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Minireview of Stereoselective Brain Imaging

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Stereoselectivity is a fundamental principle in living systems. Stereoselectivity reflects the dependence of molecular processes on the spatial orientation of constituent atoms. Stereoselective processes govern many aspects of brain function and direct the course of many psychotropic drugs. Today, modern imaging techniques such as SPECT and PET provide a means for studying stereoselective processes in the living brain. Chemists have prepared numerous radiolabelled stereoisomers for use in SPECT and PET in order to explore various molecular processes in the living brain of anesthetized laboratory animals and awake humans. The studies have demonstrated how many aspects of neurotransmission consist of crucial stereoselective events that can affect brain function in health and disease. Here, we present a brief account of those findings in hope of stimulating further interest in the vital topic.

Keywords: Brain imaging; neurotransmission; neuroreceptors; stereoisomers; SPECT; PET.

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1. INTRODUCTION

Stereoselectivity is a fundamental principle in many living systems due to their dependence on the spatial features of molecules [1,2]. Stereoselective aspects of neurotransmission can be studied in the living brain by comparing the distribution kinetics of stereoisomeric molecules appropriately radiolabeled for PET and SPECT (Table 1). The motivation for such studies comes from the desire to fully understand the molecular processes that govern brain function. This article reviews those findings.

Table 1. Summary of the main site of action, chemical name, and relative effects of the stereoisomers included in this review

Neural target	Stereoisomer	Number	Relative effect	Reference
Muscarinic acetylcholine receptor	3-Phenyl-1'-(4-iodo-phenylmethyl)-[3,4'-bipiperidine]-2,6-dione	1	(+) > (-)	[1,2]
Nicotinic acetylcholine receptor	1-methyl-2-(3-pyridyl)pyrrolidine	2	(*R*) = (*S*)	[3,4]
	Homoepibatidine	3	(1*R*,2*R*,4*S*) > (1*R*,2*R*,4*S*)	[5]
	Norchloro-fluoro-homoepibatidine	4	(+) > (-)	[6]
	7-Methyl-2-exo-(3´-iodo-5´-pyridinyl)-7-azabicyclo[2.2.1]-heptane	5	(-) > (+)	[7]
Presynaptic noradrenaline transport	2-[(2-methoxyphenoxy) phenylmethyl]morpholine	6	(*S,S*) > (*R,R*)	[8,9]
Noradrenergic α_1-receptor	1-(8-[Isobutyrylaminomethyl]-10,11-dihydrodibenzo[b,f] thiepin-10-yl)-4-methylpiperazine	7	No accumulation of (*R*) or (*S*) in brain	[10]
Noradrenergic α_2-receptor	1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c]benzazepine	8	(*S*) > (*R*) in pig (*S*) = (*R*) in human	[11] [12]
Presynaptic dopamine transport	threo-Methyl 2-phenyl-2-(2-piperidyl)ethanoate	9	(+) > (-)	[13,14]
	Methyl-3- (benzoyloxy)-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate	10	(-) > (+)	[15]
	2β-Carbomethoxy-3β-(4-iodophenyl)tropane	11	(*1R,2S,3S*) > (*1S,2R,3R*)	[16]
Dopamine D_1 receptor	8-Chloro-5-(7-benzofuranyl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine	12	(+) > (-)	[17,18]
Dopamine D_2/D_3 receptor	3,5-Dichloro-*N*-{[-1-ethylpyrrolidin-2-yl]methyl}-2-hydroxy-6-methoxybenzamide	13	(2*S*) > (2*R*)	[19]
Presynaptic serotonin transport	trans-1,2,3,5,6,10β-Hexahydro-6-[4-(methyl-thio)phenyl]-pyrrolo-[2,1-a]-isoquinoline	14	(6*S*,10b*R*) > (6*R*,10b*S*)	[20-22]
	8-Methyl-3-(4-trifluoromethyl-phenyl)8-azabicyclo[3.2.1]oct-2-ene	15	No difference between enantiomers	[23]
	8-Methyl-3-[4-trifluoromethoxyphenyl]-8-azabicyclo[3.2.1]oct-2-ene	16	No difference between enantiomers	[24]
Serotonin receptor type 1a	*N*-{2-[4-(2-Methoxyphenyl) piperazinyl]ethyl}-N-(2-pyridyl)-N-(4-fluoromethycyclohexane)carboxamide	17	*trans* > *cis*	[25]

Table 1 Continued …….

Vesicular acetylcholine transport	*trans*-8-Methyl-2-hydroxy-3-[4-[2-aminophenyl]piperizinyl]-tetralin	18	(*R*,*R*) > (*S*,*S*)	[26]
Vesicular monoamine transport	3-Isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinolin-2-ol	19	(+) > (-)	[27]
Opiate receptor	6-Deoxy-6β-fluoro-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one	20	(-) > (+)	[28,29]
	4-Methoxycarbonyl-2-[(1-pyrrolidinylmethyl]-1-[(3,4-dichlorophenyl)acetyl]-piperidine	21	(*R*) > (*S*)	[30]
Ionotropic NMDA-receptor	2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone	22	(*R*) = (*S*)	[31]
	4-Methylbenzyl-4-[(pyrimidin-2-ylamino)methyl]-3-fluoro-piperidine-1-carboxylate	23	*cis* = *trans*	[32]
Metabotropic glutamate receptor subtype 5	3-(6-Methylpyridin-2-ylethynyl)-cyclohex-2-enone-*O*-methyloxime	24	(*E*) > (*Z*)	[33]
Monoamine oxidase type B	*N,*α-Dimethyl-N-2-propynyl phenethylamine	25	(*R*) > (*S*)	[34,35]
Phosphodiesterase type 4	4-(3-Cyclopentyloxy-4-methoxy phenyl)-2-pyrrolidinone	26	(*R*) > (*S*)	[36,37]
P-glycoprotein efflux-transport	5-[*N*-(3,4-Dimethyoxyphenylethyl) methylamino]-2-(3,4-dimethoxy phenyl)-2-isopropylvaleronitrile	27	(*R*) = (*S*)	[38]

