

Functional Brain Receptor Imaging with Positron Emission Tomography

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Abstract: A new cocaine derivative for imaging the dopamine transporter has been developed. Measurements of radioligand binding of ^{11}C -(+)-McN-5652 *in vivo* with PET suggests that ecstasy interacts directly with the serotonin reuptake sites and that a single oral dose of ecstasy (1.5 mg/kg) does not cause any changes in the serotonin transporter density in the human brain. Finally, a number of epibatidine derivatives have been developed as ligands to study the central nAChRs *in vivo*, however, toxicity studies prevented further clinical use.

Keywords: Dopamine and serotonin transporters · Ecstasy · Epibatidine · PET · Pharmaceutical chemistry

Introduction

In this paper, we present the activities of two groups that are involved in the development of radioligands for imaging brain functions within the Center for Radiopharmaceutical Science of ETH, Paul Scherrer Institute and University Hospital of Zürich. The groups are the *Radio-tracer Synthesis Group* and the *Radio-pharmacy Division Group* in Zürich. The aim of both research groups is the development of selective and high affinity radioligands for diagnosis using non-invasive positron emission tomography (PET). Research is on the dopaminergic, serotonergic, glutamatergic and the nicotinic acetylcholine neurotransmission systems, however, the last two neurotransmission systems are currently the main focus of research. The research activities cover all aspects of preclinical research and BAG-approved clinical investigations. Some of our research efforts on the dopaminergic, serotonergic, and the nicotinic acetylcholine neurotrans-

mission systems are discussed here. It should be mentioned that extensive research efforts are also being made to develop PET radioligands for the glutamatergic neurotransmission system for which currently no PET radioligand exists.

PET is an imaging modality that allows the study of physiological, biochemical and pharmacological functions at a molecular level. Its strength is the ability to obtain direct quantitative information on the pharmacokinetics and pharmacodynamics of a biomolecule in living animals and humans. PET offers a specific advantage when studying biological molecules that can be labelled with positron emitting radioisotopes such as ^{11}C , ^{13}N , and ^{15}O with half-lives of 2.1, 10 and 20.3 min, respectively. Since the main constituents of biological molecules are carbon, nitrogen and oxygen, the incorporation of ^{15}O , ^{13}N or ^{11}C into pharmaceuticals leads to radiopharmaceuticals which are chemically indistinguishable from their non-labelled counterparts. Apart from negligible isotopic effects, the radiolabeled pharmaceutical possesses the same pharmacological properties as the non-labelled compound. ^{18}F with a half-life of 110 min is another frequently used positron-emitting radionuclide. However, only a few bioorganic compounds contain either a fluorine atom or a halogen. ^{18}F may be substituted for

hydrogen in many compounds with no substantial change in their biological activities. Because most PET radioligands are produced at high specific radioactivity, the PET technique employs trace amounts (few nanomoles) of the pharmaceuticals so that the risk of untoward effect or chemical toxicity remains also negligible.

Establishing the usefulness of a PET radioligand for human applications requires the expertise of several scientists including radiochemists, pharmacologists and clinicians who work in a close collaboration in an interdisciplinary environment. There are a number of criteria that must be fulfilled in order to obtain a brain receptor ligand that can be useful *in vivo*. The conditions are briefly discussed below.

High Affinity for the Receptor ($K_d \leq 10^{-9}$ M)

The equilibrium dissociation constant (K_d) of a drug receptor complex is the concentration of drug that occupies or binds to 50 % of available receptor population. By definition, affinity is the reciprocal of equilibrium dissociation constant. Considering that the concentration of binding sites (B_{max}) for most brain receptors is rather low (nano- to femtomoles per milligram tissue), PET radio-

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ligands should have binding affinities in the subnanomolar range. This can be established *in vitro* either by a direct or competitive binding assay.

Specific Radioactivity

Specific radioactivity refers to the amount of radioactivity per unit mass of a radiopharmaceutical. Unlike radioligands used only for *in vitro* binding assays, radioligands used for *in vivo* brain receptor imaging must be prepared in high specific radioactivities so that only a small percentage of the total number of available binding sites are occupied by the radioligand. Specific radioactivities

should be greater than 3700GBq/mmol. Thus, saturation of binding sites and consequent pharmacological effects are avoided.

