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Time Course of Nigrostriatal Degeneration in Parkinson's Disease*

A Detailed Study of Influential Factors in Human Brain Amine Analysis

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Summary

It could be shown that the post mortem analysis of biogenic amines, precursors, and metabolites in the human brain are influenced by various parameters.

1. The patient's medical history; long term observation of the course of the disease; age; sex.
2. Terminal illness; duration of terminal illness.
3. Previous treatment with drugs; last drugs.
4. Time interval between last drug treatment and death; time of day and date of last drug consumption.
5. Rapidity of death; time of death; duration of coma.
6. Changes occurring in tissues before death; patient's constitution during terminal illness.
7. Changes in concentration of the biogenic amines, precursors, and metabolites depending on the patient's age.
8. Time between death and necropsy.
9. Dissection of specimen.
10. Period of storage; temperature of storage.

* Dedicated to Professor *W. Birkmayer,* M.D., on the occasion of his 65th birthday.

11. Chronobiological rhythm of substances.
12. Methods of assay.
13. Homogeneity of all mentioned parameters in the control group and patient group.

For the first time it could be demonstrated that the time course of nigrostriatal degeneration, independent of the age of the parkinsonian at the beginning of the illness, is linear for the last stage and the denervation progressively increases as the duration of illness progresses.

Introduction

Frequently, one finds discrepancies in results from various laboratories in regard to the quantitative evidence of substances from catecholamine or indoleamine metabolism in the post mortem human brain. Thus, comparison of the absolute values is seldom possible. The chemical, analytical methods cannot be made solely responsible for the deviations. In some cases at least, it seems that various clinical parameters which cannot be influenced by the analyst are divergent.

Thus, this study partly presents a supplement to already available results (*Joyce*, 1962; *McLean, Nicholson, Pare* and *Stacey*, 1965; *Dowson*, 1969; *Lloyd, Farley, Deck* and *Hornykiewicz*, 1974). On the other hand, it shows the experiences and methodical guidelines in this laboratory.

Results and Discussion

In order to undertake a comparative interpretation of the results of brain analyses, one must take various parameters into consideration. These include not only the acquisition of specimens and the biochemical microanalysis, but also, in agreement with the above-mentioned authors, other important data such as:

1. The patient's medical history; long term observation of the course of the disease; age; sex.
2. Terminal illness; duration of terminal illness.
3. Previous treatment with drugs; last drugs.
4. Time interval between last drug treatment and death; time of day and date of last drug consumption.
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13. Homogeneity of all mentioned parameters in the control group and patient group.

Pre-Death and Death Data (1—6)

These points include the history of illness (sex, age, last drug[s], terminal illness, time interval between last drug and death) as well as the duration and clinical observation of the coma. Most of these data can easily be obtained. Nevertheless, the criterion for determining the metabolic alteration during the clinically manifest pre mortem interval is hard to verify. These metabolic alterations are dependent on the progression of the terminal illness and the time course of the coma, and therefore seem to be influenced by the clinically manifest physical condition and constitution of the patients. Patients who die from cardial infarcts (rapid death), for example, are considered to show normal metabolic conditions during the premortem time regarding post mortem brain analysis. This certainly no longer refers to patients who are bed-ridden for longer periods of time with progressive illness duration.

Therapeutic methods for Parkinson's disease (survey by *Birkmayer* and *Neumayer*, 1972) usually interfere directly or indirectly with the catecholamine metabolism. Administration of dopa (*Davidson, Llyod, Dankova* and *Hornykiewicz*, 1971; *Rinne, Sonninen* and *Hyyppä*, 1971), monoamine oxidase inhibitors (*Birkmayer* and *Hornykiewicz*, 1962; *Ganrot, Rosengren* and *Gottfries*, 1962; *McLean, Nicholson, Pare* and *Stacey*, 1965), antidepressants (*Marley* and *Stephenson*, 1972; *Goodwin* and *Post*, 1974) and anticholinergics (for review see *Birkmayer* and *Hornykiewicz*, 1976) alter synthesis, metabolism and/or release of biogenic amines. Additional therapy such as the application of tryptophan, for example, can also influence analysis results, even if indirectly. The indoleamine metabolism can be influenced by administration of tryptophan (*Wurtman* and *Fernstrom*, 1974). In Parkinson's disease, serotonin is reduced in almost all nuclei of the brain in comparison to the norm (*Bernheimer, Birkmayer* and *Hornykiewicz*, 1961). However, contrary to dopamine, the concentration decrease is not more than 40—50 % on the average; often even less (*Birkmayer, Danielzcyk, Neumayer* and *Riederer*, 1974). Interestingly, even after several years of administration of L-dopa + a decarboxylase inhibitor (Benserazide = 1-Dl-serin-2[2, 3, 4-trihydroxybenzyl]-hydrazine-hydrochloride) in average daily doses of

500—750 mg Madopar, no further decrease of the serotonin values could be established (Table 1), even though a competition between L-tryptophan and L-dopa for transport and uptake mechanisms could be established.

