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Seasonal Variation in Platelet [^{3}H]Paroxetine Binding in Healthy Volunteers

Relationship to Climatic Variables

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Recently, our laboratory has reported significant seasonal differences in [^{3}H]paroxetine binding to platelets in depressed subjects. This study aimed to examine the seasonal variation in [^{3}H]paroxetine binding to platelets and the relationships between [^{3}H]paroxetine binding and climatic variables in healthy volunteers. We took monthly blood samples during one calendar year from 26 healthy volunteers for assay of [^{3}H]paroxetine binding and analyzed the data by means of univariate and multivariate spectral and cosinor analyses. There was a statistically highly significant seasonal pattern in [^{3}H]paroxetine binding to platelets with significant annual, 4-monthly, and bimonthly rhythms, which were expressed as a group phenomenon. [^{3}H]Paroxetine binding to platelets was significantly lower in fall and summer than in winter and spring; lows occurred in summer and peaks in spring. The peak-trough difference in this yearly variation, expressed as a percentage of the mean, was as large as 83.7%. A large part of the variance, that is, 32.5%, in [^{3}H]paroxetine binding could be explained by weather variables, such as ambient temperature, relative humidity, and air pressure. Highly significant common annual rhythms were expressed in [^{3}H]paroxetine binding and ambient temperature or humidity (both inversely related) and changes in temperature the 2 weeks preceding blood samplings (positively related). ***[Neuropsychopharmacology 15:187–198, 1996]***

KEY WORDS: *Paroxetine binding sites; Depression; Platelets; Serotonin; Seasonality; Chronobiology*

It is thought that serotonin (5-HT) plays a role in the pathophysiology of behavioral and affective disorders, such as major depression (Maes and Meltzer 1995). Alterations in peripheral (blood platelets) and central (neurons) 5-HT reuptake mechanisms are frequently observed in affective disorders (Maes and Meltzer 1995). Blood platelets have been introduced as a model for the presynaptic serotonergic nerve-endings. Indeed, platelets are able to take up, store, and release 5-HT via mechanisms that are quite similar to those of central 5-HT neurons (Sneddon 1969, 1973; Stahl 1977). Platelets have a 5-HT uptake complex with a high affinity site for 5-HT (Sneddon 1969, 1973). The recently cloned 5-HT transporter on human platelets has been shown to be identical to the 5-HT transporter in human brain (Lesch et al. 1993).

Some, but not all, authors have found lower platelet 5-HT uptake in unmedicated patients with major depression versus normal controls (review: Meltzer and

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Arora 1991). [^{3}H]imipramine binding to platelets has been reported to be decreased in depressed patients by some investigators (Briley et al. 1980; Asarch et al. 1980; Suranyi-Cadotte et al. 1983; review: Meltzer and Arora 1991). Other groups, on the other hand, were unable to detect any difference between major depressed and normal control subjects (Whitaker et al. 1984; Tang and Morris 1985; Hrdina et al. 1985; Kanoff et al. 1987). However, [^{3}H]imipramine binding is rather heterogeneous, because this substance labels both 5-HT uptake and non-5-HT uptake sites (Laduron et al. 1982; Mellerup et al. 1983; Mellerup and Plenge 1986). Another source of bias in the [^{3}H]imipramine binding results is the significant seasonal variation, which has been observed in normal controls (Arora and Meltzer 1988; DeMet et al. 1989).

[^{3}H]Paroxetine binds with high affinity to a specific population of binding sites located on human platelet and neuronal membranes, associated with 5-HT uptake mechanisms (Mellerup et al. 1983; Habert et al. 1985; Cheetham et al. 1993). In human brain, the highest concentrations of [^{3}H]paroxetine binding were found in the substantia nigra, hypothalamus, and hippocampus (Laruelle et al. 1988). By means of autoradiography, the distribution of [^{3}H]paroxetine and [^{3}H]imipramine binding sites in human brain-tissue was found to be very similar. The distribution of paroxetine binding sites in the brain correlates well with that of serotonergic presynaptic markers (Cortés et al. 1988). It was found that the maximal binding (B_{max}) to human platelet membranes is the same for [^{3}H]paroxetine and [^{3}H]imipramine, but the affinity (Kd) is 10 times higher for [^{3}H]paroxetine (Mellerup et al. 1983). The polymers, however, on which the two binding sites are located may have different molecular weights (Mellerup et al. 1985). Therefore, it may be concluded that [^{3}H]paroxetine is a more specific and potent inhibitor of 5-HT uptake, and that this ligand is therefore more useful to examine the 5-HT transporter complex than [^{3}H]imipramine (Buus-Lassen et al. 1983; Mellerup and Plenge 1986; Cheetham et al. 1993).

Most authors were unable to find a significant difference in [^{3}H]paroxetine binding to platelet membranes between depressed patients and normal controls (Galzin et al. 1988; Lawrence et al. 1990; D'Hondt et al. 1994), although one research group reported lower [^{3}H]paroxetine binding in major depressed subjects (Nemeroff et al. 1994). A significant seasonal "variation" in platelet [^{3}H]paroxetine binding has been described in normal controls (Klompenhouwer et al. 1990) and depressed subjects (D'Hondt et al. 1994). There are also reports that there are seasonal variations in other aspects of 5-HT activity in humans, including platelet 5-HT uptake, plasma L-tryptophan availability to the brain, 5-HT levels in hypothalamus, 5-hydroxy-indoleacetic acid concentrations in cerebrospinal fluid, and neuroendocrine responses to 5-HT agonists (Carlsson et al. 1979; Wirz-Justice and Richter 1979; Swade and Coppen 1980; Arora et al. 1984; Egrise et al. 1986; Brewerton 1989; Brewerton et al. 1989; Sarrias et al. 1989; Maes et al. 1995b).