1.1 Molecular Kinetics

Several procedures are used for assessing the distribution of compounds in the living brain. Traditional methods for quantitative analysis of neuroimaging data have relied on arterial blood samples for obtaining an input function [3,4]. Arterial cannulation has, however, disadvantages such as causing pain and emotional strain in some subjects. As a result, other strategies have been developed for assessing molecular kinetics in the living brain. The reference region method, for example, requires a brain region that lacks receptors for the radioligand. By comparing the kinetics of the radioligand in the reference region to kinetics in a region with receptors for the radioligand, an estimate can be obtained of the specific binding of the radioligand [5]. Another strategy, known as the reference isomer method, uses the regional distribution of a pharmacologically inactive, radiolabelled enantiomer to obtain an estimate of the free fraction and nonspecific binding of the pharmacologically active enantiomer in brain tissue [6,7]. Thus, the reference isomer method takes advantage of stereoselective processes in order to characterize molecular events in the living brain.

2. CHOLINERGIC MUSCARINIC NEUROTRANSMISSION

2.1 Muscarinic Acetylcholine Receptor

2.1.1 (+)- and (-)-3-Phenyl-1'-(4-iodophenylmethyl)-[3,4'-bipiperidine]-2,6-dione (Iododexetimide and Iodolevetimide)

The stereoselectivity of cholinergic muscarinic neurotransmission has been explored in living human brain with iododexetimide and iodolevetimide, stereoselective antagonists of muscarinic receptors [8,9]. The distribution of radioactivity produced by [^{123}I]4-iododexetimide in the living human brain resembled the distribution of muscarinic receptors as shown by in vitro autoradiography [10] with highest levels in neostriatum and decreasing levels in neocortex, thalamus, and cerebellum. In contrast, the accumulation of [^{123}I]4-iodolevetimide failed to differ between brain regions and failed to correlate with in vitro autoradiographic findings. A subsequent study determined further the value of [^{123}I]4-iododexetimide and [^{123}I]4-iodolevetimide for brain imaging of muscarinic receptors [11]. Continuous recording of positron emission was done in living mice with a single-crystal radiation detection device using [^{123}I]4-iododexetimide.

Sasaki and coworkers found that [^{123}I]4-iododexetimide accumulated in brain whereas [^{123}I]4-iodolevetimide failed to do so. The specificity of binding of [^{123}I] 4-iododexetimide to muscarinic receptors was confirmed by displacement studies with atropine.

3. CHOLINERGIC NICOTINIC NEURO-TRANSMISSION

3.1 Nicotinic Acetylcholine Receptor

3.1.1 (*S*)- and (*R*)-1-Methyl-2-(3-pyridyl)pyrrolidine (Nicotine)

The stereoselectivity of cholinergic nicotinic neurotransmission was first investigated in living brain using (*S*)- and (*R*)-[^{11}C]nicotine for PET [12]. Under baseline conditions, the initial uptake of (*R*)-[^{11}C] nicotine was slightly greater than that of the (*S*)-form. Blockade of peripheral nicotinic receptors with trimetaphan markedly reduced the cerebral uptake of (*R*)-[^{11}C] nicotine but failed to impair the uptake of (*S*)-[^{11}C] nicotine, resulting in higher brain levels of (*S*)-[^{11}C]nicotine than of the (*R*)-form. Nordberg and coworkers measured also the cerebral uptake of (*S*)- and (*R*)-[^{11}C]nicotine using PET in Alzheimer patients and age-matched healthy controls in hope of obtaining stereoselective, diagnostic markers of pathophysiological changes in nicotinic receptor density [13]. While the uptake and distribution of (*S*)- and (*R*)-[^{11}C] nicotine were similar in the brain of healthy controls, the cerebral uptake of (*S*)-[^{11}C]nicotine was greater than that of the (*R*)-form in the brain of Alzheimer patients. Later, Nordberg and coworkers determined whether changes in the pharmacokinetics of [^{11}C]nicotine enantiomers occurred when Alzheimer patients received treatment with the cholinesterase inhibitor tacrine [14]. They found that the uptake of (*S*)-[^{11}C] nicotine in frontal and temporal cortices of Alzheimer patients was greater than that of (*R*)-[^{11}C] nicotine prior to tacrine treatment, whereas no marked difference between the uptake of (*S*)- and (*R*)-[^{11}C]nicotine in cortical regions was observed after drug treatment. The mechanism by which tacrine treatment affected stereoselective processes in the Alzheimer brain remains unknown. It is noteworthy that a subsequent PET study questioned the utility of (*S*)- and (*R*)-[^{11}C]nicotine for imaging specific nicotinic receptors in living brain [15]. The study examined binding of the enantiomers in male volunteers, some of which smoked cigarettes. Brain uptake of both (*S*)- and (*R*)-[^{11}C]nicotine was rapid and reached a peak a few minutes after tracer injection, regardless of smoker status. Because no evidence was obtained for binding of the radiotracers at specific, receptor-rich brain regions, (*S*)- and (*R*)-[^{11}C] nicotine fail to provide a valid procedure for in vivo neuroimaging of cholinergic, nicotinic receptors, despite their value for in vitro studies.