Dopa is present in the human brain in concentrations of a few nanograms/g (*Davidson, Dankova* and *Hornykiewicz*, 1971; *Birkmayer, Danielczyk, Neumayer* and *Riederer*, 1976). Doses of dopa increase not only the dopa level in the brain, but also influence the concentration of the catecholamines dopamine and noradrenaline (*Carlsson, Lindqvist, Magnusson* and *Waldeck*, 1958; *Birkmayer, Danielczyk, Neumayer* and *Riederer*, 1976; *Bartholini, Lloyd* and *Pletscher*, 1974).

Post mortem examinations of the dopa level after L-dopa therapy show that the concentration of dopa in various human brain regions vary. In the striatal nuclei, the substantia nigra, as well as the nucleus ruber, significantly higher concentrations in this amino acid could be established than in the amygdaloid nucleus, gyrus cinguli and the raphe (*Birkmayer, Danielczyk, Neumayer* and *Riederer*, 1976). This can be explained by a varyingly strong affinity of dopa to the individual nuclei of the human brain.

Table 1. *5-Hydroxy-tryptamine in L-dopa resistent and madopar treated parkinsonian patients compared to age-matched controls*

	5-Hydroxy-tryptamine (ng/g) mean ± s.d.		
	L-dopa resistant Parkinsonian patients (n = 4)	Parkinsonian patients on long term Madopar treatment (n = 5)	Controls (n = 18)
Caudate Nucleus	115 ± 14	125 ± 12	275 ± 19
Putamen	140 ± 13	135 ± 12	260 ± 21
Pallidum	135 ± 12	150 ± 14	380 ± 26
Substantia Nigra oral part	265 ± 20	280 ± 23	545 ± 32
Substantia Nigra caudal part	354 ± 24	370 ± 32	553 ± 27
N. Amygdalae	190 ± 16	215 ± 19	272 ± 15
Gyrus Cinguli	75 ± 10	63 ± 10	70 ± 12
Raphe	385 ± 35	409 ± 32	510 ± 30
Red Nucleus	410 ± 32	395 ± 35	565 ± 33

L-Dopa-resistant Parkinsonian patients: drug treatment during the last years before death: Amantadine, anticholinergics. No antiparkinson-therapy 3—7 days before death. Age of patients: 75 ± 1 years. Madopar = L-Dopa + Benserazide (4 : 1).

Madopar treated Parkinsonian patients: Madopar 3 × 250 mg daily in the mean for 5—7 years; antiparkinson-therapy 6—24 hours before death. Age of the patients: 75 ± 2 years.

Controls: age: 75 ± 3 years; 10 males, 8 females; cause of death: same pattern as in table 2 for controls.

Autopsy interval: 9 ± 3 hours for all cases; storage time of dissected material: 42 ± 5 days at —70 °C.

Method: Fluorometry according to *Ashcroft* and *Sharman* (1962).

A significant interaction with other neurotransmitter systems, especially of the indoleamines, was shown by *Bartholini, Da Prada* and *Pletscher* (1968); *Butcher* and *Engel* (1969); *Ng, Chase, Colburn* and *Kopin* (1970); *Everett* and *Borcherding* (1971), and in L-dopa psychoses of Parkinson victims by *Birkmayer, Danielczyk, Neumayer* and *Riederer* (1972, 1974).

This competition could also be shown in human blood after L-dopa, Madopar, or Sinemet doses for only a few hours after application (*Riederer*, 1976). These data and those in table 1 lead to the conclusion that even with L-dopa administration over a period of many years, the indoleamine metabolism is only temporarily influenced; the competition remains reversible, and even dopa therapy over a long period of time produces no irreversible injury to serotoninergic neurons.

Particularly problematic are post mortem examinations of biogenic amines on deceased (for example, suicides, as well) who died of overdosis or during therapy with sleeping pills, tranquilizers (diazepam, oxazepam, chlordiazepoxide), carbon monoxide, alcohol etc. Alterations in the concentration of transmitters or in the metabolism after (over)doses of this type provide no conclusion as to the deciding metabolic pattern *before* the drug consumption (for example, before suicide), and thus, no conclusion as to a biochemical mechanism (for example, depression). It could be shown that barbiturates and tranquilizers, for example, show a long-lasting excretion period and can accumulate in the tissue. An influence on the synthesis and metabolism of biogenic amines in the sense of reduction could be shown especially through tranquilizers (survey by *Dominic*, 1973). Barbiturates seem to increase the 5-hydroxytryptamine concentration (*Bonnycastle, Bonnycastle* and *Anderson*, 1962). Alcohol reduces the MAO activity in the brain (*Gottfries, Oreland, Wiberg* and *Winblad*,

1975), and increases the levels of noradrenaline and dopamine (*Post* and *Sun*, 1973).