The seasonal variation, including annual rhythms, in many human physiologic functions is probably related to endogenous or genetically determined processes, that is, these rhythms continue after removal of all periodic environmental inputs (Touitou and Haus 1992). However, these endogenous annual rhythms are probably continuously modulated by periodic events in the environment, such as the seasonal changes in the light-dark span, ambient temperature, and changes in ambient temperature during the preceding weeks (Touitou and Haus 1992; Maes et al. 1995a). Thus, short-term changes in the weather may serve as entraining agents or synchronizers for endogenous rhythms, modulating rhythm parameters, such as acrophase, mesor, or amplitude (Touitou and Haus 1992).

The aim of the present study was to examine (1) the seasonal variation in [^{3}H]paroxetine binding to platelets in healthy volunteers, and (2) the relationships between paroxetine binding and weather variables such as ambient temperature, the light-dark span, relative humidity and air pressure.

SUBJECTS

The geographic coordinates of this study were 51.2°N, 4.5°E around the city of Antwerp, Belgium. The study period extended from December 11, 1991 until December 25, 1992. Twenty-six normal volunteers (13 men, 13 women) were selected to participate in this study. The mean age was 38.7 ± 13.4 years. During 12 consecutive months a blood sample was taken from all subjects. The normal volunteers lived in Antwerp, in an area <30 km from the Meteorological Station, Royal Meteorological Institute, Deurne, Antwerp, Belgium. They were free from major medical illnesses and had a stable, settled lifestyle. All volunteers were free of any medication during the 12 month period (including anticonceptive drugs). None of the female subjects were pregnant during the time period of the study. The 26 subjects were screened for past history of psychiatric and personality disorders by means of the semi-structured clinical interview according to DSM-III-R criteria (APA 1987; Spitzer et al. 1990). None of them had suffered from psychiatric or personality disorders. They all had raw scores on the Zung Anxiety and Depression Inventories less than 32 and on the Beck Depression Inventory less than 9. Consequently, subjects suffering from psychologic stress or anxiety were excluded from this study. The volunteers were not allowed to spend more than 1 week in a geographic area other than the province of Antwerp; they

were not allowed to travel outside an area of more than 250 km from Antwerp-town. Subjects with drug use or abuse (also alcohol) were not included.

Seasons were defined by their respective solstices and equinoxes, that is, winter: December 21 to March 20; spring: March 21 to June 20; summer: June 21 to September 20; and fall: September 21 to December 20. Cross-seasons were defined as the periods between: (1) November 6 to February 5; (2) February 6 to May 5; (3) May 6 to August 5; and (4) August 6 to November 5.

METHODS

Blood collections were performed in standardized conditions in order to minimize sources of preanalytic variation (Maes et al. 1994b). Blood samples were always taken after an overnight fast at 8:00 A.M. (±30 minutes). The same two persons carried out all blood samplings throughout the study period. Each subject always had blood samplings carried out by the same investigator. Each subject had 12 consecutive monthly blood samplings. Blood samplings in men and postmenopausal women were, as much as possible, evenly spaced at monthly intervals. Blood samplings in premenopausal females were always carried out 5 to 10 days after the first day of the menstrual cycle. Blood samplings were clustered in 4 to 5 blood sampling days/month. There were, during the study period, 59 days on which blood was sampled. Sixteen subjects had their first blood sampling in December 1991, the others in January 1992.

An intravenous cannula was inserted at 8:00 A.M. (± 30 minutes) in the antecubital vein of the subjects. 2 × 10 ml of blood was collected in silated vacutainer tubes containing 0.15% K3-EDTA as anticoagulant. Within 60 minutes, platelet rich plasma was prepared by low speed centrifugation for 10 minutes at 750 g. Platelets were counted in pooled platelet rich plasma. Platelets were isolated in three centrifugation runs, pooled, and washed in 8 ml buffer (TRIS mmol/L, NaCl 150 mmol/L, EDTA 20 mmol/L, pH 7.5) according to the method of Leysen et al. (1983). The final pellet was frozen in liquid nitrogen and kept at −70°C until thawed for determination of paroxetine binding. Platelets were disrupted in 8 ml ice-cold buffer (TRIS 5 mmol/L, EDTA 5 mmol/L, pH 7.5), using an ultra-turrax homogenizer. The platelet membranes were centrifuged at 39000 *g* and washed twice in 8 ml ice-cold buffer (TRIS 70 mmol/L, pH 7.5). The final membrane pellet was suspended in ice-cold assay buffer (TRIS 50 mmol/L, NaCl 120 mmol/L, KCl 5 mmol/L, pH 7.5), in a concentration of 10^8 original platelets/ml. Incubation mixtures consisted of 500 µl platelet suspension, 25 µl [3H]paroxetine (concentration range 0.5–1:0 nmol/L, diluted in assay buffer) and 25 µl ethanol 10% or 25 µl imipramine (diluted in ethanol 10%, in a final concentration of 10^{-6} mmol/L in incubation mixture; 1000 × excess of [3H]paroxetine). After incubation for 120 minutes at room temperature, incubation was stopped by adding 4 ml ice-cold buffer. The samples were rapidly filtered through Whatman GF/B glass fiber filters under vacuum and rinsed twice with 4 ml ice-cold buffer. We used a 40-well filtration manifold. Filters were placed in vials with 5 ml scintillation-fluid (Packard Ultima-Gold), and radio-activity was counted after 12 hours of incubation in a Packard 460 scintillation counter. Specific binding (as a percentage of total binding) was 73% ± 5%.

We found that the Kd values of [3H]paroxetine binding were highly unreliable, because more than 50% of the variance in the Kd values is determined by the run in which these Kd values are determined (D'Hondt et al. 1994). Therefore, we decided not to determine the Kd values in the present study.