3.1.2 (1*R*,2*R*,4*S*)- and (1*S*,2*S*,4*R*)-Homoepibatidine

The search for an appropriate radioligand for studying nicotinic acetylcholine receptors (nAChR) in the living brain gave rise to research with radiolabelled analogs of epibatidine, a high affinity antagonist of $\alpha_2\beta_4$ nAChRs [16]. Patt and coworkers radiolabelled (1*R*,2*R*,4*S*)- and (1*S*,2*S*,4*R*)-homoepibatidine with C-11 for PET studies in two anesthetized, female pigs [17]. The (1*R*,2*R*,4*S*)-form accumulated in thalamus and cortex, whereas the (1*S*,2*S*,4*R*)-form had relatively low uptake in brain regions. Blocking and displacement experiments with cytisine, an agonist of nicotinic receptors [18], showed reversible, specific binding of only the (1*R*,2*R*,4*S*)-form. The favorable kinetic behavior and high specific binding of (1*R*,2*R*,4*S*)-*N*-[^{11}C-methyl] homoepibatidine in the living brain, combined with the non-specific binding of the (1*R*,2*R*,4*S*)-form, confirmed the stereoselectivity of $\alpha_2\beta_4$ nAChRs.

3.1.3 (+)- and (-)-Norchloro-fluoro-homoepibatidine (Flubatine)

The optical isomers of flubatine have also been studied as PET radioligands for imaging the $\alpha_2\beta_4$ subtype of nAChRs in the living brain. A thorough PET investigation on (+)- and (-)-[^{18}F]flubatine revealed clear-cut stereoselectivity of nAChRs toward the isomers in the living pig brain [19]. Brain uptake was faster for (-)-[^{18}F]flubatine than for the (+)-isomer, while lower levels of radioactivity were reached in brain regions after injection of (-)-[^{18}F]flubatine compared with the (+)-isomer. These findings underscore the stereoselectivity of transport and receptor binding by nicotinic cholinergic processes in the living brain [20].

3.1.4 (+)- and (-)-7-Methyl-2-exo-(3´-iodo-5´-pyridinyl)-7-azabicyclo[2.2.1]-heptane (NMI-EPB)

The optical isomers of NMI-EPB, radiolabelled with C-11 for PET, were used in an interesting study designed specificially to explore the

stereoselectivity of nAChRs [21]. The primary goal of the study was to determine whether separation of racemic [^{11}C]NMI-EPB into its (+)- and (-)-forms would advance PET imaging of nAChRs in the living brain. Accumulation of (-)-[^{11}C] NMI-EPB proved to be 5-fold greater than that of the antipode in the thalamus, a region rich in the nicotinic receptor $\alpha_4\beta_2$ [22]. However, failure of (-)-[^{11}C] NMI-EPB to reach steady-state within 90 min postinjection was viewed as a limitation for PET brain imaging.

4. NORADRENERGIC NEUROTRANSMISSION

4.1 Presynaptic Noradrenaline Transport

4.1.1 (*R,R*)- and (*S,S*)-2-[(2-Methoxyphenoxy)phenylmethyl]morpholine (MeNER)

MeNER inhibits the neuronal noradrenaline transporter [23]. The (*R,R*)- and (*S,S*)-isomers of MeNER have been radiolabelled with C-11 and have been used for neuroimaging in living brain. Schou and coworkers carried out PET-imaging with each enantiomer of [^{11}C]MeNER in an anesthetized cynomolgus monkey and examined the distribution of radioactivity in lower brainstem, mesencephalon, and thalamus, using the striatum as reference region for free radioligand concentration and non-specific binding [24]. Evaluation of time-radioactivity curves indicated a notably higher specific binding of (*S,S*)-[^{11}C] MeNER than of the (*R,R*)-form. Next, the pharmacokinetics of (*S,S*)- and (*R,R*)-[^{11}C] MeNER were assessed by PET in anesthetized female baboons [25]. It is to be noted, however, that the structural drawings of (*S,S*)- and (*R,R*)-[^{11}C] MeNER in that report are incorrect. Be that as it may, the clearance of (*R,R*)-[^{11}C] MeNER from brain regions rich in noradrenaline transporters was 2.5-fold faster than that of (*S,S*)-[^{11}C] MeNER. Moreover, the clearance of (*S,S*)-[^{11}C] MeNER differed between brain regions, with slower clearance of radioactivity in regions rich in noradrenaline transporters, such as thalamus and cerebellum than in noradrenaline transporter-poor regions such as striatum and cortical regions. On the other hand, (*R,R*)-[^{11}C] MeNER clearance was similar in all brain regions studied. The differences in clearance of [^{11}C] MeNER enantiomers failed, however, to cause marked differences in their distribution volumes, except for 2-fold higher values for the (*S,S*)-form than the (*R,R*)-form in thalamus. The selective blocker of noradrenaline transporters, nisoxetine, was used together with (*S,S*)- and (*R,R*)-[^{11}C]MeNER in further PET studies to determine the degree to which binding of MeNER enantiomers occurs at those sites. Pretreatment with nisoxetine markedly reduced the accumulation of (*S,S*)-[^{11}C]MeNER in thalamus and cerebellum, whereas the accumulation of (*R,R*)-[^{11}C]MeNER in brain regions was unaffected by nisoxetine, thus demonstrating the stereoselectivity of the noradrenaline transporter in the living brain and the value of radiolabelled enantiomers for quantifying the transporter in living brain. Perhaps radiolabelling of the enantiomers of MeNER with F-18 [26] can provide an improved means of optimizing PET modeling for quantifying the noradrenaline transporter in the living human brain.