Therefore, except in studies which consciously test the influence of medicaments on biochemical mechanisms, it is necessary with drugs

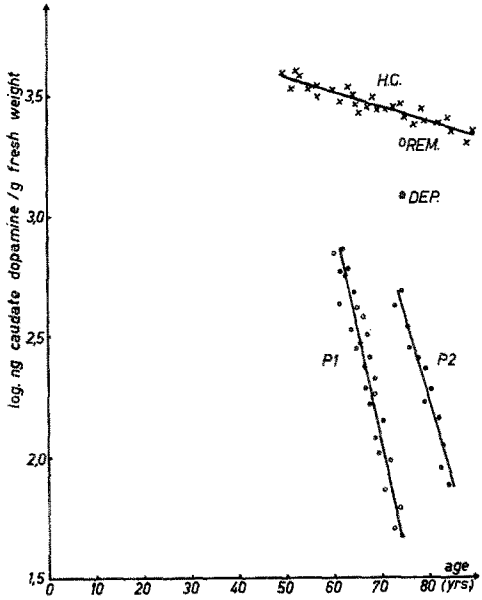

Fig. 1. Time course of nigrostriatal degeneration in parkinsonism.

H.C. (x): Healthy controls; clinical details see table 2. Statistical analysis: number of cases: 28

Chi²-test

$CC_{corr} = -0.9660$

$r = -0.0092$

$Oy = 3.88$

P1 (◯): Parkinsonian patients; begin of the disease = 60 ± 1 years; clinical details see table 2. Statistical analysis: number of cases: 27

Chi²-test

$CC_{corr} = -0.9740$

$r = -0.0956$

$Oy = 8.79$

P2 (●): Parkinsonian patients; begin of the disease = 73 ± 1 years; clinical details see table 2. Statistical analysis: number of cases: 12

Chi²-test

$CC_{corr} = -0.9768$

$r = -0.0835$

$Oy = 8.885$

DEP. (●): Depressed patients; for clinical details see *Birkmayer* and *Riederer* (1975). Number of cases: 3

REM. (O): Depressed patient during remission. Number of cases: 1

Patients	Age Group years	Loss of Dopamine in Caudate Nucleus %
H.C.	45—55 (4)	15.3
	56—65 (7)	15.7
	66—75 (8)	9.8
	76—85 (6)	10.7
	86—95 (3)	
		mean = 12.87 ± 3.05 ± s.d. pro decade
P1	61—67 (14)	28.55 ± 9.0
	67—73 (13)	46.55 ± 6.85
P2	74—80 (9)	28.3 ± 4.16
	80—84 (3)	38.6 ± 5.79

of this type that the analyst select cases—depending on the type and excretion period of the drug—where there is an interval of several days to several weeks between the last medicament consumption and death.

The extent to which the therapy with a medicament over a long period of time (for example, L-dopa, Madopar) can alter biochemical processes not only in the sense wished for, even if the drug was discontinued in time before death, cannot be estimated at present as long term experiments on animals are lacking in this regard. Long term therapy with L-dopa with or without decarboxylase inhibitors leads to an increase in side-effects as the illness progresses. Whether this can be solely attributed to the progressive degeneration of the dopaminergic nigrostriatal path, or whether years of influx of non-physiologically high dopa concentrations influences the functional ability of the presynaptic nerve endings cannot be decided at present, as long term experiments on animals are also lacking in regard to this problem. However, statistical examinations (*Birkmayer, Ambrozi, Neumayer* and *Riederer*, 1974) show that with long-term therapy with low doses of L-dopa or Madopar, the life expectation of the group of patients treated with L-dopa is exactly as high as that of the group treated mainly with anticholinergics. However, figure 1 shows that the progression of dopamine loss in the nucleus caudatus is significantly higher between the 7th and 11th year after the start of the illness than at the beginning of the illness. Therefore, the degeneration of the nigrostriatal dopaminergic path is not eliminated by the L-dopa. On the other hand, these findings explain the decrease in efficacy of dopa preparations as the illness progresses. Therefore, a

direct correlation between the appearance of socalled On-Off phases especially in the advanced stage of Parkinson's disease and the progressive degeneration of the nigrostriatal dopaminergic neurons is assumed.

Chronobiological Aspects of Parkinson's Disease (7)

Through studies by *Robinson, Davis, Nies, Ravaris, Sylwester* and *Burlington* (1971), as well as by *McGeer* and *Wada* (1971), it is known that the functional activity of monoaminergic neurons may clearly be subject to alterations depending on the age of the person. While the enzymatic activity of tyrosine-hydroxylase (*McGeer, McGeer* and *Wada*, 1971) in childhood (5—20 years of age) strongly decreases and evidently the deactivation in older age is only sligthly distinct, the activity of the monoamine oxydase increases with increased age (*Robinson, Davis, Nies, Ravaris, Sylwester* and *Burlington*, 1971). That signifies a decrease in the synthesis of dopamine and a simultaneous increase of the metabolism of this biogenic amine as well as that of noradrenaline. The concentration of serotonin and 5-hydroxyindoleacetic acid remains unchanged by the process of aging (*Robinson*, 1975). The result would be decreasing dopamine concentration in dopaminergic neurons with increasing age. Also *Carlsson* (1975) clearly showed a decrease in dopamine and methoxytyramine concentration in the striatum of the human with increasing age. Thus, an alteration in the activity of synthesizing and or destructive enzymes with increasing age must be assumed. However, *Sandler, Bonham-Carter, Cuthbert* and *Pare* (1975) do not confirm Robinson's results.