In order to compute the components of biologic variation and to determine the degree of seasonality in biologic variables, it is imperative that all samples of each subject are assayed at the same time and in the same run and that all samples of all subjects are assayed using the same batch of radioligand (Maes et al. 1994b). Therefore, we have performed [3H]paroxetine concentration binding curves using only two concentrations in the range between 0.5 and 1.0 nmol/L for each platelet sample. In this range [3H]paroxetine binding reaches its plateau value. Using this procedure we found binding capacity values that were of the same size as the Bmax values we determined in earlier experiments (D'Hondt et al. 1994). Thus, the binding capacity assayed here is a good approximation of the real Bmax. This method allowed us to assay all samples of each subject at the same time and in the same run. The same batch of radioligand (NET-869, specific activity 25 Ci/mmole) was used for all assays of all patients. The protein concentration of the platelet membrane suspension was determined in the final membrane preparation (after thawing of the platelets), using the BIO-RAD protein assay. Data were analyzed by computerized curve fitting to a rectangular hyperbola using nonlinear regression analysis (Oestreicher and Pinto 1987). Binding capacity values were expressed as fmol/mg protein or fmol/10^6 platelets (Leysen et al. 1982).

Weather data for the vicinity were taken at the Royal Meteorological Station of Deurne, Antwerp, Belgium. We measured mean daily atmospheric pressure (hPa), air temperature (°C), relative humidity (%), and minutes of sunlight/day. The daily weather data reflect measurements obtained from midnight to midnight. Weather variables are known to exhibit a highly positive autocorrelation of a one-day lag. For the study span in this study, we found positive autocorrelations for air pressure ($r = 0.84$, $p < 10^{-4}$); light-dark span ($r = 0.55$, $p < 10^{-4}$); ambient temperature ($r = 0.94$, $p < 10^{-4}$) and

humidity ($r = 0.64$, $p < 10^{-4}$). Therefore, it may be assumed that weather measurements taken from midnight to midnight reflect the climatic condition around the time that blood samples were made.

STATISTICS

Assessment of Seasonal Variation

Seasonal variation has been ascertained by means of repeated measures analysis of variance (ANOVA), single cosinor fit analysis, least squares cosine spectral analysis of a single time series or a group of time series, and multiple regression analysis (De Meyer and Vogelaere 1990; Maes et al. 1993a,b, 1994b). For a comprehensive review of the theoretical backgrounds of the procedures used in the present study, refer to De Meyer and Vogelaere (1990).

Repeated measures ANOVAs were used to assess the between-subject variability with gender and age (two groups, i.e., <35 years versus ≥35 years) effects, the intraindividual variability with seasonal, cross-seasonal or monthly differences, and the two- or three-way interactions between time × sex, time × age, and time × age × sex. Multiple a priori comparisons among treatment means, that is in the seasons or cross-seasons, were ascertained by means of the Dunn test.

Least squares spectral analysis searches for periodicities in a single time series or in a group of time series on a probabilistic basis and allows an increased scanning of the whole frequency range. The significant rhythms are identified by relatively sharp peaks rising above a continuous background. F-statistics are generated as a measure of the signal-to-noise ratio for each of the rhythms and are listed in an F-spectrum. In the present study, up to 100 frequencies (rhythms) are scanned in a range between 2 and 366 days. Group spectral analysis was carried out on the 26 time series of [^{3}H]paroxetine binding in the normal volunteers. The within F spectra were interpreted to make inferences on common rhythms expressed at the population level. Spectral analysis of a single time series was performed on the pooled time series of the 26 volunteers after normalization of the [^{3}H]paroxetine binding data relative to the yearly mean of the monthly measurements in each of the subjects. This normalization eliminates the interindividual variability in the data.

Fitting of the sinusoidal functions of known frequency is carried out by means of the single cosinor method with determination of mean amplitude, mesor, and acrophase of the fitted sinusoidal functions.

Time series are often composed of various rhythmic components. Therefore, this study identified all seasonal rhythms, that is, annual rhythms and harmonics, that may exist in the time series by means of peak F-values in the F-spectra. These terms were used to compute a multiple component model. Toward this end, multiple regression analysis was employed to determine the total amount of variance in the time series of the normalized [^{3}H]paroxetine binding data, which is explained by the various significant seasonal rhythms subtracted from the F-spectra. Therefore, we have entered the time series of [^{3}H]paroxetine binding as dependent variable and the significant seasonal rhythms as explanatory variables. The results of these multiple regression analyses were checked for autocorrelation by means of the Durbin-Watson statistic and corrected if necessary (Ostrom 1988). The predicted values are used as an index of the estimated cyclic signal in the time series (Maes et al. 1993a,b, 1994b). These values are, subsequently, extrapolated to all the days in the study period in order to display the cyclic signal subtracted from the raw data. This procedure allows us to delineate a multiple component model (the cyclic signal in the raw data) with estimates of orthophase and range from low to peak values in a time series with different rhythmic components, as well as the relative contribution of each of the significant rhythms in explaining the variance in the raw data.

Assessment of Relationships with Climatic Data

Relationships between [^{3}H]paroxetine binding and climatic variables were assessed by means of: (1) regression analysis, (2) automatic multiple regression analysis, and (3) bivariate or group cosinor analysis (De Meyer and Vogelaere 1990; Maes et al. 1993a,b, 1994b).

Regression analyses of [^{3}H]paroxetine binding data (dependent variable) on single climatic data (explanatory variables) were pooled over the 26 time series of the healthy persons, in order to eliminate the interindividual variability.

