

AGE-RELATED LOSS OF OLFACTORY SENSITIVITY: ASSOCIATION TO DOPAMINE TRANSPORTER BINDING IN PUTAMEN

M. LARSSON,^{a,b,*} L. FARDE,^{b,c} T. HUMMEL,^d M. WITT,^{d,e}
N. ERIXON LINDROTH^{b,c} AND L. BÄCKMAN^f

^aDepartment of Psychology, Stockholm University, Frescati Hagväg 14, S-106 91 Stockholm, Sweden

^bStockholm Brain Institute, Stockholm, Sweden

^cPsychiatry Section, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

^dSmell and Taste Clinic, Department of Otorhinolaryngology, University of Dresden Medical School, Dresden, Germany

^eDepartment of Neurology, University of Dresden Medical School, Dresden, Germany

^fAging Research Center, Karolinska Institutet, Stockholm, Sweden

Abstract—The relationship between age-related reductions in the binding potential for the striatal dopamine transporter (DAT) and age-related deficits in olfactory sensitivity was examined in 12 subjects ranging from 36 to 82 years of age. Positron emission tomography (PET) and the radioligand [¹¹C]β-CIT-FE were used to determine DAT binding in two striatal regions, the caudate and the putamen. The results showed age-related losses of DAT binding from early to late adulthood of similar size for caudate and putamen, and there was a pronounced age deterioration in olfactory sensitivity. Importantly, the age-related olfactory deficit was associated with reductions in DAT binding in putamen, but not caudate. Also, DAT binding in putamen added systematic variance in odor threshold after controlling for age. The findings indicate that DAT binding in putamen is related to age-related olfactory deficits, as well as to odor sensitivity independently of age. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: olfaction, dopamine, striatum, putamen, aging.

Several lines of evidence suggest a role for dopamine (DA) in olfactory processing. Recent experimental studies indicate that transgenic mice lacking the dopamine transporter (DAT) or the D2 receptor exhibit olfactory discrimination deficits (Tillerson et al., 2006), and rats injected with L-DOPA outperform controls in odor discrimination in a dose-dependent manner (Pavlis et al., 2006). Further, exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in marmosets produced a loss of olfactory sensitivity, which can be related to the degree of reduction in DA levels in striatal tissue samples (Miwa et al., 2004).

*Correspondence to: M. Larsson, Department of Psychology, Stockholm University, Frescati Hagväg 14, S-106 91 Stockholm, Sweden. Tel: +46-8-16-39-37; fax: +46-8-15-93-42.

E-mail address: maria.larsson@psychology.su.se (M. Larsson).

Abbreviations: BP, binding potential; DA, dopamine; DAT, dopamine transporter; MRI, magnetic resonance imaging; PD, Parkinson's disease; PET, positron emission tomography; ROI, region of interest.

0306-4522/09 \$ - see front matter © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2009.03.074

Additional support for the hypothesis that olfactory perception is associated with dopaminergic activity is provided in clinical research. Specifically, individuals with severe alterations of DA systems, such as patients with Parkinson's disease (PD), Huntington's disease, and schizophrenia, exhibit clear olfactory impairments (Berendse et al., 2001; Larsson et al., 2006; Rupp, 2001).

Brain imaging of the DA system in early PD patients and their relatives indicates that olfactory loss may serve as a behavioral correlate to altered striatal DA activity. One approach here is to measure the density of the DAT, which is a membrane-bound presynaptic protein that regulates the synaptic concentration of DA at nerve terminals. For example, reduced [¹²³I]β-CIT binding to the DAT has been reported in hyposmic relatives of PD patients compared to normosmic relatives (Berendse et al., 2001). The hyposmic relatives exhibited reduced striatal DAT binding in general, although the binding reduction was more pronounced in putamen than in the caudate. Related pharmacological evidence suggests that persons susceptible to extrapyramidal side effects of antipsychotic drugs, which are all D2 receptor antagonists, exhibit decreased olfactory sensitivity (Hawkes, 2006; Krüger et al., submitted for publication). Taken together, this pattern of observations suggests that striatal dopaminergic neurotransmission plays a role in olfactory functioning.