Idiopathic Parkinson (morbus Parkinson) occurs on the average between the 50th and 70th year of life. During the last 5 years, 27 patients of an average age of 60 ± 1 years at the beginning of the illness, as well as 12 patients of an average age of 73 ± 1 years at the beginning of the illness, died in this department. In both groups, there were patients who did not reach the average age of a Parkinson victim (beginning of the illness until death: $\bar{x} = 11 \pm 2$ years), and died sooner due to other causes (see Table 2). The statistical evaluation of the entire case material in this department during the years from 1960 to 1973 (*Birkmayer, Ambrozi, Neumayer* and *Riederer*, 1974) showed that basically the life expectancy of a Parkinson victim can continue for an average of 11 ± 2 years independent of age at the beginning of the illness. Naturally, the rate of death is higher in the older group than in the younger; however, this is independent of Parkinson's disease and can be traced back to other factors which also appear when the death rate of control groups for certain periods of life are

Table 2. *Description of the case material*

Patient	Sex	Age years; range	Cause of Death		Post mortem Autopsy interval hours	Last drugs before death	Storage Time Days
Controls (H.C.)	14 F 14 M	45—95	cardial infarction heart failure myocardiopathy bronchopneumonia bronchial carcinoma pulmonary embolism	(6) (4) (4) (9) (1) (4)	10 ± 3	→ Morphine until 6 hours before death	37 ± 4
Idiopathic Parkinson: begin of the disease 60 ± 1 year (P1)	15 F 12 M	61—73	bronchopneumonia myocardial infarction pulmonary embolism akinetic crisis	(12) (4) (5) (6)	11 ± 2.5	No L-dopa for 2 weeks (mean)	29 ± 3
Idiopathic Parkinson: begin of the disease 73 ± 1 year (P2)	7 F 5 M	73—85	bronchopneumonia heart failure akinetic crisis	(6) (3) (3)	10.5 ± 2	No L-dopa for 12 days (mean)	38 ± 5

In all these cases, no neuroleptics, antidepressive substances, MAO-inhibitors, or hypnotics were administered. Number of cases in parentheses.
The dissected material was stored at —70 °C.

compared. Thus, a parkinsonian who was striken ill at 60 basically lives exactly as long as a person who became ill only at the age of 70 or 75. Figure 1 shows that the progression of decrease of dopamine in the nucleus caudatus of Parkinson patients in both groups mentioned significantly differs in comparison to a control group, as well as with each-other. In the young group, patients who died 1—2 years after the beginning of the illness showed higher dopamine content than the older group. However, the life expectancy of both groups can be 11 ± 2 years. Thus, the progression of dopamine decrease in the caudate nucleus is somewhat less in the older group than in the younger (Fig. 1). In comparison with the control group, patients who died 1—2 years after the beginning of the disease, show a significant decrease in dopamine content of approximately 79 % (beginning of the illness at 60 ± 1 years) or 84 % (beginning of the illness at 73 ± 1 years). This percentage is even less than that which should be standard (66 %) for the beginning of the clinical symptomatics of Parkinson's disease according to theoretic examinations by *Carlsson* (1974). In agreement with data presented by *Carlsson* (1975), the control group shows a decrease in dopamine content in the human caudate nucleus depending on age, that is 12.8 % on the average/decade. At present, it is completely uncertain whether the progressive degeneration of the striatal dopaminergic neurons in Parkinson's disease is linear, or whether there is no linearity. In assuming a linearity, the decrease in tyrosine hydroxylase activity shown by *McGeer, McGeer* and *Wada* (1971) corresponds well with the findings in figure 1. This would mean that idiopathic Parkinson, caused by genetic false programing or other triggers, does not conform to the decrease in tyrosine hydroxylase activity which is dependent on age, and the steep decrease in tyrosine hydroxylase activity is somewhat retained between the 5th and 20th year of life in Parkinson's disease. Assumption of a linearity of this process between the 30th and 50th year of life as well, which could only be shown for the end stage of the illness by the results in figure 1, would be a decisive indication for the etiology of this neurological disorder. The assumption of linearity of nigrostriatal degeneration of dopaminergic neurons also before the time period studied would mean that Parkinson's disease begins about 20 to 30 years before the appearance of the characteristic symptoms (akinesia, tremor, and rigor).

Figure 1 also shows the increasing progression of degeneration of nigrostriatal neurons in the course of Parkinson's disease. In contrast, the dopamine content in the nucleus caudatus of the control group only decreases continuously at 12.8 % / decade. Furthermore, the depressive phases which frequently appear in the pre-clinical course

of Parkinson's disease (*Birkmayer* and *Neumayer*, 1972) lead to the conclusion that the dopamine deficit in the caudate nucleus is already manifest during this time. This hypothesis is supported by the dopamine deficit in the caudate nucleus shown by *Birkmayer* and *Riederer* (1975) and *Neumayer, Riederer, Danielczyk* and *Seemann* (1975), as well as by putamen from deceased retarded depressives (also see Fig. 1).