Metabolism and Route of Application

Whereas many drugs are given orally, PET radioligands are administered in most cases by an intravenous injection. The oral administration of a PET radiopharmaceutical would result in too rapid a metabolism and a slow uptake of radioligand into plasma. These pharmacodynamic properties would not be compatible with the relatively short half-lives of

commonly used PET radionuclides. Because PET cannot discriminate between signals from parent radioligand and radiolabeled metabolites, care must be taken to ensure that radioactive metabolites that are formed in the course of the studies do not contaminate the PET signals. It has to be verified separately for each PET radioligand whether the PET signals originate from a single radiolabeled chemical entity or not.

Blood-Brain Barrier Permeability

For a central nervous system (CNS) radiopharmaceutical to be useful as an

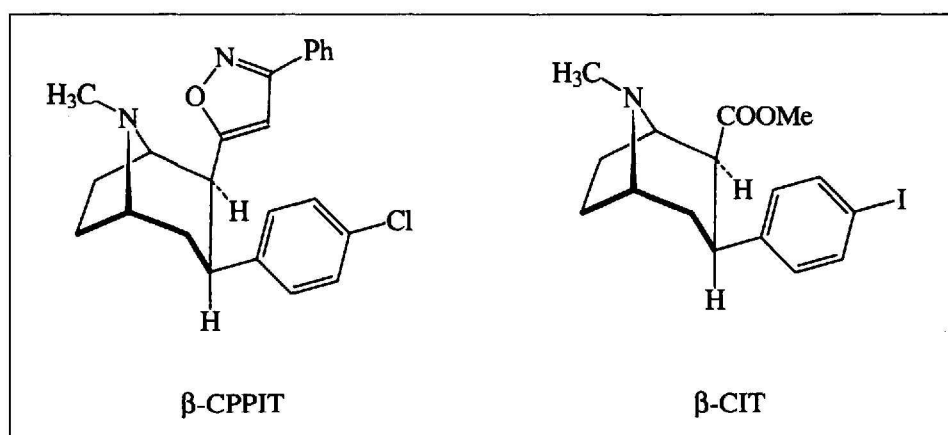


Fig. 1. Structures of β -CPPIT and β -CIT

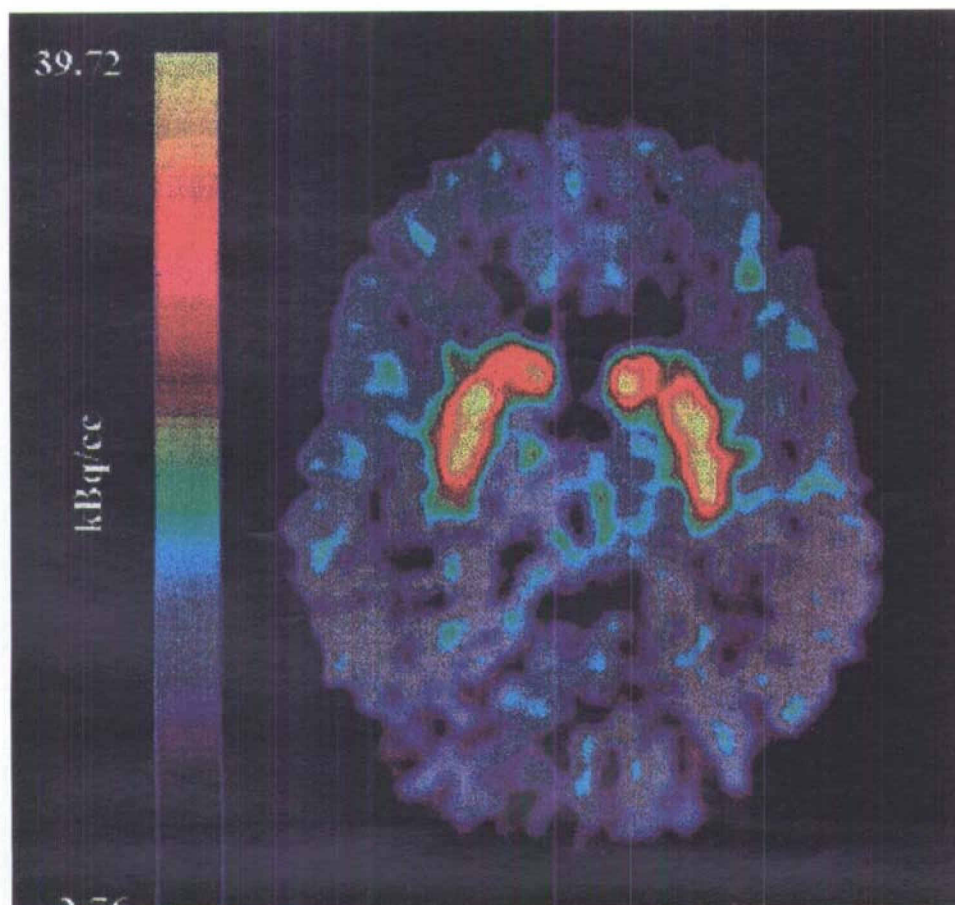


Fig. 2. PET-image at the striatal level after *i.v* injection of [^{11}C]-CPPIT into a healthy volunteer.

imaging agent, it must be lipid soluble and should pass the blood-brain barrier (BBB). The octanol/water partition coefficient, *P*, is often used as a predictor for BBB penetration. The *P* values can be computed or experimentally measured and log *P* values between 2 and 3 are generally considered optimal.

Clearance Rate and Binding to Proteins

Also of importance are rapid clearance rates from blood and non-specific binding sites. A low binding of radioligand to plasma proteins is also essential because only the unbound fraction of radioligand in plasma is available for diffusion out of the vascular tissue.

Dopaminergic System

Changes in the brain neurotransmission have been implicated in the pathophysiology of several brain disorders and therefore receptors and reuptake sites of the various neurotransmission systems have become targets for radiopharmaceutical design in the last years. With regard to the dopaminergic neurotransmitter system, we have radiolabeled cocaine analogues such as β -CIT (2 β -carbomethoxy-3 β -4-iodophenyl)tropane and FP- β -CIT (N-fluoropropyl-2 β -carbomethoxy-3 β -4-iodophenyl)nortropine with ^{11}C . Notable disadvantages associated with these compounds are non-selective binding to the serotonin transporter and the late binding equilibrium. Until recently, we have been using [^{11}C]FP-CIT on routine basis to examine changes in dopamine transporter density in Parkinson patients. A rather new compound, 3 β -(4'-chlorophenyl)-2 β -(3'-phenyloxazolone-5'-)tropane (CPPIT) (Fig. 1), a cocaine analogue that lacks the 2 β -ester group has been radiolabeled with ^{11}C by N-methylation and evaluated in mice and in five healthy volunteers. Blockade studies in mice confirmed the *in vitro* selectivity reported for CPPIT. The uptake of radioactivity in the striatum, a region with the highest densities of the dopamine transporter, was significantly reduced by preinjecting GBR 12909 (5 mg/kg), a dopamine transporter antagonist. PET studies of [^{11}C]CPPIT in healthy volunteers indicated high accumulation of the radioligand in the striatum (Fig. 2). Results obtained so far indicate that [^{11}C]CPPIT has the potential to be used as a radioligand for imaging the dopamine transporter in humans.

Serotonergic System

The compound, (+)-McN-5652 (Fig. 3), is an exceedingly potent and selective blocker of the serotonin transporter and ^{11}C labelled *trans*-(+)-McN-5652 is generally accepted as the most successful ligand for visualising the serotonin transporters *in vivo* using PET. We have recently improved on the synthesis of *trans*-(+)-McN-5652 and have also established a method to prepare the enantiomerically pure (+)-thioester precursor in high reproducible yields.

The human use of methylenedioxyamphetamine (MDMA or ecstasy) as a recreational drug is of a major concern due to the fact that ecstasy is a selective neurotoxin that causes damage to serotonin neurons. A decrease in serotonin transporter density has been demon-

strated in chronic MDMA users. Using PET and S-methyl labelled ^{11}C -(+)-McN-5652, we examined whether a single acute oral dose of ecstasy had any effects on the serotonin transporter density in healthy volunteers. Distribution volume was used as a parameter for radioligand binding.

