Distribution of $5HT_{2A}$ receptors in the human brain: comparison of data in vivo and post mortem

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Verteilung der $5HT_{2A}$-Rezeptoren im menschlichen Gehirn: Datenvergleich in vivo und post mortem

Keywords
Serotonin receptor, 5-HT_{2A}, PET, autoradiography

Summary
Aim: The study presented here firstly compars the distribution of the binding potential of the serotonin-$5HT_{2A}$ receptor as measured in vivo with data of receptor density taken from literature. Secondly, the sensitivity of the method to detect gradual differences in receptor densities is evaluated. Methods: Positron emission tomography (PET) studies were carried out in 6 healthy volunteers using the selective serotonin-$5HT_{2A}$ ligand ^{18}F-altanserin. The binding potential was quantified in 12 regions using Logan's graphical method and the equilibrium method. These data were compared to the distribution of receptor density as taken from literature. Results: The binding data in vivo correlated to autoradiography data (post mortem) with r = 0.83 (Pearson regression coefficient; p <0.0001). A difference in the receptor density between two regions could be detected with p <0.05 when it amounted at least to 18%. Conclusion: This study demonstrates a good agreement between in vivo data obtained with ^{18}F-altanserin and PET in healthy volunteers and the true autoradiographically determined distribution of $5HT_{2A}$ receptors in human brains. The in vivo method seems to be sensitive enough to detect changes in receptor density of more than 18%.

Nuklearmedizin 2002; 41: 197–201

Schlüsselwörter
Serotoninrezeptor, $5HT_{2A}$, PET, Autoradiographie

Zusammenfassung
Ziel: Vergleich der Verteilung der in vivo gemessenen Bindungspotentiale der Serotonin-$5HT_{2A}$-Rezeptoren im menschlichen Gehirn mit der aus der Literatur bekannten Verteilung und Bestimmung der Sensitivität der Methode für den Nachweis kleiner Veränderungen in der Rezeptordichte. Methoden: Sechs gesunde Probanden wurden mit dem selektiven $5HT_{2A}$-Rezeptorliganden ^{18}F-Altanserin und Positronenemissionstomographie (PET) untersucht. Die Quantifizierung des Bindungspotentials dieses Tracers erfolgte in 12 Hirnregionen sowohl nach Logans graphischer Methode als auch nach der Equilibriummethode. Ergebnisse: Die in vivo gemessenen Bindungspotentiale und die autoradiographisch ermittelten Literaturdaten korrelierten in den 12 Regionen mit r = 0,83 (Pearson-Korrelationskoeffizient; p <0,0001). Unterschiede in der Rezeptordichte zweier Regionen wurden signifikant (p <0,05), wenn sie mehr als ca. 18% betrugen. Schlussfolgerung: Diese Studie demonstriert die Übereinstimmung von in vivo mit ^{18}F-Altanserin und PET gemessenen Daten von gesunden Personen mit der autoradiographisch bestimmten Verteilung zerebraler $5HT_{2A}$-Rezeptoren. Unterschiede der Rezeptordichte von mehr als 18% können mit der In-vivo-Methode detektiert werden.

The serotonergic system of the brain modulates a considerable spectrum of human behaviour including attention, learning, memory, emotion, nutrition, and sexuality (10). One of the most important receptors is the $5HT_{2A}$ site, which is present in high concentrations in the entire cortex and in subcortical structures. The $5HT_{2A}$ receptor is involved in the pathogenesis of psychiatric diseases, e. g. anxiety disorder and depression (8).

In the past decades, the distribution of the $5HT_{2A}$ receptors was examined post mortem with quantitative receptor autoradiography. Pazos et al. (11) mapped the $5HT_{2A}$ receptor distribution post mortem using ^{3}H-ketanserin autoradiography. Recently, nuclear medicine imaging methods have increasingly become available for in vivo studies of $5HT_{2A}$ receptor distribution. As first ligand ^{11}C-methylspiperone was applied (3) binding predominantly to the striatal dopamine D_2 receptors, but also with considerable affinity to cortical $5HT_{2A}$ receptors. Thus, cortical methylspiperone accumulation was regarded as indicative for $5HT_{2A}$ receptor binding. A ligand with similar pharmacological properties is ^{18}F-fluoroethylspiperone (16). Selective $5HT_{2A}$ receptor ligands without a remarkable affinity for dopaminergic sites are ^{18}F-setoperone (1) and ^{18}F-altanserin (7).