Time between Death and Necropsy (8)

The time between death and necropsy is of decisive importance. The activities of the synthetic and of the destructive enzymes have been shown to differ in various areas of post mortem animal brains (*Bogdanski, Weissbach* and *Udenfriend*, 1957). As different authors demonstrated (*Ganrot, Rosengren* and *Gottfries*, 1962; *Gottfries, Orelan, Wiberg* and *Winblad*, 1975; *Birkmayer, Riederer, Youdim* and *Linauer*, 1975), monoamineoxydase is stable during the first hours after death. Extensive studies by *McGeer, McGeer* and *Wada* (1971) clearly showed a dependence of tyrosine-hydroxylase activity on storage time. Post mortem alterations in the activities of tyrosine-hydroxylase, dopadecarboxylase, and dopamine-β-hydroxylase were examined in human brain areas (*Black* and *Geen*, 1975). It could be shown that tyrosine-hydroxylase has a half time of about 2—4 hours; Dopadecarboxylase activity decay was about 20 % after 5 hours, and dropped rapidly after this time. Dopamine-β-hydroxylase was remarkably stable after death. *Carlsson, Lindqvist* and *Kehr* (1974) showed that dopamine degradates to methoxytyramine dependent on time and temperature of post mortem storage. *Perry, Berry, Hansen, Diamond* and *Mok* (1971) reported on postmortem changes in the free amino acid pool of autopsied human brains. As it is usual to cool the bodies to 4 °C between death and autopsy, it therefore seems possible that both types of enzymes, monoamine synthetisizing and monoamine destructive, work with different kinetics during this time. To exclude this possibility, it is important to deep freeze the material (—70 °C) as soon as possible after death. Enzyme activity and the content of biogenic amines are different in various areas of the human brain. Therefore, it is possible that post mortem alterations in enzyme activity or biogenic amine concentration differ from area to area in the brain. Table 3 a—c do not show any significant alteration in dopamine, noradrenaline, and 5-hydroxytryptamine values between 3—5 hours and 16—20 hours post mortem.

Dissection of Specimen (9)

The technique of dissection should be performed according to standardized methods always by the same pathologist.

In the study presented, the severous cerebral nuclei were always withdrawn by one examiner (pathologist). During removal of the brain from the skull, care must be taken that the brain stem and therewith the mesencephalon do not tear. Should the brain stem be torn off, preparation of the large mesencephalic nuclei, the substantia nigra, and the n. ruber can only be accomplished with difficulty. In this study, 9 nuclei of the brain or brain regions were studied, that is, the n. caudatus, putamen, pallidum, amygdaloid nucleus, g. cinguli, oral part of the substantia nigra, caudal part of the substantia nigra and raphe region of the rhombencephalon. A skilled person has no particular difficulty in dissecting and removing these regions of the brain. The amygdaloid nucleus is easiest to remove in a frontal section from the middle of the uncus gyri hippocampi, whereby the border between the hippocampus and cortex must be carefully dissected. For the putamen and pallidum, frontal incisions towards the base of the brain are useful, because in the somewhat inclined brain sections, both nuclei can be separated and removed without difficulty by cutting with a scissor on the border area towards each other and away from the white substance. The oral and caudal part of the substantia nigra are first separated by an incision in the middle of the crura cerebri and then each part is prepared separately. Both halves of the separated n. ruber can be enucleated as hemispheric segments by the same incision. Starting from the rhombencephalon, a strip of tissue averaging 3 mm is cut out from the pons and medulla oblongata along the raphe, which, however, contains white and gray substance of the reticular formation mixed indiscriminantly. Isolation of nuclei with the aid of a dissecting microscope was not undertaken by us. The frontal section of the gyrus cinguli is removed immediately next to the corpus callosum.

Period of Storage; Temperature of Storage (10)

The extent of the alteration in biogenic amines also depends on the temperature and the conditions of storage of tissues. Although *Lloyd, Farley, Deck* and *Hornykiewicz* (1974) and *McLean, Nicholson, Pare* and *Stacey* (1965) noted no significant changes in 5-hydroxytryptamine concentration between 6 and 33 hours and

Table 3 a. *The dependence of dopamine content of various regions of the human brain (controls) on the interval between death and autopsy*

Brain area	Dopamine (ng/g fresh weight)*				
	3—5 hours (n = 4)	5—8 hours (n = 5)	8—12 hours (n = 5)	12—16 hours (n = 5)	16—20 hours (n = 4)
Caudate Nucleus	3721 ± 315	3675 ± 295	3700 ± 251	3630 ± 234	3600 ± 265
Putamen	4220 ± 365	4150 ± 321	4180 ± 304	4050 ± 285	4005 ± 220
Pallidum	870 ± 95	845 ± 45	880 ± 52	832 ± 65	825 ± 42
Substantia Nigra oral part	520 ± 50	505 ± 35	532 ± 38	497 ± 39	487 ± 41
Substantia Nigra caudal part	630 ± 55	652 ± 48	627 ± 43	617 ± 50	605 ± 40
N. Amygdalae	175 ± 14	155 ± 13	175 ± 11	160 ± 10	156 ± 9
Gyrus Cinguli**	25— 40	25— 38	25— 41	25— 35	25— 31
Raphe	165 ± 15	142 ± 14	146 ± 13	150 ± 11	143 ± 12
Red Nucleus	810 ± 75	835 ± 62	822 ± 49	792 ± 53	758 ± 41

* Dopamine has been measured according *Anton* and *Sayre* (1964); calculations by internal standards; means ± s.d.; n = number of cases.