The results (Fig. 4) showed that a single oral dose of ecstasy (1.5 mg/kg) given 90 min prior to PET measurement, significantly reduced ^{11}C -(+)-McN-5652 binding in subcortical regions compared to baseline scan. The reduction in ^{11}C -(+)-McN-5652 binding suggests that ecstasy interacts directly with the serotonin reuptake sites in the human brain. A second question of the study was whether four weeks after a single oral dose of ecstasy, probable alterations in the density of the serotonin transporter would persist and still be detectable. Four weeks after the second scan, a third PET scan was performed in the same volunteers. No significant effects of the single oral dose of ecstasy on the binding of ^{11}C -(+)-McN-5652 were observed. These results corroborate and extend numerous findings in animals that one or a few single doses of ecstasy (1.5 mg/kg) are unlikely to cause alterations in serotonin transporter density indicative for serotonergic neurotoxicity.

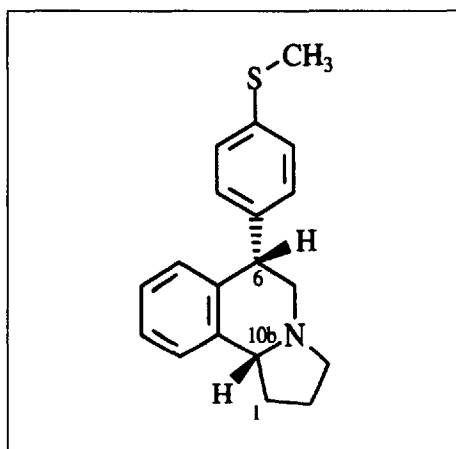


Fig. 3. Structure of (+)-McN-5652

Neuronal Nicotinic Acetylcholine Receptors (nAChRs)

Nicotinic receptors are involved in a broad variety of physiological and patho-

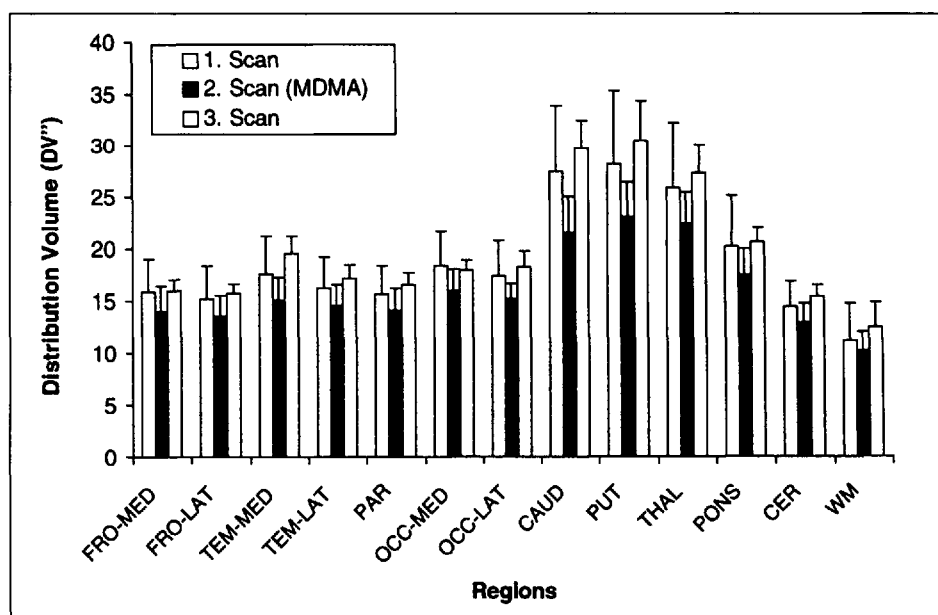


Fig. 4. Distribution volumes in different brain regions derived from three PET scans using [^{11}C](+)-McN-5652 (one-tissue compartment model). Scan 1: baseline scan, scan 2: after ecstasy pretreatment, scan 3: scan after one month. Data are shown as means \pm SD (*n* = 5).