A prerequisite for the application of a newly developed radiotracer is the determination of its sensitivity for detecting changes within the respective biochemical system (5). Thus, a radioligand capable to trace a change in receptor density of 10% is preferable to one requiring a difference of 50% in order to yield B_{max} values significantly differing from the normal distribution.

In the study presented here, we firstly aimed at comparing the distribution of ^{18}F-altanserin binding to the $5HT_{2A}$ receptors as measured in the living human brain to the autoradiographically obtained ^{3}H-ketanserin binding data (11). Secondly, the sensitivity of altanserin for gradual differences in receptor densities was evaluated in cortex and subcortical structures.

Patients and methods

Six healthy volunteers (4 women, 2 men; mean age: 35 years; range: 31-43 years) without known neurological or psychiatric disorders were investigated. Informed consent was obtained from each of them prior to the investigation. The study protocol was approved by the federal authorities

Received: November 29, 2001; in revised form: April 4, 2002

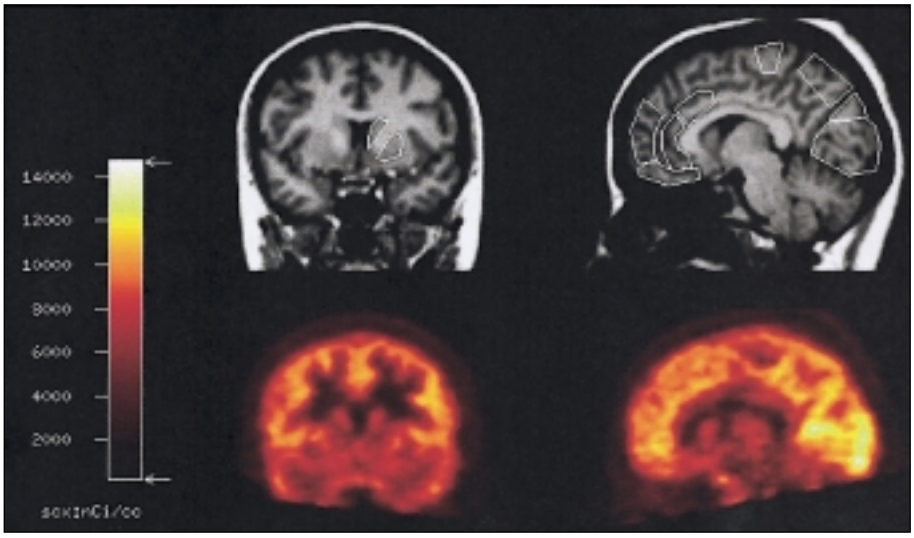

Fig. 1 Coronal and paramedian sagittal slice of a T_1-weighted MRI image with delineated ROI-templates (upper row) and the corresponding ^{18}F-altanserin PET images (bottom row)

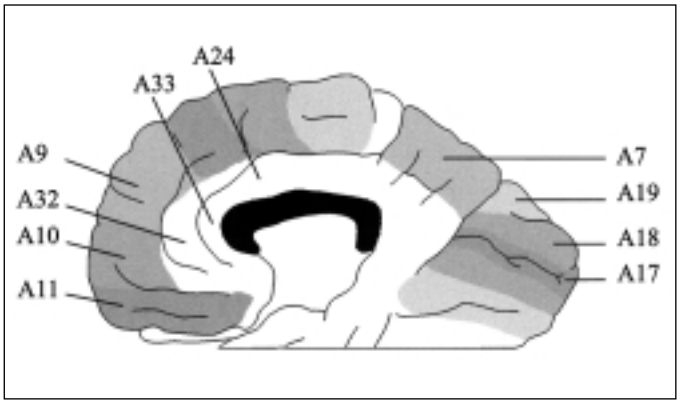

Fig. 2 Schematic view of a brain with delineated Brodman's areas

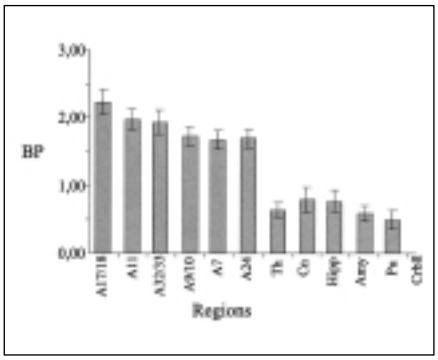

Fig. 3 ^{18}F-altanserin binding potential (BP) in cortical and subcortical regions (Th: thalamus, Cn: caudate nucleus, Hipp: hippocampus, Amy: amygdala, Pu: putamen, Crbll: cerebellum). The bars represent the mean and the vertical lines the standard deviation of six persons