When evaluating the impact of climatic data on [^{3}H]paroxetine binding data, it may be assumed that the latter may respond to a set of climatic variables which work in concert rather than individually and that it takes a period of time (e.g., some weeks) for some climatic factors to affect paroxetine binding, that is, the so-called memory effect. In order to investigate these possible synergistic effects between two or more climatic variables and the memory effects of the climate variables, we have used a multiple regression model whereby present together with lagged climatic data are entered as explanatory variables in a distributed lag model (Ostrom 1988; Maes et al. 1994b). Time lags of the climatic variables of 1 to 3 weeks or the changes in the climatic data the weeks preceding the blood samplings (e.g., Δ temperature = present ambient temperature minus temperature of the preceding weeks) are entered as additional explanatory variables in an automatic multiple regression model (Maes et al. 1993a, 1994b, 1995a). In order to prevent interpretational problems due to multicollinearity, we have performed multiple regression

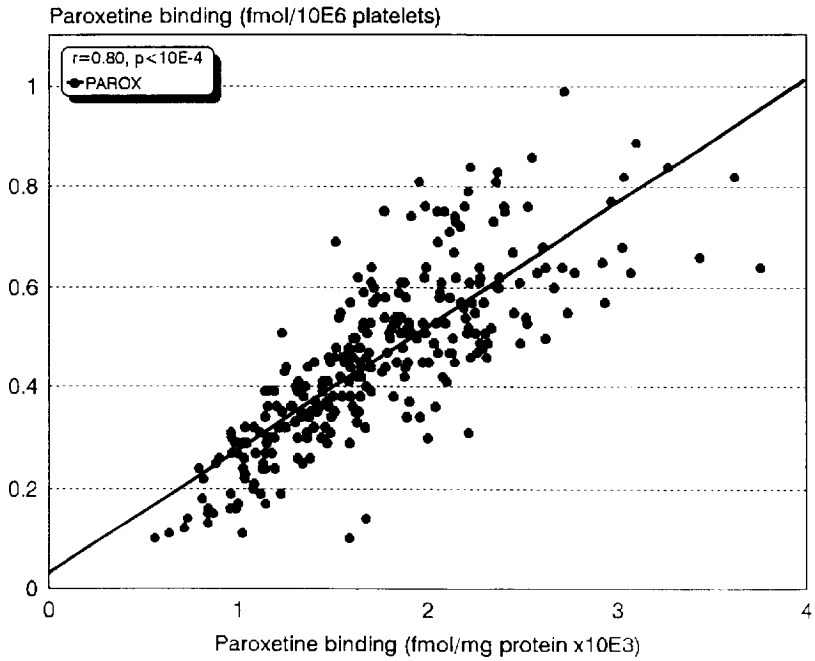

Figure 1. Correlation between paroxetine binding to platelets (●) expressed as fmol/mg protein or as fmol/10^6 platelets in 26 normal volunteers (r = 0.80; $p < 10E\text{-}4$).

analyses whereby the weather variables are entered in a forward stepwise (step-up) automatic inclusion method (with an F to enter of $p = .05$) with inspection of the determinant.

The degree of synchronization between the time series of [3H]paroxetine binding and climatic data was investigated by means of bivariate or multivariate cosinor analysis with assessment of the coherence between the time series and computation of the common acrophase of two or more time series (Maes et al. 1994b).

RESULTS

We found highly significant and positive relationship between [3H]paroxetine binding expressed as fmol/mg protein and as fmol/10^6 platelets (r = 0.80, $p < 10^{-5}$). Figure 1 shows the relationship between both measurements in the 26 subjects during the study span. The total variability expressed as a percentage (i.e., analytic + biologic variability = CVt = SD/mean × 100), in the [3H]paroxetine binding data expressed as fmol/mg protein (i.e. 32.07%) and as fmol/10^6 platelets (i.e. 37.46%) were comparable. As both measures are highly related and as the spectral and cosinor analyses performed on both terms yielded comparable results, we present here only the data of paroxetine binding expressed as fmol/10^6 platelets.

Assessment of Seasonal Variation

Results of ANOVAs. Figure 2 shows the mean monthly measurements of [3H]paroxetine binding in the 26 subjects. By means of repeated measures ANOVA (with age-groups and sex as additional groups), significant differences between the months were found (F = 11.2, $df = 11/217$, $p < 10^{-4}$). The effects of age (F = 0.3, $df = 1/22$, $p = .6$) and sex (F = .9, $df = 1/22$, $p = .7$) were not significant. There were no significant interactions between time and age (F = 1.4, $df = 11/217$, $p = .2$), time × sex (F = .9, $df = 11/217$, $p = .5$) or time × age × sex (F = .6, $df = 11/217$, $p = .8$).

By means of a repeated measures ANOVA a significant seasonal difference was found (F = 24.4, $df = 3/249$, $p < 10^{-4}$): Dunn tests ($t = 7.8$, $p < 10^{-4}$) showed that [3H]paroxetine binding was significantly higher in winter (0.508 ± 0.148 fmol/10^6 platelets) and spring (0.557 ± 0.155 fmol/10^6 platelets) than in fall (0.407 ± 0.172 fmol/10^6 platelets) and summer (0.367 ± 0.146 fmol/10^6 platelets). There were no significant differences either between fall or summer ($t = 1.8$, $p = .06$) or between winter and spring ($t = 1.2$, $p = .2$). The cross-seasonal differences (F = 14.2, $df = 3/249$, $p < 10^{-4}$) in [3H]paroxetine binding were not as significant as the seasonal differences.

Results of Spectral Analyses. By means of group spectral analysis performed on the grouped time series of the 26 subjects, we found significant (annual or harmonical) rhythms at 366 days (F = 36.78, $p < 10^{-4}$), 122 days (F = 7.1, $p < .01$) and 60 days (F = 5.7, $p < .01$).

Results of spectral analysis (i.e., the F spectrum) performed on the pooled time series of the normalized [3H]paroxetine binding data in each of the 26 normal volunteers showed three significant rhythms (at the $p = .05$ level); peaks in these rhythms were found at 366

