ vs. NORM).

[c]State probability differed significantly between groups (χ^2 = 70 08, df = 1, p = 0 0000, contrast of DEPR + SCHZ vs. NORM), with a higher probability of an IRI being in the burst state in both patient groups vs. normals The difference between DEPR and SCHZ is also statistically significant (p < 0.001), but the absolute difference is very small.

Table 3. Summary Table from Categorical Log-Linear Model Analysis (CATMOD)

Source	DF	χ^2	p
Intercept	1	1940.59	0.0000
Initial-state (burst, isolated)	1	114.01	0.0000
Group	2	7.75	0.02
REM period (#1, 2, 3, or 4)	3	3.00	0.39
Initial-state × group	2	16.65	0.0002
Initial-state × REM period	3	5.41	0.144
Group × REM period	6	16.35	0.012
Residual	6	4.13	0.659

The dependent variable in this analysis is destination state probability. The CATMOD table is designed to resemble an ANOVA table, but uses χ^2 instead of F ratios. These results indicate that the destination state is significantly dependent on the initial state; i.e., there is a strong tendency for a given IRI to be a member of the same state as the IRI that preceded it. The three groups differ significantly on the probability of destination state; furthermore, this is of a significantly different pattern in different groups (initial state by group interaction). The significant group by REM period interaction indicates that groups also differ in their pattern of destination state probability over the four REM periods (see Table 4). The nonsignificant residual indicates a good fit of the statistical model to the observed data.

effects and 2-way interactions only. It is the latter model that forms the basis of the tables. Adequate goodness-of-fit of the model resulted in a nonsignificant residual χ^2 (Table 3). CATMOD was also used to estimate the STPMs for group × REM period (Table 4). Contrasts were used in a subsequent CATMOD to break down significant differences.

Results

In all, 181 REM periods were recorded. After removing those with fewer than 75 IRI, 149 remained. All subjects had STPMs that were significantly different from a renewal STPM with the exception of one REM period of one subject in the NORM group. This is in accord with Boukadoum and Ktonas (1988) who found 89% passed when a 5 × 5 STPM was used. Thus, transitions between the two states were not random.

Probability density function estimates resulting from a single-process model of IRI generation are shown with the raw IRI data in Figure 1a. Using the same raw data, linear estimates for the burst and isolated IRI density functions of a two-process model are shown in Figure 1b. The latter is a better physiological explanation. It also fully supports the Markovian assumption of a quantitization of IRIs into burst and isolated groups. Results for our NORM group closely matched those of Boukadoum and Ktonas (1988) (see Figure 1 legend for numerical values).

In Table 2, STPM data are displayed by group. It can be seen that the raw data in the upper third of the table produce slightly higher values for the burst-to-burst transition STPM cell than do the data in the middle third which have been adjusted to remove IRI less than 200 msec. Overall, the pattern in the NORM group is similar to that reported by Boukadoum and Ktonas (1988) for normals. The top two-thirds of the table are calculated by Boukadoum's group arithmetic mean method, and the bottom third of the table shows CATMOD's logit estimates of the STPMs. The latter are the highest probabilities in the table, and also theoretically the best estimates of these probabilities. The groups differed significantly. Considering the initial state "burst," the destination state transition probabilities of the DEPR and SCHZ groups were significantly higher than NORM group (top left cell) in a contrast. The DEPR and SCHZ groups also were

Table 4. Markovian State Transition Probabilities, by Group and REM Period

Group	Initial state	REM period #1 Destination state <5 sec	>5 sec	REM period #2 Destination state <5 sec	>5 sec	REM period #3 Destination state <5 sec	>5 sec	REM period #4 Destination state <5 sec	>5 sec	REM period effect df	p	Initial state effect df	p
NORM	<5 sec	0.826	0.173	0.859	0.140	0.878	0.121	0.890	0.109	3	0.37	1	0.02
	>5 sec	0.875	0.175	0.775	0.225	0.785	0.214	0.837	0.162				
SCHZ	<5 sec	0.886	0.113	0.900	0.100	0.937	0.062	0.926	0.073	3	0.24	1	0.0000
	>5 sec	0.802	0.197	0.800	0.200	0.814	0.185	0.798	0.201				
DEPR	<5 sec	0.919	0.080	0.934	0.065	0.939	0.060	0.912	0.087	3	0.052	1	0.0000
	>5 sec	0.778	0.222	0.815	0.155	0.805	0.194	0.731	0.268				