** Just above baseline value of the method (20 ng/g); two to three Gyrus cinguli were pooled for determination.

Storage in sealed polyethylen plastic bags at —70 °C until analysis, which has been performed within on month after dissection of specimen.

Table 3 b. *The dependence of the noradrenaline content of various regions of the human brain (controls) on the interval between death and autopsy*

Brain area	Noradrenaline (ng/g fresh weight)*				
	3—5 hours (n = 5)	5—8 hours (n = 4)	8—12 hours (n = 6)	12—16 hours (n = 4)	16—20 hours (n = 3)
Caudate Nucleus	90 ± 11	85 ± 12	92 ± 11	81 ± 10	75 ± 9
Putamen	60 ± 8	64 ± 7	70 ± 7	63 ± 8	55 ± 6
Pallidum	40 ± 7	42 ± 4	35 ± 5	35 ± 4	30 ± 4
Substantia Nigra oral part	105 ± 16	95 ± 11	101 ± 9	91 ± 9	90 ± 10
Substantia Nigra caudal part	98 ± 13	100 ± 9	95 ± 10	88 ± 9	85 ± 8
N. Amygdalae	65 ± 7	60 ± 6	67 ± 5	62 ± 5	59 ± 6
Gyrus Cinguli	50 ± 7	52 ± 4	50 ± 4	47 ± 5	44 ± 5
Raphe**	30—39	30—41	35—43	29—34	25—32
Red Nucleus	260 ± 22	270 ± 19	255 ± 20	250 ± 18	242 ± 19

* Noradrenaline has been measured according to *Bertler, Carlsson* and *Rosengren* (1958); calculations by internal standards; means ± s.d.; n = number of cases.

** Just above baseline value of the method; two to three raphe preparations were pooled for determination.

Storage in sealed polyethylen plastic bags at —70 °C until analysis, which has been performed within one month after dissection of specimen.

Table 3 c. *The dependence of 5-hydroxy-tryptamine content of various regions of the human brain (controls) on the interval between death and autopsy*

Brain area	5-Hydroxy-tryptamine (ng/g fresh weight)*				
	3—5 hours (n = 5)	5—8 hours (n = 7)	8—12 hours (n = 6)	12—16 hours (n = 5)	16—20 hours (n = 4)
Caudate Nucleus	292 ± 25	304 ± 17	286 ± 19	279 ± 21	274 ± 18
Putamen	270 ± 22	261 ± 13	267 ± 16	259 ± 18	254 ± 17
Pallidum	375 ± 30	384 ± 21	371 ± 23	351 ± 28	357 ± 24
Substantia Nigra oral part	590 ± 43	617 ± 39	623 ± 41	587 ± 37	564 ± 42
Substantia Nigra caudal part	575 ± 48	613 ± 52	598 ± 27	580 ± 31	571 ± 43
N. Amygdalae	285 ± 24	269 ± 19	302 ± 17	276 ± 20	291 ± 22
Gyrus Cinguli	65 ± 11	74 ± 7	71 ± 8	61 ± 6	57 ± 4
Raphe	530 ± 40	555 ± 42	548 ± 37	519 ± 29	507 ± 31
Red Nucleus	595 ± 44	572 ± 40	619 ± 34	562 ± 37	572 ± 42

* 5-Hydroxy-tryptamine has been measured according to *Ashcroft* and *Sharman* (1962), modified by *Quay* (1963); calculations by internal standards; means ± s.d.; n = number of cases.

Storage in sealed polyethylen plastic bags at —70 °C until analysis, which has been performed within one month after dissection of specimen.

Table 4. *The dependence of the dopamine, noradrenaline and 5-hydroxy-tryptamine content on the storagetime at —70 °C**

	Dopamine (ng/g fresh weight) n = 5				Noradrenaline (ng/g fresh weight) n = 7				5-Hydroxy-tryptamine (ng/g fresh weight) n = 7			
Brain area	1 day	1 week	4 weeks	12 weeks	1 day	1 week	4 weeks	12 weeks	1 day	1 week	4 weeks	12 weeks
Caudate N.	3560 ± 280	3530 ± 295	3575 ± 300	3520 ± 275	92 ± 12	95 ± 10	90 ± 11	85 ± 10	280 ± 17	295 ± 20	285 ± 14	260 ± 22
Subst. Nigra	585 ± 45	590 ± 50	550 ± 45	570 ± 52	103 ± 14	105 ± 13	95 ± 11	90 ± 10	560 ± 43	550 ± 35	575 ± 40	555 ± 30
N. Amygdalae	175 ± 15	180 ± 17	170 ± 15	155 ± 14	65 ± 8	65 ± 9	70 ± 8	63 ± 7	275 ± 20	260 ± 21	270 ± 18	255 ± 21
Red Nucleus	810 ± 70	815 ± 65	790 ± 55	798 ± 64	220 ± 22	215 ± 20	205 ± 18	213 ± 20	610 ± 50	595 ± 45	600 ± 35	590 ± 41

* Analysis of biogenic amines have been performed by dividing brain areas of controls into 4 tissue preparations of about equal fresh weight. After storage at —70 °C biochemical analysis then were carried out later: 1 day, 1 week, 4 weeks and 12 weeks respectively.