4.2 Noradrenergic α_1 Receptor

4.2.1 (*R*)- and (*S*)-1-(8-[Isobutyrylaminomethyl]-10,11-dihydrodibenzo[b,f] thiepin-10-yl)-4-methylpiperazine (LuAE43936)

Noradrenergic α_1 receptors are widely distributed in the brain and are believed to affect both cerebral circulation and neuropsychiatric disorders [27,28]. The enantiomers of [^{11}C] LuAE43936 have been used in a recent PET study directed toward imaging α_1-noradrenergic receptors in the living brain [29]. (*R*)- and (*S*)-[^{11}C] Lu AE43936 failed, however, to accumulate in regions of the anesthetized pig brain, apparently due to activity of the P-glycoprotein efflux pump [30]. Thus, the stereoselectivity of α_1-noradrenergic receptors remains to be explored in the living brain.

4.3 Noradrenergic α_2 Receptor

4.3.1 (*R*)- and (*S*)-1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c]benzazepine (Mirtazapine)

Racemic mirtazapine is an antidepressant drug with potent inhibitory actions at α_2-noradrenergic autoreceptors and heteroceptors [31]. The accumulation of (*S*)-[^{11}C]mirtazapine was greater than that of the antipode in the cerebral cortex and thalamus of anesthetized pigs [32]. However, in awake humans, no reliable differences were noted between the distribution kinetics of (*R*)- and (*S*)-[^{11}C] mirtazapine in the brain [33].

5. DOPAMINERGIC NEUROTRANSMISSION

5.1 Presynaptic Dopamine Transport

5.1.1 (+)- and (-)-threo-Methyl 2-phenyl-2-(2-piperidyl)ethanoate (Methylphenidate)

Racemic methylphenidate binds reversibly to the dopamine transporter and inhibits dopamine reuptake and enhances levels of synaptic dopamine [34]. The enantiomers of [^{11}C] methylphenidate given intravenously showed marked differences in cerebral pharmacokinetics, with higher uptake of the (+)-form than of the antipode in the striatum of an anesthetized baboon [35]. In a subsequent study, the regional cerebral uptake and clearance of (+)- and (-)-[^{11}C]methylphenidate was examined both in healthy, awake humans and in anesthetized baboons [36]. Stereoselectivity was demonstrated by a 3-fold greater accumulation of (+)-[^{11}C] methylphenidate compared with the antipode in the striatum. In humans, clear-cut differences were also observed between levels of (+)- and (-)-[^{11}C]methylphenidate in brain regions, with markedly higher values for the (+)-form. Thus, the PET findings on (+)- and (-)-[^{11}C]methylphenidate in the living brain confirm the stereoselectivity of the dopamine transporter. In a subsequent study, PET was used to study the brain kinetics of (+)- and (-)-[^{11}C]methylphenidate after oral administration in anesthetized baboons and rats [37]. Uptake of (+)- and (-)-[^{11}C]methylphenidate in brain reached peak values 30 – 40 minutes after oral administration, with higher whole brain levels for the (-)-form than of the (+)-form, in marked contrast to the results obtained after intravenous injection of the radiolabelled enantiomers. Such findings reflect the role of peripheral metabolism in the ultimate fate of orally administered stereoisomeric drugs [38] and emphasize the importance of assessing the role of stereoselective metabolism in imaging studies designed to explore central neurotransmission in the living brain.

5.1.2 (+)- and (-)-Methyl-3- (benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (Cocaine)

Psychostimulant actions of racemic cocaine have been related to blocking of presynaptic uptake sites for dopamine, with the (-)-form being several fold more potent than the antipode [39]. A PET study showed (-)-[*N*-methyl-^{11}C]cocaine to accumulate in the basal ganglia of baboons, whereas (+)-[*N*-methyl-^{11}C]cocaine failed to do so, apparently due to rapid, stereoselective metabolism of the (+)-enantiomer in the bloodstream [40]. Such findings emphasize the importance of determining the impact of stereoselective peripheral metabolism in studies on pharmacokinetics of enantiomeric radiotracers in the living brain [41].

5.1.3 (1*R*,2*S*,3*S*)- and (1*S*,2*R*,3*R*)-2β-Carbomethoxy-3β-(4-iodophenyl)tropane (β-CIT)

β-CIT is an analog of cocaine and binds to both dopamine and serotonin transporters [42]. Early SPECT studies of (*1R,2S,3S*)-[^{123}I]β-CIT indicated that striatal binding probably reflected mainly dopamine transporters whereas extrastriatal binding was associated primarily with serotonin transporters [43]. A subsequent SPECT study was carried out using (*1R,2S,3S*)- and (*1S,2R,3R*)-[^{123}I]β-CIT to see whether the reference isomer method could quantify monoamine transporters in the living brain [44]. In striatal regions, accumulation of (*1R,2S,3S*)-[^{123}I]β-CIT was two-fold greater than that of the antipode, which is consistent with stereoselective binding by the dopamine transporter. Displacement studies with unlabelled (*1R,2S,3S*)-β-CIT showed that mainly nonstereoselective, nonspecific binding could be measured in brain regions by (*1S,2R,3R*)-[^{123}I]β-CIT. The findings confirmed that neuroimaging procedures could be improved for quantifying binding at dopamine transporters in living brain by using (*1R,2S,3S*)- and (*1S,2R,3R*)-[^{123}I]β-CIT.