CATMOD estimates of 2 × 2 STPMs. Significance values come from three subsequent CATMOD analyses of initial states and REM periods, one for each group, to further elaborate the significant group × REM period effect of Table 3. In this design, there were no significant interaction effects of initial state × REM period. The small number of subjects gave low statistical power for these comparisons. Only in the DEPR group is there nearly a significant difference in destination state transition probability due to REM period. This is likely the cause of the significant group × REM period interaction in Table 3.
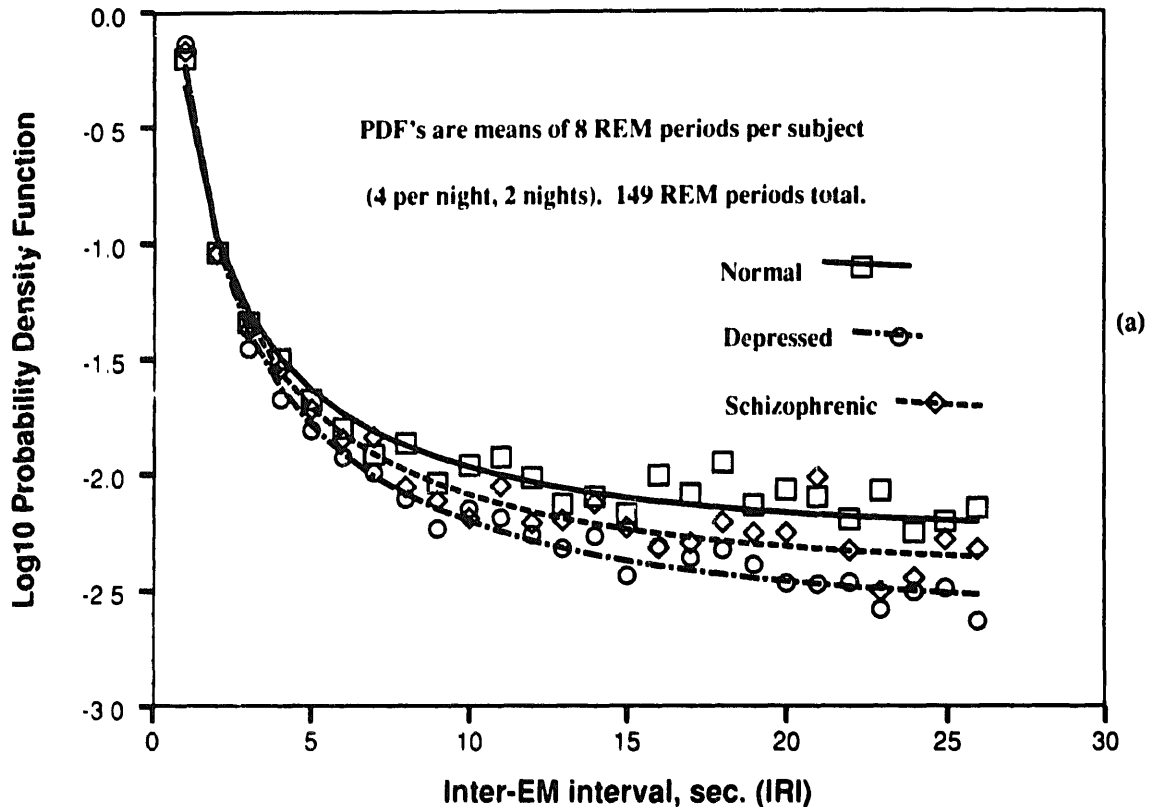
Figure 1.(a) Estimated probability density func'ions (PDF) of IRIs for the three groups, using a single-process model of eye-movement (EM) generation. Data are replotted from Douglass et al (1985). Single-process model requires an unusual nonlinear regression of logarithmic data which is difficult to explain physiologically.

statistically significantly different from each other, but the absolute difference was very small. Considering the initial state "isolated," the destination state transition probabilities did not differ significantly among the three groups.