Methods of assay: Dopamine according to *Anton* and *Sayre* (1964); Noradrenaline according to *Bertler, Carlsson* and *Rosengren* (1958); 5-Hydroxy-tryptamine according to *Ashcroft* (1962); modification according to *Quay* (1963).

Age of controls: 72 ± 3 years; Values: means ± s.d.: calculations by internal standards; n = number of cases.

12—92 hours respectively, *Joyce* (1962) found 5-hydroxytryptamine concentrations in the frontal cortex of man decreased up to 18.5 % during 48 hours when compared to zero-time. Storage in sealed plastic bags at —17 °C resulted in a significant decrease of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentration between 1 and 26 days storage. 5-Hydroxytryptamine was reduced to 34 % and 5-hydroxyindoleacetic acid to 44 % during this time of storage (*Dowson*, 1969). 5-Hydroxytryptamine, dopamine, and noradrenaline, as well as precursors and metabolites are stable for months if stored at —70 °C (Table 4). The above mentioned discrepancies also seem to be influenced not only by the storage temperature but also by the interval between death and necropsy. Most of all brains studies were carried out with few specimens and very discrepant time intervals between death and autopsy.

Chronobiological Rhythm of Substances (11)

Part of this subject was already discussed under (7) with special regard to Parkinson's disease. Figure 2 clearly shows the circadian rhythmic distribution of the times of death of a control group as well as of the deceased Parkinson's victims. Noticeable is that a clear increase of cases of death during the night hours can be observed in parkinsonians.

In the group of patients with Parkinson's disease (n = 68), 43 (= 63.3 %) patients died during the night hours (8 p.m.—8 a.m.), and 25 (= 36.7 %) between 8 a.m. and 8 p.m.

In contrast, 66 (= 38.4 %) patients with other neurological illnesses (n = 172) died during the night (8 p.m.—8 a.m.), and 106 (= 61.6 %) during the daytime (8 a.m.—8 p.m.) (Fig. 2).

Reinberg (1974) cited that cardiac infarcts cumulate mainly in late afternoon and early evening hours. In contrast, infectious diseases lead to death significantly more often in the early morning hours. Statistical analysis of all cases of death in the circadian course produced a maximum during the daytime in his material. The results in Fig. 2 A confirm this trend. The fact that Parkinson's victims tend to die during the night leads to the conclusion that there is a basic connection with the degeneration of the dopaminergic nigrostriatal nerve paths, which appears first in this disease. Furthermore, Parkinson's disease is also characterized by a deficit in noradrenaline (n. caudatus —54 %, substantia nigra, oral —87 %, substantia nigra, caudal —59 %, amygdaloid nucleus —60 %, gyrus cinguli —52 %,

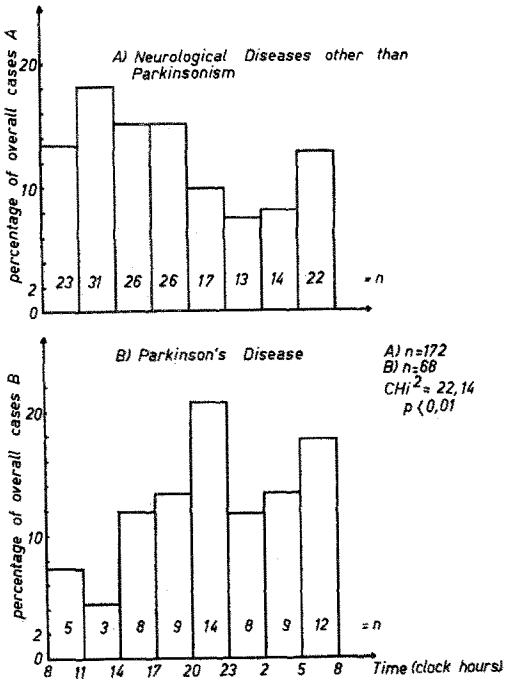

Fig. 2. Circadian rhythm in human mortality.

A. Patients with different neurological diseases (number of cases = 172): Huntington's chorea (4), endogene depression (6), multiple sclerosis (45), amyotrophic lateral sclerosis (7), syringomyelia (6), arteriosclerosis universalis (19), encephalomalacia (10), encephalitis (3), apoplexy (14), myelopathy (8), spastic hemiparesis (16), org. psycho-syndrome (18), other neurological diseases (16; single cases).

B. Patients suffering from Parkinson's disease: idiopathic Parkinson (58), post-encephalitic Parkinson (5), arteriosclerotic Parkinson (5).

Number of patients in parentheses: N = total patients in the group, time interval of morbidity study: 1. 1. 1974—31. 12. 1975. Age of group A: 69 ± 4 years, age of group B: 75 ± 3 years.

Statistical analysis correlating A and B:

Chi^2-test: $CC_{corr} = 22.14$

$P < 0.01$

nucleus ruber —67 %, in comparison to mean values in control persons; *Birkmayer, Danielczyk, Neumayer* and *Riederer*, 1974). A correlation between reduced sympathetic activity, predominance of parasympathetic function during the night, and the statistically demonstrable accumulation of deaths of parkinsonians during the night, seems to be possible.