5.2 Dopamine D_1 Receptor

5.2.1 (+)- and (-)- 8-Chloro-5-(7-benzofuranyl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine (NNC 112)

(+)-NNC 112 selectively inhibits dopamine D_1 receptors that are implicated in severe cognitive disorders [45], whereas (-)-NNC 122 lacks affinity for that receptor type [46]. (+)- and (-)-[11C]NNC 112 were used for PET brain imaging in anesthetized monkeys and awake human volunteers [46]. The analysis of PET data

focused on the distribution of (+)- and (-)-[^{11}C]NNC 112 in basal ganglia and neocortex, which are regions rich in dopamine D_1 receptors, whereas the cerebellar cortex can serve as a low-binding reference region [47]. Much higher levels of (+)-[^{11}C]NNC 112 were obtained in striatum than in cerebellum of monkey brain, whereas no marked regional differences appeared for the (-)-form. The findings demonstrated, once again, the role of stereoselective processes in the quantification of neuroreceptors in the living brain. Recently, (+)- and (-)-[^{11}C]NNC 112 were used for PET brain imaging of monkeys in a study on the occupany of dopamine D_1 receptors achieved by the antipsychotic drug clozapine [48]. (+)-[^{11}C]NNC 112 reached 4 – 5 fold higher levels in the striatum than in the cerebellum, while levels of (-)-[^{11}C]NNC 112 failed to show regional differences. Dopamine D_1 receptor occupancy produced by intravenous clozapine ranged between 32 – 83%, as estimated using (+)-[^{11}C]NNC 112.

5.3 Dopamine D_2/D_3 Receptor

5.3.1 (2*S*)- and (2*R*)-3,5-Dichloro-N-{[-1-ethylpyrrolidin-2-yl]methyl}-2-hydroxy-6-methoxybenzamide (Raclopride and FLB472, respectively)

Raclopride binds reversibly and selectively to postsynaptic dopamine receptors that are implicated in actions of antipsychotic drugs [49]. The antipode of raclopride, FLB472, is pharmacologically inactive as antagonist of dopamine receptors, which has encouraged its use for the reference isomer method to distinguish between specific and nonspecific binding at D_2/D_3-sites. [^{11}C]-Raclopride and [^{11}C]-FLB472 have been studied by PET in the living human brain [49]. Radioactivity derived from [^{11}C]raclopride reached 4-fold higher levels in putamen than in other brain regions, whereas radioactivity from [^{11}C]FLB472 failed to accumulate in the brain. The findings demonstrated that D_2/D_3 receptors are stereoselective and indicated that PET studies carried out with [^{11}C] FLB472 can provide an estimate of free ligand concentration and non-specific binding at D2-sites.

6. SEROTONERGIC NEUROTRANSMISSION

6.1 Presynaptic Serotonin Transport

6.1.1 (6*S*,10b*R*)- and (6*R*,10b*S*)- trans-1,2,3,5,6,10β-Hexahydro-6-[4-(methyl-thio)phenyl]-pyrrolo-[2,1-a]-isoquinoline (McN-5652)

McN-5652 is a diastereomeric compound with potent, stereoselective, inhibitory actions on serotonin transport, with the (6*S*,10b*R*)-form being several hundred-fold more potent than the antipode [50]. The enantiomers of [^{11}C] McN-5652 have been popular tools for PET studies on serotonin uptake [51-53]. (6*S*,10b*R*)-[^{11}C]McN-5652 shows high uptake in the hypothalamus, thalamus, striatum, and pons, intermediate uptake in the cerebral cortex, and minimal uptake in cerebellum and white matter. In contrast, uptake of (6*R*,10b*S*)-[^{11}C]McN-5652 appears similarly low in brain regions. PET findings on the enantiomers of [^{11}C] McN-5652 confirm that stereoselective processes govern serotonin uptake in living brain [54].

In a pioneering study, (6*R*,10b*S*)- and (6*S*,10b*R*)-[^{11}C]McN-5652 were used together with the reference isomer method for determining whether the psychoactive drug MDMA ("Ecstasy") affects serotonin transport [55]. Dynamic PET images were obtained at several times after intravenous injection of each enantiomer of [^{11}C] McN-5652. Prior to treatment with MDMA, radioactivity levels in pons, hypothalamus, thalamus, caudate and putamen were higher for (6*S*,10b*R*)-[^{11}C]McN-5652 than for the (6*R*,10b*S*)-form. Treatment with MDMA reduced the accumulation of (6*S*,10b*R*)-[^{11}C] McN-5652 in selected brain regions, while it failed to affect the accumulation of (6*R*,10b*S*)-[^{11}C] McN-5652. Such findings provide clear-cut evidence for effects of MDMA on serotonin uptake in the living brain.

Another PET study used the reference isomer method with (6*S*,10b*R*)- and (6*R*,10b*S*)-[^{11}C] McN-5652 in young and old monkeys to determine by PET whether the availability of serotonin transporters changes with age [56]. As in other studies, relatively high levels of radioactivity were observed in thalamus and striatum after injection of (6*S*, 10b*R*)-[^{11}C]McN-5652 in both age groups, whereas no region-specific differences in radioactivity were seen after injection of (6*R*,10b*S*)-[^{11}C]McN-5652.