The summary table from CATMOD is shown in Table 3. There was a significant effect of initial state, group, and state × group interaction. There was no significant REM period effect, but there was a significant group × REM period interaction. The predicted STPM probabilities for a second set of CATMOD analyses are shown group × REM period × state in Table 4 to further illustrate this point. Though all three groups have a significant difference in destination state probability due to the effect of initial state, it appears that the DEPR group has, in addition, an effect due to REM period which approaches significance. The number of subjects gives insufficient power for this comparison, which is likely to be the cause of the significant group × REM period interaction effect of Table 3. (This interesting result suggests that if the study were re-done with a larger number of subjects, it might show that DEPR subjects have a different pattern of destination state probability over the REM periods of the night, whereas NORM and
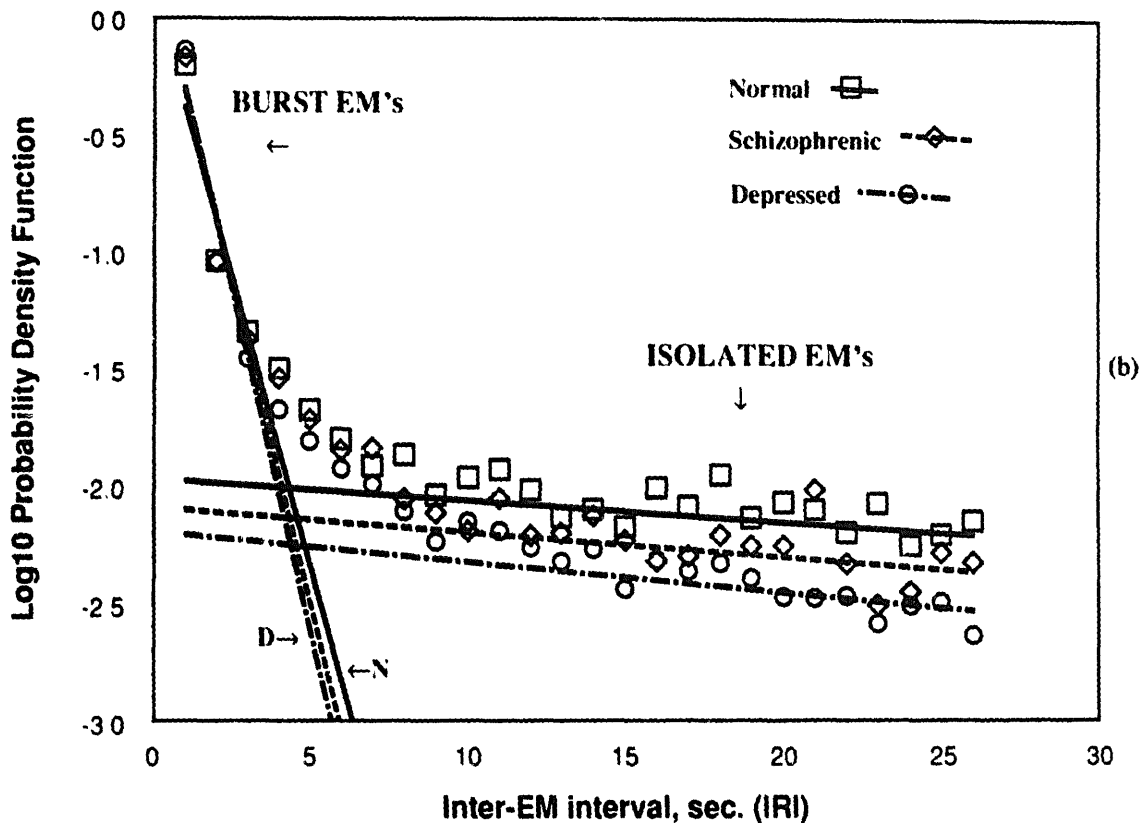
Figure 1.(b) PDFs using the two-process model of Boukadoum and Ktonas (1988). Simple linear regressions were fitted separately to burst and isolated IRI segments of the curves in (a). Burst fit was from 1 to 3 sec IRI; isolated fit was from 15 to 25 sec IRI. Regression equation $y = mX + b$ on semi-log axes. Correlation is Pearson product-moment r.