The relationship between physiological variability in striatal dopaminergic activity and olfactory proficiency is not fully understood in normal subjects. Besides a considerable interindividual variability in the density of markers for the DA system (Farde et al., 1995, 2000), both post-mortem and positron emission tomography (PET) studies indicate a gradual loss across the adult lifespan for pre- and postsynaptic striatal DA markers ranging from 5% to 10% per decade from the 20s and onwards (Bäckman et al., 2006; Reeves et al., 2002). In addition, there is pervasive evidence that olfactory perception declines in old age (e.g. Cain and Gent, 1991; Larsson et al., 2004). The fact that DA is implicated in odor functioning suggests that age-related losses of DA markers, such as the DAT, may be associated with age-related olfactory deficits.

The purpose of the current PET study was to examine the relationship between age-related losses in DAT binding and age-related olfactory deficits. DAT binding in caudate and putamen was quantified in a sample of 12 healthy subjects from early through late adulthood using the radioligand [¹¹C] β-CIT-FE (Farde et al., 2000). Olfactory functioning was assessed using an odor threshold task (Murphy et al., 1991).

EXPERIMENTAL PROCEDURES

Subjects

The study was approved by the Ethics and Radiation Safety Committees of the Karolinska Hospital, Stockholm. All subjects were provided with written and oral information about the study and signed written informed consent prior to participation. Twelve subjects (six women and six men) between 36 and 82 years of age participated. They were healthy according to physical examination, blood, and urine analysis, and no subject had a history of psychostimulant use or psychiatric or neurological disorder.

Olfactory threshold test

N-butyl alcohol is a standard in research on olfactory sensitivity and was used to test odor sensitivity. The test includes a series of concentrations ranging from 8 ml (strongest) to 0.1 μm (weakest) of n-butanol/200 ml of distilled water (Larsson and Bäckman, 1993; Murphy et al., 1991). Eleven dilutions were prepared, where each successive dilution decreased the concentration of the odorant by a factor of 3. The experimenter presented a pair of bottles, one with butanol solution and one blank containing distilled water only. The instruction was to smell each bottle and decide which one contained an odor. The presentation of the odorant and the blank was randomized. The assessment started with the weakest concentration and if the subject was able to pick out the odorant from the blank, the experimenter presented the same concentration again until the criterion of five consecutive correct responses was met (Murphy et al., 1991). If the subject failed, the experimenter presented a stronger concentration until the criterion was met. To prevent effects of adaptation, 30 s elapsed between each presentation. If a subject could detect the weakest concentration, the highest score (10) was given; if the second-weakest concentration was detected, a score of 9 was given, and so forth. It is worth noting that higher concentrations of butanol may stimulate the trigeminal system (Wysocki et al., 2003). However, given that the series of butanol dilutions comprised very low concentrations, it is highly unlikely that the test activated the trigeminal sensory system (Cometto-Muniz and Cain, 1995).

Brain imaging procedure

Magnetic resonance imaging (MRI). The MRI system used was GE Signa, 1.5 T. T_2 -weighted and proton density MR images of the brain were obtained for all subjects. The positioning of the head and the series of sections were the same as in the subsequent PET measurements (see below). A head fixation system with an individual plaster helmet was used in both the MRI and PET measurements to allow the same head positioning in the two imaging modalities (Bergström et al., 1981).

Radiochemistry. The precursor for synthesis of [^{11}C] β -CIT-FE was supplied by Research Biomedicals International (Natick, MA, USA). [^{11}C] β -CIT-FE was prepared by O-methylation of the corresponding free acids with [^{11}C]methyl iodine or [^{11}C]methyl triflate, according to procedures that have been described in detail elsewhere (Halldin et al., 1996; Lundkvist et al., 1998).