Displacement studies with fluvoxamine, a specific serotonin reuptake inhibitor, indicated that the difference between levels of (6*S*,10b*R*)- and (6*R*,10b*S*)-[^{11}C]McN-5652 in brain regions reflects specific binding to serotonin transporters. Specific binding at serotonin transporters turned out to be markedly lower in old animals than in young ones in brain regions other than cerebellum and cingulate gyrus. These findings show how attention to stereoselectivity can serve to disclose effects of aging on neurotransmission in the living brain. And by replacing C-11 with F-18, the kinetics of (6*R*,10b*S*)- and (6*S*,10b*R*)-McN-5652 can be studied by PET in the living brain during an extended time period, which favors a pseudo-equilibrium [57]. Under those conditions, brain regions rich in serotonin transporters accumulated more (6*S*,10b*R*)-[^{18}F]McN-5652 than of the antipode, in accordance with the stereoselectivity of serotonin transport.

6.1.2 (+)- and (-)-8-Methyl-3-(4-trifluoromethyl-phenyl)8-azabicyclo[3.2.1]oct-2-ene (NS2381)

Racemic [^{11}C]NS2381 is a potent and selective inhibitor of serotonin uptake that has been used for PET brain imaging in anesthetized pigs [58]. According to Pfeiffer's rule [59,60], one would expect differences in potency between two enantiomers to be linearly related to the potency of the racemate. However, when the enantiomers of [^{11}C] NS2381 were studied by PET, they failed to show marked differences in brain kinetics [58]. Evidently, the mirror-image differences in spatial features of the enantionmers of [^{11}C] NS2381 had little impact on the stereoselectivity of the serotonin transporter.

6.1.3 (+)- and (-)-8-Methyl-3-[4-trifluoromethoxyphenyl]-8-azabicyclo[3.2.1]oct-2-ene (NS2456)

Rcemic NS2456 is another compound with potent, selective, inhibitory actions on serotonin uptake, and PET brain imaging has been carried out with the enantiomers of [^{11}C]NS2456 in anesthetized pigs [61]. No reliable difference were noted between the binding potentials of [^{11}C]NS2456 enantiomers in the living brain, which suggests that the stereoselective requirements of the serotonin transport fail to apply for the enantiomers of NS2456.

6.2 Serotonin Receptor Type 1a

6.2.1 cis- and trans-*N*-{2-[4-(2-Methoxyphenyl)piperazinyl]ethyl}-*N*-(2-pyridyl)-*N*-(4-fluoromethycyclohexane)carboxamide (Mefway)

Mefway has been radiolabeled for PET and used in a remarkable study to explore the impact of conformational features on binding at serotonin type 1a receptors in the living brain [62]. Initial studies had shown that trans-mefway was several times more potent than the cis-form as antagonist at serotonin type 1a receptors [63]. The cis- and trans-[^{18}F]mefway were studied separately in a microPET scanner, and data were recorded with high resolution from cortical, subcortical, and brainstem regions. The binding potential of trans-[^{18}F]mefway toward serotonin type 1a receptors in cortical and midbrain regions was more than 10-fold greater than that of the cis-form [64]. Thus, the spatial orientation of F-18 on the cyclohexane ring of mefway markedly affected the distribution of the radiotracer in the living brain.

7. VESICULAR TRANSPORT

7.1 Vesicular Acetylcholine Transport

7.1.1 (*R,R*)- and (*S,S*)-trans-8-Methyl-2-hydroxy-3-[4-[2-iminophenyl]piperizinyl]-tetralin (HAPT)

Imaging of vesicular transport of acetylcholine in the central nervous system may serve as a marker of presynaptic terminals that may be defective in neurodegenerative disorders [65]. Two stereoisomeric antagonists of vesicular acetylcholine transport, (*R,R*)- and (*S,S*)-HAPT, were recently radiolabelled with C-11 and studied by PET in the living monkey brain [66]. The PET findings confirmed the stereoselectivity of the vesicular acetylcholine transporter towards (*R,R*)- and (*S,S*)-[^{11}C]HAPT; only the (*R,R*)-form bound selectively to sites in the striatum, a brain region with a high transporter density [67]. The PET findings make (*R,R*)-[^{11}C]HAPT a likely candidate for further studies of vesicular acetylcholine transport in the living brain.

7.2 Vesicular Monoamine Transport

7.2.1 (+)- and (-)-3-Isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinolin-2-ol (Dihydrotetrabenazine)

Radiolabelled dihydrotetrabenazine has been used to study the stereoselectivity of the vesicular monoamine transporter because the (+)-form has 1000-fold higher affinity than the (-)-form for the binding site [68]. PET scanning with (+)- and (-)-[^{11}C] dihydrotetrabenazine in healthy subjects showed highest binding of the (+)-form in striatum and lowest binding in cerebral cortices [69]. Shortcomings in the use of (+)- and (-)-[^{11}C] dihydrotetrabenazine for PET studies of the vesicular monoamine transporter have, however, called for other high-affinity, mirror-image radioligands to explore the stereoselectivity of vesicular monoamine transport in neuropsychiatric disorders [70].