in radiation protection and by the ethics committee of the Heinrich-Heine-Universität Düsseldorf.

Details of the PET examinations are published elsewhere (6). Briefly, a GE PC4096+ camera was used. Altanserin was labelled with ^{18}F in analogy to the procedure described (7) and injected in a dose of 3.7 MBq per kg body weight. The cerebral radioactivity concentration was measured dynamically for 90 minutes. At each time point, 15 transversal slices starting from 1 cm above the meatoorbital line were reconstructed with a slice thickness of 6.4 mm each using measured attenuation correction. The spatial resolution of the reconstructed images amounted to 7 mm. In order to obtain metabolite corrected input curves, arterialized venous blood samples were drawn from the arm contralateral to the injection side.

After co-registration of the PET data with individual MRI scans (Siemens Magnetom Vision, Erlangen; 1.5 tesla), Brodman areas A7, A9/10, A11, A17/18, A19, A24 as well as frontal cingulate, insula, and temporal gyri were defined as cortical ROIs on coronal and sagittal slices; putamen, caudate nuclei, amygdala, and thalamus were defined as subcortical ROIs. Regions A9/10 and A17/18 were not further differentiated. The frontal cingulate consisted of A32/A33 and A25 (4). Cerebellum, subcortical nuclei, insula, temporal, and – partly – parietal lobe were delineated on coronal, all further areas on sagittal slices. Only those areas examined by Pazos et al. (11) were included in the investigation (Fig. 1 and 2).

The decay-corrected radioactivity concentrations in the co-registered PET-ROIs were determined and plotted against time. Because of the small sizes of the subcortical nuclei, these data underwent a partial volume correction. For this purpose, the size of the structure was determined as the arithmetic mean of its diameters. Comparison to the theoretic recovery curve yielded the respective correction factor. The resulting time-activity curves as well as the respective input curves were used for the graphical determination of the binding potential (BP), which equals the ratio of receptor density to affinity $B_{max}/_{KD}$ (9).

The BPs obtained in 12 brain regions (Fig. 3) were compared to the respective B_{max} values reported in literature (11). In this autoradiographic study cortical B_{max} was not determined for the cortex as a whole. Instead, separate B_{max} values were obtained for the single anatomical layers. Given the limited spatial resolution, PET data in fact are averaged over all cortical layers. Therefore, for comparison of autoradiography and PET data the literature values for B_{max} were averaged, too. Considering that the single layers not only present with different B_{max} values (range: 110-370 fmol/mg), but also vary within one order of magnitude as to their thickness

Tab. 1 Binding potential (BP) of ^{18}F-altanserin determined with Logan's graphical analysis in twelve brain regions (means of six persons). B_{max}°: $5HT_{2A}$ receptor density after arithmetic averaging of the data for each cortical layer; B_{max}´: thickness-weighted mean of $5HT_{2A}$ receptor density according to (11); receptor density in fmol/mg protein; data after partial volume correction in brackets

Region	BP	B_{max}°	B_{max}´
A17/18	2.24	251	246
A32/33	1.94	227	168
A24	1.62	174	123
A11	1.98	268	246
A7	1.68	211	214
A9/10	1.73	195	203
Hippocampus	0.57 (0.77)	135	135
Caudate nucleus	0.57 (0.79)	154	154
Putamen	0.47 (0.50)	103	103
Amygdala	0.53 (0.59)	140	140
Thalamus	0.64 (0.64)	66	66
Cerebellum	0.00	37	37

Tab. 2 Percentual differences in B_{max}´ between the cortical regions. The data are marked when the corresponding binding potential as measured with ^{18}F-altanserin differed significantly ($p < 0.05$; Student's paired t-test)