PET assessment. The PET examinations were performed using a siemens ECAT Exact HR 47 in three-dimensional mode. The reconstructed volume was displayed as 47 sections with a center to center distance of 3.125 mm and a slice thickness of 4 mm. The in-plane and axial resolutions are approximately 3.8 and 4.0 mm, full width at half-maximum (Weinhard et al., 1994).

In each PET measurement, the subject was placed recumbent with the head in the PET system. A cannula was inserted into the antebachial vein on both arms. A sterile physiological phosphate buffer solution (pH=7.4) containing [^{11}C] β -CIT-FE (196–323 MBq) was injected intravenously as a bolus during 2 s. The

specific radioactivity was 300–3299 Ci/mmol (11–122 GBq/ μmol) at the time of injection.

Brain radioactivity was measured in a series of consecutive time frames up to 57 min. The frame sequence consisted of three 1-min frames followed by four 3-min frames and seven 6-min frames.

Image analysis. Regions of interest (ROIs) were drawn on three horizontal MRI sections through the central part of the putamen and the head of the caudate, respectively. The top and bottom of the putamen and caudate were not included, because these parts may be subject to considerable partial-volume effects. Three horizontal sections covering the middle part of the cerebellum defined this region. The ROIs were transferred to the corresponding reconstructed PET images. To obtain regional time-activity curves, average radioactivity in each ROI was calculated for each frame, corrected for decay and plotted versus time.

Calculations. The binding potential (BP) reflects the ratio between receptor density (B_{max}) and affinity (K_d). To calculate regional BP values, the simplified reference tissue model (Gunn et al., 1997; Lammertsma et al., 1996) was applied, as described previously (Farde et al., 2000). In this model, the time-activity curve for the cerebellum, a reference region assumed to be devoid of DAT, was used as to approximate the plasma input function.

RESULTS

Descriptive statistics for the olfactory threshold task and DAT binding are shown in Table 1. In the first step, we examined the correlations among the three key variables: age, DAT binding, and olfactory performance. The results indicate that advancing age is associated with decreased DAT binding in both putamen ($r=-0.67$, $P<0.02$) and caudate ($r=-0.64$, $P<0.02$). Chronological age was also negatively related to olfactory sensitivity ($r=-0.73$, $P<0.01$). DAT binding in putamen was strongly and positively related to odor threshold ($r=0.80$, $P<0.01$), whereas the correlation between DAT binding in caudate and odor threshold was considerably weaker ($r=0.44$, $P>0.15$). The DAT by threshold correlation is illustrated in Fig. 1.

To determine the relative importance of age and DAT binding to odor threshold, two sets of step-wise hierarchical regression analyses were performed and applied to caudate and putamen, respectively. The results from these analyses are provided in Table 2. The relative variance accounted for by age and DAT binding in each ordinal position reflects the strength of association with the olfactory criterion. For the caudate, the analysis showed that age accounted for 52% of the predictive variance when entered first in the model, and that DAT binding entered in the second step did not explain any additional variance in odor threshold. When entered first in the regression model,

Table 1. Descriptive statistics for the odor threshold and DAT measures

Measure	M	SD	Range
Odor threshold ^a	7.67	1.37	6–10
DAT binding			
Caudate	4.73	0.74	3.71–6.05
Putamen	4.93	0.56	4.07–5.79

^a 1 Anosmia; 10 very sensitive.

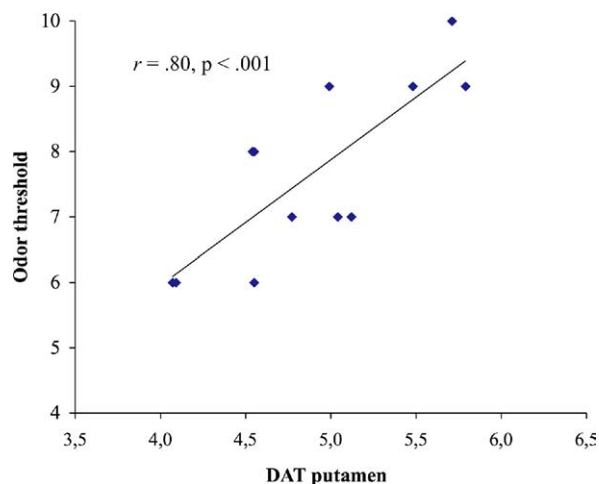


Fig. 1. Correlation between DAT binding in the putamen and odor threshold.