Isol. fit:	NORM	$y = (-0.0092 \, X) - 1.970;$	$r^2 = 0.963$
	SCHZ	$y = (-0.0106 \, X) - 2.093;$	$r^2 = 0.964$
	DEPR	$y = (-0.0130 \, X) - 2.192;$	$r^2 = 0.971$
Burst fit:	NORM	$y = (-0.4895 \, X) + 0.1159;$	$r^2 = 0.961$
	SCHZ	$y = (-0.5458 \, X) + 0.2410;$	$r^2 = 0.962$
	DEPR	$y = (-0.5785 \, X) + 0.2881;$	$r^2 = 0.962$

The intersection of the burst and isolated functions for each group occurred at the following IRI (X-axis) points, in sec. This value is the best single choice (quantitization threshold) to separate burst from isolated IRIs.

NORM = 4.25

SCHZ = 4.36

DEPR = 4.38

The same point as determined for normals by Boukadoum and Ktonas (1988) was 4.3 sec IRI, although they used 5.00 sec in their publication. We used 5.00 sec as the threshold point in all our Markov calculations, for consistency with the above authors.

SCHZ groups are stationary on such a measure.) The intercorrelation matrix of the conventional REM measures (Table 5) showed many large correlations, suggesting considerable redundancy in these variables. ANOVAs on a subset of these variables (Table 6) showed a significant excess of #IRI and BURIRI/HR but no difference in #MIN of REM sleep, in both depressed and schizophrenic groups compared with normals. Of particular interest was the ISOLIRI/HR, which did not differ between groups. This immediately suggests why the lines fitting the isolated IRI in Figure 1b are approximately parallel but offset (see Discussion). Fragmentation was worst among depressed patients.

Discussion

To our knowledge, this is the first application of Markovian STPMs to the REM sleep of normals and psychiatric patients. Schizophrenic and depressed patients are clearly differentiated from normals; thus, the null hypothesis is rejected. However, the two patient groups are not easily distinguished from each other on absolute value, so the Markovian results parallel those of RD (Benson and Zarcone 1991).

A major limitation of the present study results from the DEPR group being significantly older than the SCHZ or NORM groups. Because young schizophrenics are compared with older depressed patients, there is no way of telling whether the results observed are due to age or diagnosis or both.

Our demonstration that the STPM is stationary over nights and REM periods in normals confirms the findings of Boukadoum and Ktonas (1988). We now extend this observation to schizophrenic and, provisionally, to depressed patients, although the latter were the only subjects to approach significant difference over REM periods. The STPM is indeed a consistent quantification of the phasic events of REM sleep. This is in direct contrast to EM density, which increases with successive REM periods during the night (Benson and Zarcone 1991). A practical implication is that a single REM period with over 20 EMs would suffice to calculate a valid estimate of an individual's characteristic 2 × 2 STPM, as the sampling error of the STPM decreases to a plateau at this point (Jansen and Cheng 1988).

Both the probability distribution analysis of IRIs and the Markovian analysis support a two-process model of the generation of eye movements in REM sleep, one process causing the emission of isolated IRIs of long duration, the other causing burst IRIs of very short duration. This explains why a simple negative exponential frequency distribution, a single process model, does not fit the observed data (Spreng et al 1968).