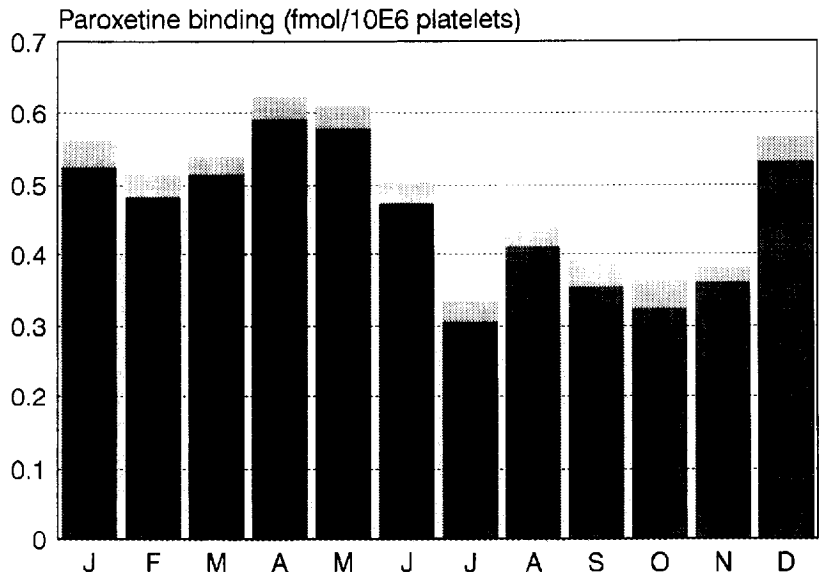

Figure 2. Monthly mean differences (*dark section of bars*) (SEM) (*lighter top section of bars*) in paroxetine binding to platelets (expressed as fmol/10^6 platelets) of normal volunteers.

days, 133 days (significant between 115 to 162 days and 61.2 days (significant between 56.6 to 61.2 days). Taken together, group spectral analysis and spectral analysis of the pooled time series showed three significant seasonal rhythms, that is, annual (366 days), 4-monthly (122 days), and bimonthly (61 days) rhythms.

Results of Single Cosinor. Table 1 (regressions 1 to 3) shows the results of single cosinor analyses performed on the pooled time series of the normalized [^{3}H]paroxetine binding data in each of the 26 subjects with fitted sinusoidal functions of annual, 4-monthly, and bimonthly rhythms. It was found that the annual, 4-monthly, and bimonthly rhythms explained 23.6%, 5.1%, and 5.3%, respectively, of the variance in the pooled [^{3}H]paroxetine binding data. The acrophase of the annual rhythm in [^{3}H]paroxetine binding occurred around March 19 and the amplitude of this fitted annual rhythm was 0.109 fmol/10^6 platelets.

Results of Multiple Regression Analyses. Table 1 (regression 4) shows the results of the multiple regression analysis of [3H]paroxetine binding on the three above seasonal rhythms. It was found that 34.3% of the variance in the [^{3}H]paroxetine binding data could be explained by the regression on these three rhythms. The

Table 1. Results of Regression Analyses of Paroxetine Binding to Platelets on Seasonal Rhythms and Climatic Data in 26 Normal Volunteers

No.	Explanatory Variables (with *p*-value between brackets)	R^2 (%)	F-Statistic	*df*	*p*
1	Annual (366 days)	23.6	43.9	2/284	$<10^{-4}$
2	Four-monthly (122 days)	5.1	7.6	2/284	0.0009
3	Bimonthly (61 days)	5.3	8.0	2/284	$<10^{-4}$
4	366 days [$<10^{-4}$]; 122 days [$<10^{-4}$]; 61 days [$<10^{-4}$]	34.3	24.3	6/280	$<10^{-4}$
5	Air pressure (Ap)	9.7	30.6	1/285	$<10^{-4}$
6	Temperature (T)	1.4	4.0	1/285	0.04
7	Humidity (H)	10.8	34.5	1/285	$<10^{-4}$
8	Δ Temperature (ΔT)	10.5	33.4	1/285	$<10^{-4}$
9	Δ Humidity (ΔH)	9.2	28.9	1/285	$<10^{-4}$
10	+Ap [0.0003]; −T [$<10^{-4}$]; −H [$<10^{-4}$]; +ΔT [$<10^{-4}$]; +ΔH [0.009]	32.5	27.0	5/281	$<10^{-4}$
11	366 days [$<10^{-4}$]; 122 days [$<10^{-4}$]; 61 days [0.0002], −T [0.0003]	37.6	28.0	7/279	$<10^{-4}$

This table shows the results of 11 different (multiple) regression analyses. Regressions 1 to 3 show the results of single cosinor analyses, with the significant rhythms (as determined by means of spectral analysis) as explanatory variables, i.e., 366 days (annual), 122 days (four-monthly) and 61 days (bimonthly). Regression 4 shows the total variance in paroxetine binding explained by the annual, four-monthly, and bimonthly rhythms combined. Regressions 5 to 9 show the variance in paroxetine binding explained by the climatic variables. These regressions were pooled over the 26 time series of the normal volunteers in order to eliminate inter-individual variability. Δ: denotes present T or H minus T or H two weeks prior to the blood samplings. Regression 10 shows the results of an automatic (step-up) multiple regression analysis of paroxetine binding on all climatic data (pooled over the 26 time series of the normal volunteers). ±: denotes the sign of the climatic variables in the regression equation. Regression 11 shows the results of the multiple regression analysis of paroxetine binding on the significant rhythms and climatic variables.