DAT binding in caudate accounted for 20% of the variance in odor threshold, and chronological age explained an additional 32% of the variation when entered second.

A quite different pattern of results emerged in the analyses for putamen. As shown in Table 2, initial entry of age accounted for 52% of the variation and DAT binding entered in the second step explained an additional 18% of the threshold variance. However, when entered first, DAT binding accounted for 64% of the variance and eliminated the influence of age on odor threshold. Specifically, age accounted for 52% of the variation in odor threshold when entered first, but explained only 6% of the variance when the DAT variation was statistically controlled. Binding to the DAT in caudate and putamen in two age-matched subjects (63 and 65 years) with low and high olfactory threshold is shown in Fig. 2.

DISCUSSION

We examined the relationship between age-related losses in striatal DAT binding and age-related olfactory losses in an age-heterogeneous sample. The results show strong relationships among adult age, DAT binding in the putamen, and olfactory proficiency. The current findings extend previous clinical work by showing that variation of putaminal DAT binding in a normal sample is related to olfactory sensitivity, an association that proved to be independent of age.

Although age was strongly related to olfactory performance when entered first in the model, DAT binding in

putamen still shared a substantial amount of variance with olfactory acuity when entered second. However, the reverse was not true. Initial entry of the putaminal DAT variable accounted for most of the olfactory variance, and eliminated the influence of age on performance. Thus, the observation that there was a reliable amount of interindividual variability in DAT binding after statistical control of age suggests that dopaminergic activity in putamen is related to olfactory proficiency. It is important to note that the relationships among age, putaminal DAT binding, and odor proficiency were based on correlational data. Thus, as suggestive as the data may be, we are unable to make any causal inferences.

The finding that variation in olfactory sensitivity was related to DAT binding in putamen, but not in caudate, is intriguing. The human striatum is a heterogeneous structure comprising different anatomical and functional subdivisions. Based on projections from the midbrain and connections in corticostriatal–thalamo-cortical loops, the striatum may functionally be organized into limbic, associative, and sensorimotor compartments (Martinez et al., 2003). This subdivision has originally been suggested from animal studies and the complexity of the functional striatal neuroanatomy in humans is poorly understood. Interestingly, however, a recent PET study on associations between D2 receptor binding and cognitive functioning provided direct biochemical support for a functional subdivision (Cervenka et al., 2008).

With regard to olfactory functioning, there is also support for a subdivision of the striatum. Importantly, emerging literature suggests that putamen may play a key role for the integrity of olfactory functioning. Using odor identification as an index of olfactory functioning, Siderowf et al. (2005) reported reliable relationships between DAT imaging abnormalities and odor identification proficiency in early PD. Specifically, DAT binding in putamen was strongly related to odor identification ($r=0.74$), whereas the corresponding relationship for the caudate was considerably weaker ($r=0.36$; Westermann et al., 2008). This difference in degree of association between the two striatal structures resembles closely those observed for normal persons in the current study. A differential role of the putamen in the processing of olfactory information has also been hypothesized by Mueller et al. (2006), who reported a relationship between structural pathology of the putamen, as determined by MRI, and olfactory impairment in patients with Wilson's disease.

A limitation of the present study is that only two dopaminergic brain regions were examined. The PET system

Table 2. Amount of variance (R^2) in odor threshold accounted for by age and DAT in putamen and caudate as a function of order of entry

	Putamen				Caudate			
	<i>R</i>	R^2	<i>F</i>	<i>P</i>	<i>R</i>	R^2	<i>F</i>	<i>P</i>
Age	0.73	0.52	11.12	0.008	0.73	0.52	11.12	0.008
DAT	0.84	0.70	5.49	0.044	0.72	0.52	0.02	0.894
DAT	0.80	0.64	17.78	0.002	0.44	0.20	2.45	0.148
Age	0.84	0.70	2.02	0.189	0.73	0.52	6.29	0.033