8. OPIOID NEUROTRANSMISSION

8.1 Opiate Receptor

8.1.1 (+)- and (-)-6-Deoxy-6β-fluoro-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one (Cyclofoxy)

Racemic cyclofoxy blocks opiate receptors: (-)-cyclofoxy has high affinity at *μ*- and *κ*-receptors, whereas (+)-cyclofoxy has virtually no affinity at those sites [71]. In a PET study, a four-fold difference was observed between the accumulation of the enantiomers of [^{18}F]cyclofoxy in thalamus and caudate of the living monkey brain, in favor of the (-)-form [72]. Subsequent work has focused on kinetic models for analysis of PET data obtained with (+)- and (-)-radiolabeled cyclofoxy in order to establish the reference isomer procedure for quantifying opiate receptors in living brain [73,74]. Thus, a subsequent study assessed kinetic data obtained using tracer amounts of (-)-[^{18}F]cyclofoxy and (+)-[^{3}H]cyclofoxy co-infused with the unlabelled antipode [75]. The results indicated that (+)-cyclofoxy can be used to estimate the non-specific component of (-)-cyclofoxy binding in brain.

8.1.2 (*R*)- and (*S*)-4-Methoxycarbonyl-2-[(1-pyrrolidinylmethyl]-1-[(3,4-dichlorophenyl)acetyl]-piperidine

The (*R*)-enantiomer of 4-methoxycarbonyl-2-[(1-pyrrolidinylmethyl]-1-[(3,4-dichlorophenyl)acetyl]-piperidine, commonly known as GR103545, is a potent and selective agonist of κ-opioid receptors, whereas the (*S*)-enantiomer is markedly less active [76]. (*R*)- and (*S*)-[^{11}C]4-Methoxycarbonyl-2-[(1-pyrrolidinylmethyl]-1-[(3,4-dichlorophenyl)acetyl]-piperidine have been used for PET imaging to explore the stereoselectivity κ-opioid receptors in the living brain [77]. The volume of distribution of [^{11}C]GR103545 was high in cingulate cortex, striatrum, and thalamus, moderate in frontal, temporal and parietal cortices, and low value in occipital cortex and brain stem. In contrast, the antipode failed to accumulate in any region, reflecting only homogeneous, nonspecific uptake in the living brain. Thus, the PET results demonstrate the stereoselectivity of κ-opioid receptors in the living brain.

9. GLUTAMATERGIC NEUROTRANSMISSION

9.1 Ionotropic NMDA Receptor

9.1.1 (*R*)- and (*S*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone (Ketamine)

Racemic ketamine binds noncompetitively to the NMDA-glutamatergic receptor [78]. (*R*)- and (*S*)-[^{11}C]Ketamine have been used as PET radioligands for studying the stereoselectivity of NMDA receptors in the living brain [79]. The enantiomers were retained to a similar extent at central sites, with regional binding potentials of 0.25 – 0.55 for (*S*)-[^{11}C]ketamine and of 0.15 – 0.42 for the (*R*)-form. PET displacement studies showed that (*S*)- and (*R*)-[^{11}C]ketamine competed for the same binding sites. Evidently, the NMDA-glutamatergic receptor lacks stereoselectivity toward the enantiomers of ketamine in the living brain.

9.1.2 cis- and trans-4-Methylbenzyl-4-[(pyrimidin-2-ylamino)methyl]-3-fluoro-piperidine-1-carboxylate

PET radiotracers have been prepared in efforts directed towards imaging of the NR2B subunit of NMDA receptors [80,81], an extrasynaptic biomolecule thought to play a role in glutamate-mediated neuronal death [82]. Autoradiographic studies carried out with rat brain showed relatively high levels of specific binding by both cis- and trans-4-methylbenzyl-4-[(pyrimidin-2-ylamino)methyl]-3-[^{18}F]fluoropiperidine-1-

carboxylate in hippocampus, striatum, olfactory tubercle, cortex, and thalamus [80]. Subsequent microPET studies showed, however, that the [^{18}F]-labelled diastereomers were rapidly cleared from the brain, with little or no stereoselectivity for regional brain uptake [81].

9.2 Metabotropic Glutamate Receptor Subtype 5

9.2.1 3-(6-Methylpyridin-2-ylethynyl)-cyclohex-2-enone-*O*-methyloxime (ABP688)

Metabotropic glutamate receptor subtype 5 (mGlu5 receptors) may be linked with mental disorders [83]. The stereoselectivity of mGlu5 receptors was explored recently in an excellent PET study that compared the binding of (*E*)- and (*Z*)-[^{11}C]ABP688 in the living rat brain [84]. Marked stereoselectivity of mGlu5 receptors was noted toward the enantiomers of [^{11}C]ABP688 in that only the (*E*)-form accumulated in relevant brain regions. It is noteworthy that co-administration of (*Z*)- and (*E*)-[^{11}C]ABP688 reduced cerebral accumulation of the (*E*)-form, which serves as a warning against the use of racemic mixtures for imaging of mGlu5 receptors in the living brain.

10. MONOAMINE OXIDASE

10.1 Type B MAO

10.1.1 (*R*)- and (*S*)-*N*,α-Dimethyl-*N*-2-propynylphenethylamine (Deprenyl)

The stereoselectivity of MAO type B has been studied in the living brain with the enantiomers of [^{11}C] deprenyl. (*R*)-[^{11}C] Deprenyl showed greater retention than (*S*)-[^{11}C] deprenyl in brain, accumulating primarily in striatum [85]. A subsequent study compared the kinetics of (*R*)- and (*S*)-[*N*-^{11}C-methyl]-4-fluorodeprenyl in the brain of anesthetized baboons [86]. Accumulation of the (*R*)-form in brain regions exceeded that of the antipode in striatum, thalamus and cerebellum. It is noteworthy that co-adminstration of (*R*)- and (*S*)-[*N*-^{11}C-methyl]-4-fluorodeprenyl markedly lowered the total accumulation of radioactivity to a level below that produced by the (*R*)-form alone. The findings highlight the importance of studying individual components of stereoisomeric mixtures in order to achieve an understanding of the mechanisms governing the kinetic behavior of radiolabelled compounds in the living brain.