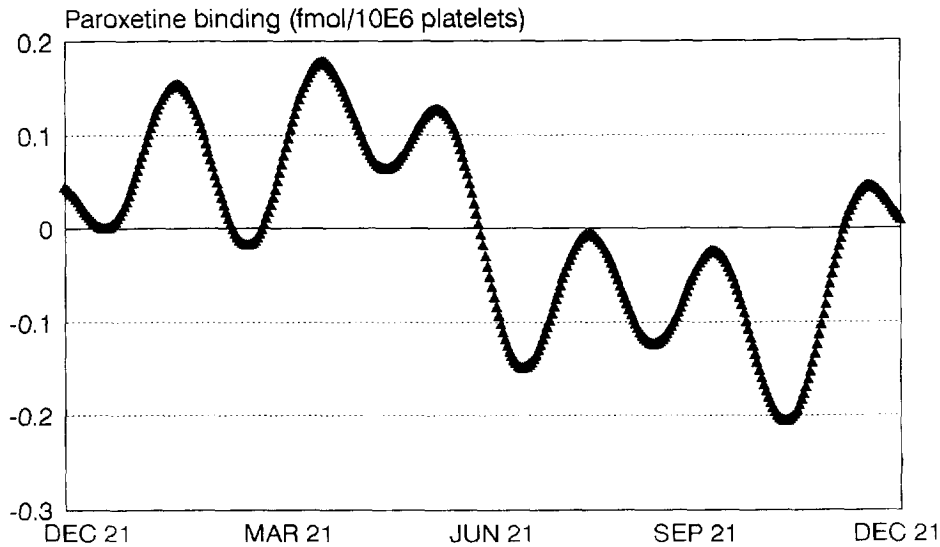

Figure 3. Yearly variation in paroxetine binding to platelets in 26 normal persons. This chronogram shows the cyclic signal in paroxetine binding values as determined by means of multiple regression analysis: 34.3% of the variance in paroxetine binding is determined by means of three rhythms, i.e., 366 days, 122 days, and 61 days. These rhythms are subtracted by means of spectral analysis on the pooled time series of the normalized paroxetine binding data (i.e., normalized relative to the yearly mean values in each subject).

Durbin-Watson statistic was not significant in this analysis; the autocorrelation coefficient was -0.138.

Figure 3 shows the cyclic signal in [^{3}H]paroxetine binding computed by means of the above seasonal rhythms. The peak-trough difference in this seasonal variation expressed relative to the yearly mean value was 83.7%. The orthophase (peak value) in this seasonal variation occurred around April 6, whereas trough values were observed around October 31.

By means of spectral analysis (to determine the significant seasonal rhythms) in conjunction with multiple regression analysis, a significant seasonal variation was found in the climatic data. Up to 22.8% of the variance ($F = 11.1$, $df = 8/299$, $p < 10^{-4}$) in air pressure was explained by rhythms of 366 days ($p = .002$), 183, 122, and 61 days (all: $p < 10^{-4}$); 87.0% of the variance ($F = 199$, $df = 10/297$, $p < 10^{-4}$) in ambient temperature was explained by rhythms of 366, 183, 122, 91, and 61 days (all: $p < 10^{-4}$); 48.2% of the variance ($F = 34.7$, $df = 8/299$, $p < 10^{-4}$) in hours of sunlight/day was explained by rhythms of 366, 122, and 61 days (all: $p < 10^{-4}$), and 91 days ($p = .008$); 44.3% of the variance ($F = 29.8$, $df = 8/299$, $p < 10^{-4}$) in relative humidity was explained by rhythms of 366, 183, 122, and 61 days (all: $p < 10^{-4}$); and 63.7% of the variance ($F = 88.0$, $df = 6/301$) in Δ temperature was explained by three rhythms of 366, 122, and 61 days; ($F = 88.0$, $df = 6/301$, $p < 10^{-4}$).

Relationships with Climatic Data

Regression Analyses. Table 1 (regressions 5 to 9) shows the results of regression analyses pooled over the 26 time series of the normal volunteers with [^{3}H]paroxetine binding as dependent variable and climatic data as explanatory variables. Highly significant relationships were found between [^{3}H]paroxetine binding and air pressure (positive), humidity (negative), Δ humidity (positive), and Δ temperature.

Table 1 (regression 10) lists the results of an automatic step-up multiple regression analysis with [^{3}H]paroxetine binding as dependent variable and all present and past climatic data as explanatory variables. It was found that 32.5% of the variance in [^{3}H]paroxetine binding was explained by the regression on air pressure (positive), temperature and humidity (both negative), Δ temperature, and Δ humidity (both positive).

Finally, Table 1 (regression 11) shows the results of multiple regression analysis with the significant rhythms as well as the climatic data as dependent variables. It was found that 37.6% of the variance in [^{3}H]paroxetine binding was explained by the three seasonal rhythms and ambient temperature combined.

Results of Bivariate and Multivariate Cosinor Analyses. Bivariate cosinor analysis showed a common annual rhythm in the time series of [^{3}H]paroxetine binding and Δ temperature ($F = 83.7$, $p < 10^{-4}$; coherence: $F = 0.00$, $df = 1/593$, $p = .96$; common acrophase around April 8). After rotation of the time series of temperature and humidity by 180°, bivariate cosinor analyses showed that significant annual rhythms were expressed in the time series of [^{3}H]paroxetine binding and either temperature ($F = 97.0$, $p < 10^{-4}$; coherence: $F = 0.01$, $df = 1/593$, $p = .91$) or relative humidity ($F = 46.2$, $p < 10^{-4}$; coherence: $F = 0.00$, $df = 1/593$, $p = .98$). By means of multivariate cosinor analysis, a common annual rhythm was found in the time series of [^{3}H]paroxetine binding, Δ temperature, temperature and relative humidity (the time series of temperature and humidity were rotated by 180°; $F = 33.8$, $p < 10^{-4}$; coherence: $F = .01$, $df = 3/1207$, $p = .99$; the common peaks in [^{3}H]paroxetine binding and Δ temperature and lows in temperature and humidity occurred around March 15).

Figure 4 shows the inverse relationship between the cyclic signals in [^{3}H]paroxetine binding and in relative