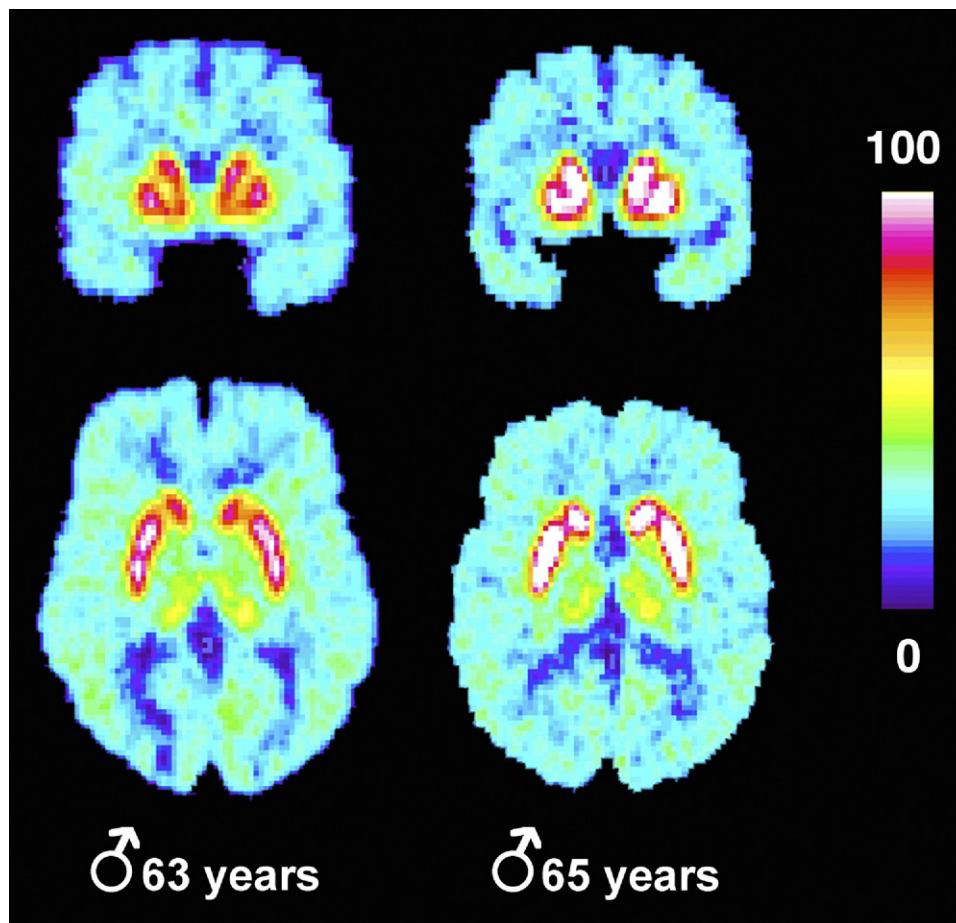


Fig. 2. PET sections of [^{11}C] β -CIT-FE binding to the DAT in caudate and putamen in two age-matched subjects (63 and 65 years) with low (left) and high (right) olfactory threshold. Color-coded horizontal PET images are through the striatal level of the human brain. The images represent integrated radioactivity from 9 to 51 min after radioligand injection. The color scale shows the relative relationship between color and radioactivity. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

used and the radioligand β -CIT-FE do not allow detailed examination of DAT binding in midbrain or other extrastriatal regions. For instance, close to the ventral striatum there is a diffuse irregular area named substantia innominata, within the substantia innominata, olfactory inputs may travel from the small cells of Calleja, an important dopaminergic cell group that is thought to play a role in olfactory modulation of macrosomatics (Fallon et al., 1983; Gottfried, 2006; Hurd et al., 2001). The more recent development of improved radioligands for DAT, such as PE2I (Jucaite et al., 2006), and implementation of high resolution PET systems (de Jong et al., 2007) pave the way for a more detailed examination of dopaminergic markers of potential relevance to olfaction within and outside the striatum. Another caveat of the present study is that the sample size was relatively small. That said, a reliable and biologically plausible relationship was observed between putaminal DAT binding and odor threshold. Future research should extend the generality of the present findings to other olfactory parameters, such as discrimination, identification, and episodic memory.