A17/18	A32/33	A24	A11	A7	A9/10	
X	31.3	50.0	0.0	13.0	17.5	A17/18
	X	27.2	31.3	21.0	16.8	A32/33
		X	50.0	42.5	39.4	A24
			X	13.0	17.5	A11
				X	5.1	A7
					X	A9/10

(range: 0.1-1.3 mm; Fig. 4) two methods of averaging are conceivable. Firstly, B_{max} values in the cortical layers of the respective region were added and divided by the number of layers. This yields an arithmetic mean B_{max}° ignoring the difference in layer thickness. Secondly, B_{max} in the cortical layers were multiplied with the thickness of the layer, values of all layers were added and divided by the total thickness of the cortex in the respective region (Fig. 5).

$$B_{max}' = \frac{B_{max\,(V1)}D_{(V1)} + B_{max\,(V2)}D_{(V2)} + \ldots + B_{max\,(V6)}D_{(V6)}}{D_{(V1)} + D_{(V2)} + \ldots + D_{(V6)}}$$

$B_{max(V1\ldots6)}$ corresponds to the receptor concentration, $D_{(V1\ldots6)}$ to the thickness of the respective cortical layer (15). We considered all cortical layers, when measurable receptor concentrations were known from literature. The latter method yields a thickness-weighted mean of receptor density (B_{max}´).

Arithmetic as well as thickness-weighted mean of autoradiographically obtained $5HT_{2A}$ receptor densities were correlated with in vivo data (Pearson's linear regression). The sensitivity of the measurement of BP with ^{18}F-altanserin and PET in vivo to detect differences in $5HT_{2A}$ receptor density within cortical or subcortical structures was determined with an analysis of variance (ANOVA) for repeated measures. Additionally, the differences between the single areas were assessed with Student's t-test.

Results

The distribution of the BP values measured with PET, B_{max}° and B_{max}´ are displayed in Fig. 3 and Table 1. The highest BP values were found in primary visual cortex (A17/18) and gyrus rectus (A11). BP was lower in the subcortical nuclei. In Logan's graphical analysis the cerebellar BP is fixed to 0. The correlation between BP and the autoradiographically obtained B_{max}° and B_{max}´ values yielded an r of 0.91 and 0.83, respectively ($p < 0.0001$, Fig. 6).

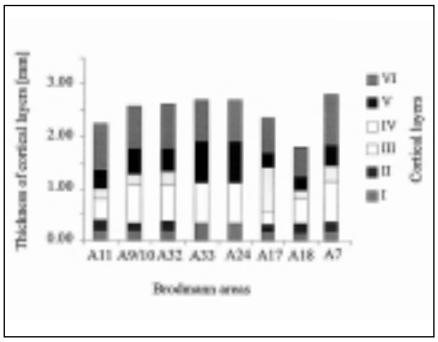

Fig. 4 Thickness of the cortical layers I-VI in the examined regions according to (15)

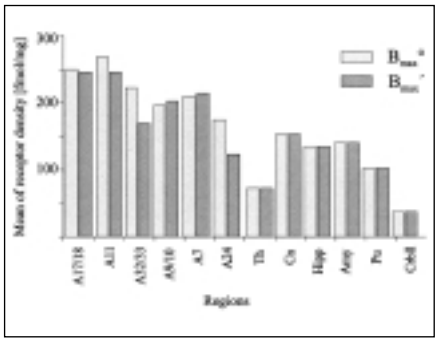

Fig. 5 Receptor density in different brain regions (Th: thalamus, Cn: caudate nucleus, Hipp: hippocampus, Amy: amygdala, Pu: putamen, CrbII: cerebellum) according to (11). The mean of the cortical layers was determined arithmetically (B_{max}°) and in a thickness-weighted manner (B_{max}´). This is relevant only for cortical structures

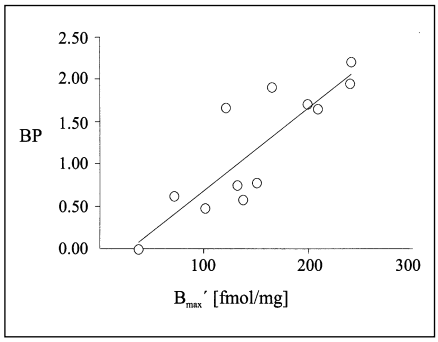

Fig. 6 Plot of binding potential (BP) against receptor density (B_{max}´); linear regression with r = 0.83 ($p < 0.0001$)