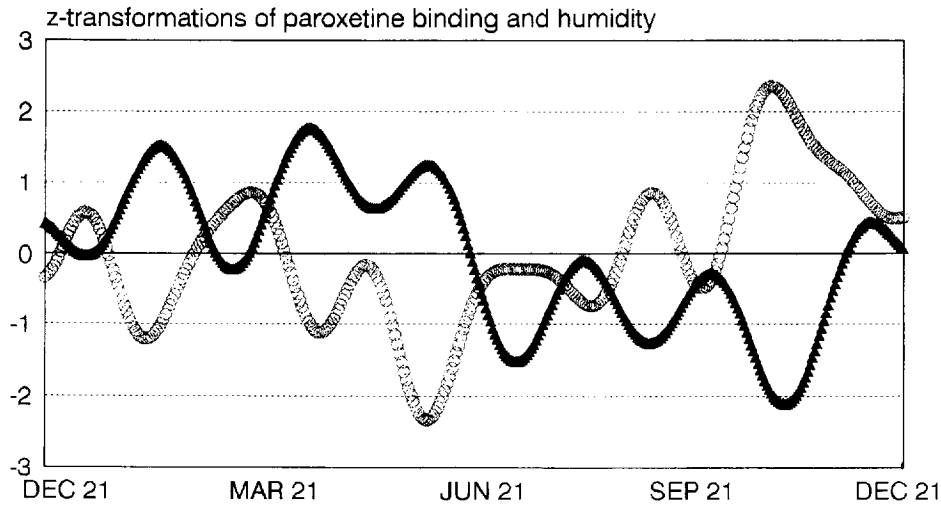

Figure 4. This figure shows the time-relationship between paroxetine binding to platelets (▲) in 26 normal volunteers and relative humidity (○) (both variables are in z-transformation). The cyclic signal in paroxetine binding is determined as described in the legends to Figure 3. 44.3% of the variance in relative humidity is explained by the regression on four rhythms, i.e., 366 days, 183 days, 122 days, and 61 days. There is a significant negative relationship between the cyclic signals in both variables ($r = 0.73$, $p < 10^{-4}$).

humidity. Regression analysis showed that the cyclic signals in both variables were significantly correlated ($r = 0.73$, $p < 10^{-4}$). Figure 5 shows the positive relationship between the cyclic signals in the time series of [^{3}H]paroxetine binding and Δ temperature. Regression analysis showed that the cyclic signals in both variables were significantly correlated ($r = 0.83$, $p < 10^{-4}$).

DISCUSSION

The major finding of the present study is that [^{3}H]paroxetine binding to platelets exhibits statistically highly significant seasonal variation in normal persons, with significant annual, 4-monthly, and bimonthly rhythms, which are expressed as a group phenomenon. It was found that a significant part of the intraindividual variability (±34.3%) in the [^{3}H]paroxetine binding values could be explained by seasonal rhythms, such as annual, 4-monthly, and bimonthly rhythms. The peak-trough difference in this seasonal variation expressed relative to the yearly mean value was as high as 83.7%. [^{3}H]Paroxetine binding to platelets was significantly higher in winter and spring than in fall and summer, with lows occurring in summer and peaks in spring. These findings concur with a previous study reporting significant seasonal differences in [^{3}H]paroxetine binding to platelets in unipolar depressed patients, that is, peak [^{3}H]paroxetine binding was observed in spring (D'Hondt et al. 1994). Therefore, it may be suggested that depressed patients and normal volunteers exhibit a similar seasonal pattern of alterations in [^{3}H]paroxetine

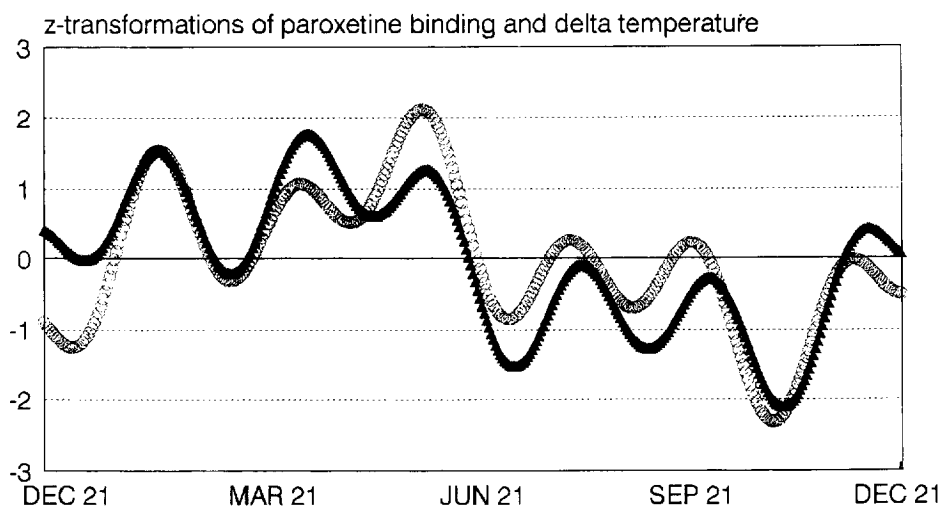

Figure 5. This figure shows the time-relationship between paroxetine binding to platelets (▲) in 26 normal volunteers and changes in ambient temperature (○) the 2 weeks preceding blood samplings (both variables are in z-transformation). The cyclic signal in paroxetine binding was determined as described in the legends to Figure 3. 63.7% of the variance in Δ temperature is explained by the regression on three rhythms, i.e., 366 days, 122 days, and 61 days. There is a significant positive relationship between the cyclic signals in both variables ($r = .83$, $p < 10^{-4}$).

binding to platelets. Klompenhouwer et al. (1990), on the other hand, reported that the highest B_{max} values occurred in June. However, the last-mentioned study is difficult to interpret because the delineation of "seasonal variation" should not rely on differences between certain periods of the year (i.e., five time points) as ascertained by means of repeated measures ANOVA, but rather on the detection of seasonal rhythms, such as annual rhythms or harmonics, as checked by means of least squares cosine spectral analysis in conjunction with cosinor fit and multiple regression analysis (De Meyer and Vogelaere 1990; Maes et al. 1993a,b; 1994b). Indeed, these methods allow: (1) to identify all significant seasonal rhythms that may exist in a group of time series; (2) to determine the relative contribution of each rhythm to the total intraindividual variance in the time series; (3) to compute a multiple component model with estimates of orthophase (peak values) and range of change from low to peak values in a time series with different rhythmic components; and (4) to check whether these rhythms are expressed as a group phenomenon. Moreover, Klompenhouwer et al. (1990) did not specify whether all assays of one and the same subject were determined at the same time, in the same run, and using a same batch of radioligand. These methods are prerequisites in order to minimize the analytical variability and to allow for a more adequate evaluation of the biological intraindividual variability (Maes et al. 1994b; Fraser 1994). Seasonal "differences" in B_{max} of imipramine binding in healthy volunteers with peak binding in summer and fall or in September were observed by Egrise et al. (1983, 1986) and Arora and Meltzer (1988), respectively. Other groups, on the other hand, were unable to find significant seasonal "differences" in imipramine binding between some periods of the year (Tang and Morris 1985; Galzin et al. 1986). However, comparison of the binding characteristics of both paroxetine and imipramine may be hazardous. In accordance with our study, Klompenhouwer et al (1990) did not observe significant gender or age-related effects on the seasonal "variation" of [3H]paroxetine binding in normal controls.