Our results also suggest some conclusions about the choice of parameters for STPM analysis. The linear regression of our NORM burst function in Figure 1b has coefficients similar to that reported by Boukadoum and Ktonas (1988), despite the inclusion of about 20% more IRIs, and all in the 60–200 msec range. This suggests that IRI emission in the range 60–200 msec is merely an extension of the function fitted to IRI greater than 200 msec, and need not always be excluded. Also, in future work of this type, 4.3 sec rather than 5.00 sec would seem to be a more objective point at which to divide the burst from the isolated IRIs, as Boukadoum's normals and all three of our experimental groups showed threshold values very close to 4.3 sec.

What exactly is shown to be abnormal by our analysis that is not evident from older forms of phasic event analysis? The IRI probability distributions of Figure 1b are striking in that the linear fits to the burst IRI distribution are virtually identical in the three experimental groups, whereas the fits to the isolated IRI distribution are parallel but offset

Table 5. Correlation of Conventional REM Measures

	#IRI	#MIN	BURIRI/HR	BUR%TM	ISOLIRI/HR	ISOL%TM	BURCNT/HR	IRI/BUR	FRAG	RD
#IRI	1.000									
#MIN	0.3892	1.0000								
BURIRI/HR	0.9331	0.1738	1.0000							
BUR%TM	0.9192	0.1385	0.9900	1.0000						
ISOLIRI/HR	0.7715	0.0292	0.8362	0.8500	1.0000					
ISOL%TM	-0.9331	-0.1646	-0.9946	-0.9954	-0.8354	1.0000				
BURCNT/HR	0.8123	0.2808	0.8500	0.5508	0.6885	-0.8623	1.0000			
IRI/BUR	0.8738	0.2131	0.9215	0.9000	0.6062	-0.9154	0.7446	1.0000		
FRAG	-0.4154	-0.1585	-0.3785	-0.4023	-0.3900	0.4015	-0.3238	-0.3254	1.0000	
RD	0.9346	0.1792	0.9969	0.9877	0.8546	-0.9931	0.8500	0.9000	-0.4069	1.0000

Intercorrelation matrix of sleep variables, Spearman's rho; $n = 25$ Any correlation >0.411 is significant at the 0.05 level. See text under "Statistical Analysis" for definition of variables. Note that the bursts in these conventional statistics are defined as a sequential group of IRIs where each member of the burst is ≤ 2.00 sec; this differs from the <5.00 sec definition used in the Markovian calculations The reason for the difference is that these are the values commonly used by a number of previous authors when reporting their calculations of the two very different types of statistical analysis used in this paper. We designed the present report to be easily comparable to previous reports.

Table 6. ANOVA Using Selected Variables from Table 5

Variable	Source	df	F	p	Means
#IRI	Group	2	5 57	0.0047	NORM 407 DEPR 636[a] SCHZ 686[a]
	REM period	3	7.01	0.0002	RP1 408 RP2 422 RP3 770[a] RP4 616[a]
#MIN	Group	2	1.15	N.S.	NORM 26.5 DEPR 24.7 SCHZ 29.0
	REM period	3	7.78	0.0001	RP1 20.5 RP2 22.7 RP3 34.4[a] RP4 26.5[a]
BURIRI/HR	Group	2	13.39	0.0001	NORM 729 DEPR 1405[a] SCHZ 1163[a]
	REM period	3	1.69	N.S.	RP1 947 RP2 1056 RP3 1166 RP4 1128
ISOLIRI/HR	Group	2	1.69	N.S.	NORM 212 DEPR 234 SCHZ 232
	REM period	3	2.51	N.S.	RP1 221 RP2 216 RP3 218 RP4 253
FRAG	Group	2	7.70	0.0007	NORM 90.3[a] DEPR 80.1 SCHZ 89.4[a]
	REM period	3	2.38	N.S.	RP1 88.7 RP2 88.4 RP3 87.7 RP4 81.3

RP1 = REM period number one, etc Three-way ANOVAs were initially performed, but the night variable was never significant. Accordingly, 2-way ANOVAs are reported here, pooling both nights together. SAS version 6.03 GLM procedure. Bonferroni post-hoc comparisons of means were done at the 0.95 confidence interval.