Further investigations are certainly necessary to clarify this question. However, the fact that the parkinsonians in our clinic are treated with Madopar almost without exception, and the last daily therapy is administered between 5 and 6 p.m. on the average, could be of decisive significance. That means that the dopaminergic and noradrenergic deficit is eliminated medicinally during the daytime. In contrast, during the nighttime—especially during the second half—it is certainly manifest as L-dopa-plasma level tests, which were performed between 7 and 8 a.m., produced values which hardly differ from the blank test control values. Therefore, it does not seem impossible that the primary disturbance demonstrable in the catecholamine metabolism in Parkinson's disease leads to alterations in the susceptibility or chronosusceptibility (*Reinberg*, 1974) in regard to the point of time of death.

Many substances out of the catecholamine or indoleamine metabolism such as phenylalanine, tyrosine, noradrenaline, homovanillic acid, vanillylmandelic acid, methoxyhydroxyphenylglycol, serotonin (for references see *Birkmayer, Riederer, Youdim* and *Linauer*, 1975), free tryptophan (*Tagliamonte, Gessa, Biggio, Vargiu* and *Gessa*, 1974), as well as the enzymes monoamine oxydase (*Birkmayer, Riederer, Youdim* and *Linauer*, 1975), and dopamine-β-hydroxylase (*Okada, Fujity, Ohta, Kato, Ikuta* and *Nagatsu*, 1974) show circadian rhythm (survey by *Pengelley*, 1974; *Riederer, Birkmayer, Neumayer, Ambrozi* and *Linauer*, 1974; *Aschoff, Ceresa* and *Halberg*, 1974). Circadian rhythms of the tyrosine transaminase activity and tryptophan pyrrolase activity in the liver were described by *Wurtman* (1974).

Circadian rhythms are also established for acetylcholine in the brain of the rat (*Saito, Yamashita, Yamazati, Okada, Satomi* and *Fujieda*, 1975), as well as for serotonin, and melatonin in pineal glands (*Quay*, 1964).

The majority of these substances show circadian rhythm; some alter their concentration or activity in the course of weeks, months, or years. Up to now, only a few investigations of this type were performed on the human brain, however, the knowledge acquired up to the present indicate that rhythmic alterations of this type can also appear in the human brain.

Experiments on animals on the circadian course of tryptophan, 5-hydroxytryptamine, and 5-hydroxyindoleacetic acid (*Wurtman* and *Fernstrom*, 1974) as well as the monoamine oxydase activity in various nuclei in the post mortem human brain (*Birkmayer, Riederer, Youdim* and *Linauer*, 1975) show that clarification of this question is important regarding the result's significance.

Methods of Assay (12)

The analytic chemical methods should be adjusted to the concentration of the substances to be demonstrated, as the overall error of the method in a less sensitive procedure can often be considered smaller than in a hypersensible procedure of analysis of the expected concentration region. Particular errors in sample taking can thus be avoided.

In connection with this, it should be mentioned that different regions of the brain show slight variations in regard to specific weight (*Blinkow* and *Glezer*, 1968). If one determines the fluid content of samples from various areas of the brain, clear differences are also seen (Table 5). Thus, for exact comparison of the measurement results of different areas of the brain, it seems more advantageous to base the analysis value on 1 g dry weight. A further advantage of this base amount is the equalization of variations in water content which are possible through longer periods of storage.

Table 5. *Content of fugacious substances in various areas of the human brain*

Brain area	% Fugacious Portions mean ± s.d.
Caudate Nucleus	80.0 ± 4.04 (6)
Putamen	76.8 ± 6.3 (6)
Pallidum	72.0 ± 5.56 (6)
Substantia Nigra oral part	69.2 ± 10.9 (6)
Substantia Nigra caudal part	69.6 ± 2.61 (6)
Amygdaloid Nucleus	74.5 ± 5.32 (6)
Gyrus Cinguli	79.2 ± 3.49 (6)
Raphe	68.8 ± 2.40 (6)
Nucleus Ruber	65.8 ± 9.36 (6)

Method: Freeze-dried for 24 hours in a high vacuum (10^{-2} torr; —50 °C). Under these conditions, mainly water and lighter fugacious substances such as certain lipids and lipoids were evaporated.

0.2—0.3 g respectively were weighed in fresh weight. The number of estimations is given in parentheses. The values should be understood as mean value ± standard deviation.

In regard to gas chromatography and the fluorometry as the most frequently used analytical methods at present, we would like to point to the comprehensive and extensive studies by *Kaiser* (1964) (gas chromatography) and *Udenfriend* (1962, 1969) (fluorometry). How-

ever, it should be mentioned that international ring studies on the same sample with the analysis mentioned produced differences of ± 10 % in the measurement results. Therefore, from the methodic standpoint as well, standardization of the methods is absolutely necessary.

Effects of the nutritional condition, diagnostic measures, medicamentous side-effects, as well as the influence of medications on chemical measurement results were treated in depth by *Lang, Rick* and *Roka* (1973), and should be taken into consideration in the evaluation of measurement results.

Homogeneity (13)

The homogeneity of the control group compared to the patient group should include all mentioned parameters.

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