11. SECOND MESSENGER NEURO-TRANSMISSION

11.1 Phosphodiesterase Type 4

11.1.1 (*R*)- and (*S*)-4-(3-Cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidinone (Rolipram)

Rolipram is a racemate that inhibits phosphodiesterase type 4 (PDE4), an enzyme that terminates the action of cAMP in brain [87]. The enantiomers of rolipram, radiolabelled for PET, have been used to estimate the concentration of PDE4 in living brain [88]. The study involved baseline scans with intravenous injection of either (*R*)-[^{11}C]rolipram or (*S*)-[^{11}C]rolipram followed by scans with increasing intravenous doses of unlabelled (*R*)- or (*S*)-rolipram prior to injection of the radiolabelled compounds in anesthetized pigs. Stereoselectivity of PDE4 in the living brain was confirmed by the fact that the accumulation of (*R*)-[^{11}C]rolipram was several fold greater than that of (*S*)-[^{11}C]rolipram. A subsequent study used (*R*)-[^{11}C]rolipram and (*S*)-[^{11}C]rolipram of high specific activity for PET brain imaging in anesthetized rats [89]. (*R*)-[^{11}C]Rolipram reached higher peak values and remained longer in brain tissue than the (*S*)-form; only the (*R*)-form of [^{11}C]rolipram showed regional differences in distribution volume, with highest levels in frontal cortex and lowest levels in midbrain. Such findings highlight the stereoselectivity of enzymatic processes in the living brain.

12. BLOOD BRAIN EFFLUX TRANSPORT

12.1 P-Glycoprotein Efflux Transport

12.1.1 (*R*)- and (*S*)-5-[*N*-(3,4-Dimethyoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (Verapamil)

Verapamil is a substrate for the P-glycoprotein efflux transporter. The enantiomers of [^{11}C] verapamil have been used for PET to assess the P-glycoprotein efflux transporter in the living brain [90]. No reliable difference was observed between the accumulation of (*R*)- and (*S*)-

[^{11}C]verapamil in the brain of wild type and knock-out mice. Evidently, the P-glycoprotein efflux transporter lacks stereoselectivity toward the spatial features of (*R*)- and (*S*)-verapamil.

13. DISCUSSION

Imaging techniques such as PET and SPECT enable us to assess the stereoselectivity of neurotransmission in the living brain. Three main motives have encouraged the use of PET and SPECT for that purpose. Firstly, scientists desire optimal conditions for quantifying the kinetics of neuroreceptor events. Secondly, drug companies desire detailed information for selecting optimal molecules for patient care. Thirdly, health authorities desire optimal procedures for diagnosing brain disorders and for evaluating treatment procedures.

We have highlighted the clinical significance of stereoselective PET and SPECT radioligands for diagnostic imaging to accomplish personalized treatment. Stereoselectivity is of fundamental importance in living systems due to the dependence of molecular processes on the spatial orientation of constituent atoms. Various CNS functions are governed by stereoselectivity of psychotropic drugs. In vivo, non-invasive PET and SPECT imaging systems provide unique opportunities to study these stereoselective processes in the brain.

Recently, radiochemists have synthesized several stereoisomers for PET/SPECT imaging to investigate molecular events in the living brains of lab animals and humans. These studies have illustrated that various aspects of neurotransmission are associated with stereoselective events, which can significantly impact brain function in health and disease. Currently, neuroscientists and drug developers are faced with the challenge of synthesizing relatively more effective and less toxic treatments for neurological disorders. Hence, further investigations on molecular imaging employing stereoselectivity principles will confer valuable information for the differential diagnosis and effective treatment. It is, however, noteworthy that some studies have failed to detect stereoselectivity in certain neuronal events. For example, reliable stereoselective differences failed to appear between (*S*)- and (*R*)-[^{11}C]nicotine studied by PET neuroimaging, despite the marked stereoselectivity of the enantiomers in some other test systems [91,92]. Likewise, glutamatergic receptors showed negligible stereoselectivity toward (*S*)- and (*R*)-[^{11}C]ketamine studied by PET in the living brain, while marked differences have often been reported between the enantiomers of ketamine studied in vitro [93].

Several factors may limit the capacity of PET and SPECT imaging to demonstrate reliable differences between central effects of mirror-image radioligands. First, if the signal-to-noise ratio of radioactivity in brain tissue is low, then small differences between the pharmacokinetics of radiolabelled mirror-image molecules may go undetected. Second, if a variety of non-stereoselective central and peripheral mechanisms are involved in the metabolism, distribution, and binding of radiolabelled, mirror-image compounds, then stereoselective events may be difficult to identify [69]. Third, if an inappropriate kinetic model is applied to data obtained by PET and SPECT, then differences in the dynamics of radioligands can be missed. Fourth, if potent anesthetics are used with PET and SPECT imaging, then stereoselective neuronal processes may be disrupted.

14. CONCLUSION

Today, neuroscientists and drug developers continue to face the challenge of producing more effective, less toxic treatments for brain disorders. In our view, further attention to molecular imaging of stereoselective processes in the living brain, in health and disease, will provide valuable information for determining the causes, consequences, and cures of brain disorders.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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