There were significant interregional differences of BP (ANOVA, n = 6, α = 0.05, p <0.0003, power = 0.9). Explorative t-tests revealed that cortical regions, whose autoradiographically obtained B_{max} values differed by 18%, generally also displayed significant differences in vivo (Tab. 2). In the five subcortical regions, the differences of the measured BPs were not significant (ANOVA, n = 6, α = 0.05, p = 0.24, power = 0.14) although literature data for B_{max}° and B_{max}´ differed up to 32%. Only after partial volume correction significant interregional differences between the subcortical structures were obtained (p <0.0001, power = 0.9). Subsequent t-tests showed that regional differences become detectable in vivo, when the autoradiographically obtained receptor density between two structures varies by about 30%.

Discussion

Our results show that the binding potential of ^{18}F-altanserin in different cortical and subcortical regions measured in vivo and the $5HT_{2A}$ receptor density measured post mortem with quantitative receptor autoradiography exhibit a strong positive correlation. Hence, ^{18}F-altanserin-PET in conjunction with Logan's graphical analysis provides a suitable tool for the examination of $5HT_{2A}$ receptors in the human brain. Furthermore, our data show that this method is sufficiently sensitive to detect interregional cortical differences, if $5HT_{2A}$ receptor density varies by about 18%. The analysis of subcortical structures, which display considerably lower $5HT_{2A}$ receptor densities, requires additional anatomical information. When partial volume correction is performed, differences between these regions become apparent, too. However, changes of $5HT_{2A}$ receptor density exceeding those in cortical structures seem to be necessary in order to obtain significant results.

Our data correspond to the results of other scientific groups (2, 12, 13). In their previous work the arithmetic mean B_{max}° of $5HT_{2A}$ receptor density in the cortical layers was used for comparison with BP. We determined a correlation coefficient of 0.91. In the studies cited, results were in the same order of magnitude (r = 0.97; p <0.001). Additionally, the variations of cortical layer thickness and $5HT_{2A}$ receptor concentrations were taken into account in our study, yielding a thickness-weighted mean of the $5HT_{2A}$ receptor density (B_{max}´), which correlated equally well with the measured BP (r = 0.83; Fig. 6). This especially applies to the cortex with its high density of $5HT_{2A}$ receptors. However, subcortical areas require the performance of partial volume correction. Upon correction, correlations of 0.53 and 0.71, respectively, were obtained. Thus, the present investigation shows that the results of former studies remain valid even if the mean cortical receptor density was determined more elaborately.

The correlation coefficient of the autoradiographical data and the binding potential as measured with PET approaches 0.9 in this study as well as in previously published papers. Better correlations between these two methods cannot be expected, since PET and autoradiographical data are subject to multiple sources of error. The PET measurements base on ROIs, which are drawn in cortical and subcortical structures. Because these ROIs contain not only grey but also white matter and cerebrospinal fluid, a decrease of the real binding potential is measured. Even if these compartments could be omitted completely, they still would be of influence because of the partial volume effect. In contrast, autoradiographical data are subject to variations due to several merely logistic circumstances, e. g. the delay of tissue preparation after death, the cause of death. Even more variation is added to PET and autoradiography because the individual difference of persons submitted to both methods with respect to standard reference values (15) is unknown.

The second aim of our study was to estimate the sensitivity of the ^{18}F-altanserin-PET method for detection of changes of the $5HT_{2A}$ receptor density. This sensitivity depends on the magnitude of B_{max}´ because this value marks the signal-to-noise relationship. In the cortex, B_{max}´ lies between 246 and 123 fmol/mg. This variability is reflected by the significant interregional differences of BP as evidenced by the results of ANOVA. Moreover, more direct comparisons between the cortical regions display significant differences, too. However, this must be considered cautiously because most significant differences would not withstand Bonferroni's correction for multiple testing. Circumstances in the subcortical areas differ from those in cortical regions: B_{max} is lower (66-154 fmol/mg) so that an unfavourable signal-to-noise ratio may be inferred. Actually, ANOVA fails to display any substantial regional differences of BP. This is caused by the relatively large structures (e.g. thalamus and putamen) showing a low $5HT_{2A}$ receptor density, whereas relatively small structures like the caudate nucleus exhibit high $5HT_{2A}$ receptor concentrations. As a consequence, differences between subcortical regions fail to become apparent without partial volume correction.