CONCLUSION

In conclusion, the present findings suggest that DAT binding in putamen is related to age-related deficits in olfactory sensitivity, as well as to odor perception irrespective of age.

Acknowledgments—The present research was supported by grants from the Swedish Research Council to Maria Larsson, and by grants from the Swedish Research Council and Swedish Brain Power to Lars Bäckman.

REFERENCES

- Bäckman L, Nyberg L, Lindenberger U, Li S-C, Farde L (2006) The correlative triad among aging, dopamine, and cognition: current status and future prospects. *Neurosci Biobehav Rev* 30:791–807.
- Berendse HW, Booij J, Francot CMJE, Bergmans PLM, Hijman R, Stoof JC, Wolters EC (2001) Subclinical dopaminergic dysfunction in asymptomatic Parkinson's disease patients' relatives with a decreased sense of smell. *Ann Neurol* 50:34–41.
- Bergström M, Boethius J, Eriksson L, Greitz T, Ribbe T, Widén L (1981) Head fixation device for reproducible position alignment in transmission CT and positron emission tomography. *J Comput Assoc Tomogr* 5:136–141.

- Cain WS, Gent JF (1991) Olfactory sensitivity: reliability, generality, and association with aging. *Exp J Hum Percept Psychol* 17:382–391.
- Cervenka S, Bäckman L, Cselenyi Z, Halldin C, Farde L (2008) Associations between dopamine D2-receptor binding and cognitive performance indicate functional compartmentalization of the human striatum. *Neuroimage* 40:1287–1295.
- Cometto-Muniz JE, Cain WS (1995) Relative sensitivity of the ocular trigeminal, nasal trigeminal and olfactory systems to airborne chemicals. *Chem Sens* 20:191–198.
- de Jong HW, van Velden FH, Kloet RW, Buijs FL, Boellaard R, Lammertsma AA (2007) Performance evaluation of the ECAT HRRT: an LSO-LYSO double layer high resolution, high sensitivity scanner. *Phys Med Biol* 52:1505–1526.
- Fallon JH, Loughlin SE, Ribak CE (1983) The islands of Calleja complex of rat basal forebrain. III. Histochemical evidence for a striatopallidal system. *J Comp Neurol* 218:91–120.
- Farde L, Hall H, Pauli S, Halldin C (1995) Variability in D₂ dopamine receptor density and affinity: a PET study with [¹¹C] raclopride in man. *Synapse* 20:200–208.
- Farde L, Ginovart N, Halldin C, Chou Y, Olsson H, Swahn CA (2000) PET-study of [¹¹C] β-CIT-FE binding to the dopamine transporter in the monkey and human brain. *Int J Clin Neuropsychopharmacol* 3:203–214.
- Gottfried JA (2006) Smell: central nervous processing. *Adv Otorhinolaryngol* 63:44–69.
- Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997) Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage* 6:279–287.
- Halldin C, Farde L, Lundkvist C, Ginovart N, Nakashima Y, Karlsson P, Swahn CG (1996) [¹¹C]β-CIT-FE, a radioligand for quantitation of the dopamine transporter in the living brain using positron emission tomography. *Synapse* 4:386–390.
- Hawkes C (2006) Olfaction in neurodegenerative disorder. *Adv Otorhinolaryngol* 63:133–151.
- Hurd YL, Suzuki M, Sedvall GC (2001) D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. *J Chem Neuroanat* 22:127–137.
- Jucaite A, Odano I, Olsson H, Pauli S, Halldin C, Farde L (2006) Quantitative analyses of regional (C-11)PE2I binding to the dopamine transporter in the human brain: a PET study. *Eur J Nucl Med Mol Imaging* 33:657–668.