[a]Means that did not differ among themselves, but that differed significantly from other means in the same stratum.

considerably. This suggests a conclusion of theoretical importance: that the physiological process emitting the isolated IRIs is *identical* in NORM, DEPR, and SCHZ groups, the offset being due only to the greater number of IRIs in the patient groups (the divisor). In further confirmation, the bottom third of Table 2 shows no significant differences in the transition probability of isolated-to-isolated IRIs and Table 6 shows no significant group differences in the absolute number of these IRIs.

The corollary is that the process that emits burst IRIs might be the source of the observed significant group differences. This is indeed the case. Whereas the probability distribution of the burst IRIs is very similar in all three groups, there are significant differences in the *number* of BURIRI/HR, as DEPR and SCHZ groups have nearly double the number found in NORM. The burst-to-burst transition probability (Table 2) is also significantly higher in the SCHZ and DEPR groups than NORM, whereas the isolated-to-burst and isolated-to-isolated transition probabilities do not differ between groups. This suggests that the entry into a new burst from a given isolated IRI is no more likely to occur in patients than normals. Once in the burst, however, the probability of the next IRI remaining in the burst is significantly higher in the SCHZ and DEPR groups versus NORM. Physiologically, this implies that the patients have an intact and normal burst IRI generator which is either being driven excessively by some other system, or which has lost some inhibitory input. The caveat regarding mean group ages applies to this conclusion also: it is possible that aging itself causes these effects.

A possible source of excess drive could be the vestibular system. Ornitz et al (1973) demonstrated in normals that vestibular or auditory stimulation during REM sleep increased burst IRI activity. Also there is a tenfold increase over preflight values in the number of burst IRIs when astronauts sleep in the weightless conditions of earth orbit (Petre-Quadens and Dequae 1987).

Biochemically, some preliminary data (Douglass et al 1990) suggest that the M1 muscarinic (cholinergic) system might be involved in the overactive burst generator in the patients. The M1 antagonist biperiden failed to alter the Markovian probabilities in normals, but in schizophrenics it reduced the abnormally high burst-to-burst transition probability while leaving the isolated-to-isolated transition probability unchanged. Biperiden had a similar effect on RD, lowering it in schizophrenics but not altering it in normals. This dissociation of the effect of M1 blockade on burst versus isolated IRI production suggests that excess burst IRIs in schizophrenics are controlled by M1 cholinergic systems, although M2 pontine systems seem to control the onset of the REM state as a whole. This is in accord with Hobson's (1990) observation of the effect of cholinomimetic drugs applied directly to the pons in the cat, wherein the PGO (pontine-geniculate-occipital) burst cells of the region were stimulated to produce PGO waves. PGO waves have not been observed in the human, as they are usually measured by implanted brain electrodes. They are believed to be the cause of the rapid eye movements of REM sleep.

The present findings suggest that the Markovian burst-to-burst transition probability might be a reflection of the activity of the PGO-wave-generating cells of the peribrachial pons. The reason why this activity should be increased in schizophrenia and depression is not yet clear, but it seems to involve 5HT and cholinergic mechanisms. Benson et al (1983) found an inverse correlation between cerebrospinal fluid levels of the serotonin metabolite 5-HIAA and burst EM measures in psychiatric patients. Combining this observation with the biperiden data suggests the possibility of an imbalance between cholinergic and serotonergic systems in schizophrenics and depressives as the cause of the increased burst-to-burst transition probability. This hypothesis should be amenable to pharmacological testing in humans and animals.

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