Generally, it is assumed that annual variations in human biochemical, immune, hematologic, and neurophysiologic features are genetically determined, whereas cyclic environmental factors, for example, changes in the weather, may modulate or entrain rhythm parameters, such as amplitude and acrophase (Touitou and Haus 1992). In this respect, the present study showed that an important part of the intraindividual variability (i.e., 32.5%) in [3H]paroxetine binding to platelets was explained by weather variables, such as changes in ambient temperature, relative humidity, and air pressure. Moreover, highly significant common annual rhythms were expressed in [3H]paroxetine binding and ambient temperature or humidity (both inversely related) and changes in temperature the 2 weeks preceding blood samplings (positively related). In addition, it was found that ambient temperature explained a significant part of the variance in [3H]paroxetine binding, which could not be explained by the annual, bimonthly, or 4-monthly rhythms. These findings suggest that short-term alterations in climatic variables modulate the seasonal rhythms in [3H]paroxetine binding to platelets and that a change in humiture (i.e., effects of humidity combined with temperature) is an important weather variable. At present there is no further information how these weather variables (e.g., changes in humiture) may affect [3H]paroxetine binding. In this respect, it has been reported that a 7-day period of treatment with light significantly increased platelet [3H]imipramine binding in patients with winter depression but not in normal controls (Szadoczky et al. 1989). In the present study, however, no significant relationship was detected between the annual variations in light-dark span and [3H]paroxetine binding. Although hypothetical, seasonal changes in ambient temperature may affect cholesterol metabolism thereby influencing the viscosity of the platelet membrane that, in turn, could induce alterations in the specific binding of serotonin (Heron et al. 1980; Maes et al. 1994a). Some studies reported a clear seasonality in total plasma cholesterol levels, which was negatively correlated with mean monthly air temperature (Robinson et al. 1993).

Although our study clearly shows that [3H]paroxetine binding to platelets is organized along a multifrequency seasonal time structure, the results do not provide further information on the nature of this yearly variation. The seasonal rhythms observed in this study may be due to: (1) a seasonal variation in the daily mean values of [3H]paroxetine binding to platelets or (2) a putative seasonal modulation of putative circadian rhythms in [3H]paroxetine binding. A seasonal modulation of circadian rhythms has been observed in some other human functions organized along a daily and seasonal time structure, for example, immune functions (Lévi et al. 1988). Further investigations should examine whether a seasonal modulation of circadian rhythms may be detected in [3H]paroxetine binding to platelets.

Another question is whether this peripheral finding also occurs within the central nervous system. It is known that [3H]paroxetine binds with high affinity to a specific population of binding sites located on human platelet and neuronal membranes, associated with 5-HT uptake mechanisms (Mellerup et al. 1983; Habert et al. 1985; Cheetham et al. 1993), and that the cloned 5-HT transporter on human platelets is identical to the 5-HT transporter in human brain (Lesch et al. 1993). In this respect, it has been shown that patients with posttraumatic stress disorder (PTSD), who showed a positive response to fluoxetine pharmacotherapy, had lower plate-

let paroxetine binding characteristics than non-responders (Fichtner et al. 1994). These findings suggest that peripheral findings on paroxetine binding may be relevant for central 5-HT activity. Further research should examine seasonal variation in brain 5-HT transporter in experimental animals.

The present study on seasonal differences in [^{3}H]paroxetine binding adds to the literature on seasonal differences in various serotonergic variables, such as plasma L-tryptophan (L-TRP) levels, cerebrospinal fluid (CSF) 5-hydroxy-indoleacetic acid (5-HIAA) concentrations, and neuroendocrine responses to 5-HT precursors or agonists, such as L-TRP, 5-hydroxy-tryptophan, and m-chlorophenylpiperazine (Carlsson et al. 1979; Swade and Coppen 1980; Brewerton et al. 1988; Brewerton 1989; Maes et al. 1995). It is interesting to note that in spring, when peak [^{3}H]paroxetine binding was found in the present study, CSF 5-HIAA and plasma L-TRP concentrations measured in previous studies were at their lowest. Interestingly, violent suicide has been shown to occur more frequently in spring, whereas dysfunctions in 5-HT activity may be related to violent suicide (review: Maes et al. 1993b). For those reasons, we hypothesize that the previous seasonal fluctuations in 5-HT activity, with a possible altered serotonergic function in spring, could put suicide-prone subjects at a higher risk in spring.

The findings of a highly significant yearly variation in [^{3}H]paroxetine binding to platelets may have relevance for the clinical efficacy of antidepressive drugs, such as the selective 5-HT reuptake inhibitors (SSRIs). Indeed, it is known that the effects of various therapeutical drugs, such as heparin, corticosteroids, anticancerous and anti-inflammatory drugs, and bronchodilators may vary according to their time of administration (Bruguerolle 1992). Recently, Fichtner et al. (1994) reported that decreased platelet [^{3}H]paroxetine binding in PTSD is associated with a positive therapeutic response to subsequent treatment with fluoxetine, that is, responders had lower pretreatment K_d values and a trend toward lower pretreatment B_{max} values. Because part of the effects of SSRIs are due to their activity at the 5-HT uptake site, it may be hypothesized that the multiplicity of seasonal rhythms in paroxetine binding sites involves modifications of the efficacy of SSRIs according to month of administration. Future chronopharmacologic research should examine the time-dependency in the chronopharmacologic responses to SSRIs in relation to measures of [^{3}H]paroxetine binding.

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