- Lammertsma AA, Bench CJ, Hume SP, Osman S, Gunn K, Brooks DJ, Frackowiak RS (1996) Comparison of methods for analysis of clinical [¹¹C]raclopride studies. *J Cereb Blood Flow Metab* 16:42–52.
- Larsson M, Bäckman L (1993) Semantic activation and episodic odor recognition in young and older adults. *Psychol Aging* 8:582–588.
- Larsson M, Lundin A, Robins Wahlin T-B (2006) Olfactory functions in asymptomatic carriers of the Huntington disease mutation. *J Clin Exp Neuropsychol* 28:1373–1380.
- Larsson M, Nilsson L-G, Olofsson J, Nordin S (2004) Demographic and cognitive predictors of odor identification: evidence from a population-based study. *Chem Sens* 29:547–554.
- Lundkvist C, Sandell J, Nägren K, Pike VW, Halldin C (1998) Improved synthesis of the PET radioligands, [¹¹C]FLβ457, [¹¹C]MDL100907 and [¹¹C]β-CIT-FE, by the use of [¹¹C]methyl triflate. *J Label Compd Radiopharm* 41:545–556.
- Martinez D, Slifstein M, Broft A, Mawlawi T, Hwang DR, Huang YY, Cooper T, Kegeles L, Zarahn E, Abi-Dargham A, Haber SN, Laruelle T (2003) Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. *J Cereb Blood Flow Metab* 23:285–300.
- Miwa T, Watanabe A, Mitsumoto Y, Furukawa M, Fukushima N, Morizumi T (2004) Olfactory impairment and Parkinson's disease-like symptoms observed in the common marmoset following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Acta Otolaryngol Suppl* 553:80–84.
- Mueller A, Reuner U, Landis B, Kitzler R, Reichmann H, Hummel T (2006) Extrapiramidal symptoms in Wilson's disease are associated with olfactory dysfunction. *Mov Disord* 21:1311–1316.
- Murphy C, Cain WS, Gilmore MM, Skinner RB (1991) Sensory and semantic factors in recognition memory for odors and graphic stimuli: elderly vs. young persons. *Am J Psychol* 104:161–192.
- Pavlis M, Feretti C, Levy A, Gupta N, Linster C (2006) L-DOPA improved odor discrimination learning in rats. *Physiol Behav* 87:109–113.
- Reeves S, Bench C, Howard R (2002) Aging and the nigrostriatal dopamine system. *Int J Geriatr Psychiatry* 17:359–370.
- Rupp CI (2001) Dysfunctions in olfactory processing in schizophrenia. *Curr Opin Psychiatry* 16:181–185.
- Siderowf A, Newberg A, Chou KL, Lloyd M, Colcher A, Hurtig HI, Stern MB, Doty RL, Mozley PD, Wintering N, Duda JE, Weintraub D, Moberg PJ (2005) [^{99m}Tc]TRODAT-1 SPECT imaging correlates with odor identification in early Parkinson disease. *Neurology* 64:1716–1720.
- Tillerson JL, Caudle WM, Parent JM, Gong C, Schallert T, Miller GW (2006) Olfactory discrimination deficits in mice lacking the dopamine transporter or the D2 dopamine receptor. *Behav Brain Res* 172:97–105.
- Weinhard K, Dahlbom M, Eriksson L, Michel C, Bruckbauer T, Pietrzyk U, Heiss W (1994) The ECAT exact HR: performance of a new high resolution positron scanner. *J Comput Assoc Tomogr* 18:110–118.
- Westermann B, Wattendorf E, Schwerdtfeger U, Husner A, Fuhr P, Gratzl O, Hummel T, Bilecen D, Welge-Luessen A (2008) Functional imaging of the cerebral olfactory system in patients with Parkinson's disease. *J Neurol Neurosurg Psychiatry* 79:19–24.
- Wysocki CJ, Cowart BJ, Radil T (2003) Nasal trigeminal chemosensitivity across the adult life span. *Percept Psychophys* 65:115–122.