The ^{18}F-altanserin PET measurements were carried out as single bolus studies and Logan's graphical method was used for quantification of the binding potential. It turned out that this approach yields data in good agreement with the results of a kinetic analysis using a compartment model (2). The determination of BP under so-called true equilibrium conditions using a constant infusion paradigm (14) requires an elaborate experimental setup. Therefore, with routine clinical conditions it is unlikely to become feasible.

Acknowledgements
This study was supported by a grant from the Pinguin-Stiftung. We furthermore acknowledge Prof. Dr.-Ing. H. R. Herzog, Institut für Medizin (Forschungszentrum Jülich GmbH, Director: Prof. Dr. K. Zilles), for his assistance during the PET measurements as well as for his helpful comments on this manuscript.

References

1. Blin J, Pappata S, Kiyosawa M et al. [^{18}F]setoperone: a new high-affinity ligand for positron emission tomography study of the serotonin-2 receptors in baboon brain in vivo. Eur J Pharmacol 1988; 147: 73-82.
2. Biver F, Goldman S, Luxen A et al. Multicompartmental study of fluorine-18 altanserin

binding to brain 5HT2 receptors in humans using positron emission tomography. Eur J Nucl Med. 1994; 21: 937-46.

3. Burns HD, Dannals RF, Langstrom B et al. (3-N-[^{11}C]methyl)spiperone, a ligand binding to dopamine receptors: radiochemical synthesis and biodistribution studies in mice. J Nucl Med 1984; 25: 1222-7.
4. Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 2000; 4: 215-22.
5. Eckelman WC. Sensitivity of new radiopharmaceuticals. Nucl Med Biol 1998; 25: 169-73.
6. Larisch R, Klimke A, Mayoral F et al. Disturbance of serotonin $5HT_2$ receptors in remitted patients suffering from hereditary depressive disorder. Nucl Med 2001; 40: 129-34.
7. Lemaire C, Cantineau R, Guillaume M et al. Fluorine-18-altanserin: a radioligand for the study of serotonin receptors with PET: radiolabeling and in vivo biologic behavior in rats. J Nucl Med 1991; 32: 2266-72.
8. Leonard BE. Serotonin receptors and their function in sleep, anxiety disorders and depression. Psychother Psychosom 1996; 65: 66-75.
9. Logan J, Fowler JS, Volkow ND et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-^{11}C-methyl]-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 1990; 10: 740-7.
10. Maesden CA. The neuropharmacology of serotonin in the central nervous system. In: Feighner JP, Boyer WF (eds). Selective serotonin re-uptake inhibitors. Chichester, New York, Brisbane, Toronto, Singapore: John Wiley & Sons 1996; 1-34.
11. Pazos A, Probst A, Palacios JM. Serotonin receptors in the human brain-IV. Autoradiographic mapping of serotonin-2 receptors. Neuroscience 1987; 21: 123-39.
12. Sadzot B, Lemaire C, Maquet P et al. Serotonin $5HT_2$ receptor imaging in the human brain using positron emission tomography and a new radioligand, [^{18}F]altanserin: results in young normal controls. J Cereb Blood Flow Metab 1995; 15: 787-97.
13. Smith GS, Price JC, Lopresti BJ et al. Test-retest variability of serotonin 5-HT_{2A} receptor binding measured with positron emission tomography and [^{18}F]altanserin in the human brain. Synapse 1998; 30: 380-92.
14. van Dyck CH, Tan PZ, Baldwin RM et al. PET quantification of 5-HT_{2A} receptors in the human brain: a constant infusion paradigm with [^{18}F]altanserin. J Nucl Med 2000; 41: 234-41.
15. Economo v. CF, Koskinas GN. Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen. Wien, Berlin: Springer 1925.
16. Wienhard K, Coenen HH, Pawlik G et al. PET studies of dopamine receptor distribution using [^{18}F]fluoroethylspiperone: findings in disorders related to the dopaminergic system. J Neural Transm Gen Sect 1990; 81: 195-213.

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