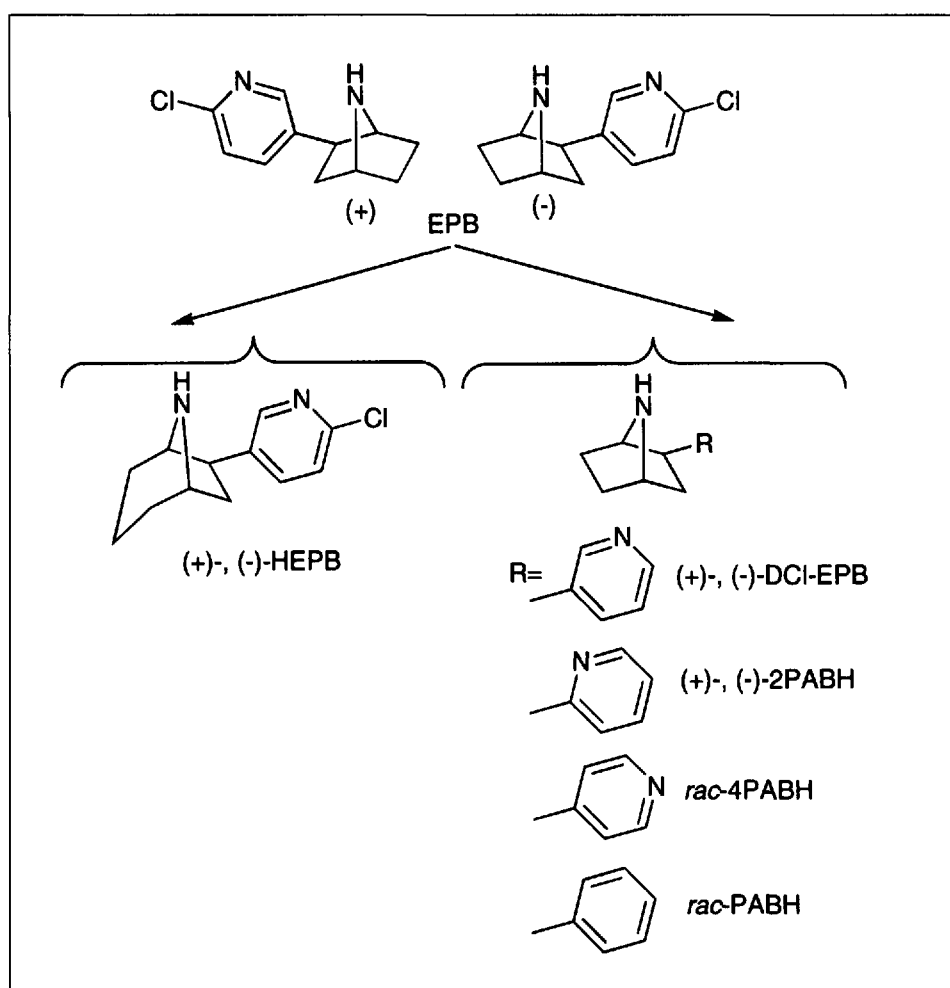


Fig. 5. Structures of EPB analogues

physiological phenomena in the CNS. The development of new drugs acting as cholinergic channel modulators might be beneficial for the treatment of Alzheimer's disease, Parkinson's disease, attention deficit hyperactivity disorder, Tourette's syndrome, schizophrenia, depression, anxiety, smoking cessation, and for the treatment of pain, and neurogenic inflammation. Furthermore, nicotinic receptor subtypes are involved in learning, memory, and cognitive performance. Subtype selective ligands of nicotinic nAChR may be useful in the treatment of these CNS disorders. Beside a possible benefit as therapeutically useful substances, the new ligands also might be useful as radiotracers in biological research and for the diagnosis of above mentioned CNS disorders.

The syntheses of all ligands followed a convergent path: the azabicyclo[2.2.1]heptene system was constructed and the pyridine moiety was attached (Heck reaction). Variations about the pyridine ring of EPB and modification of the bicyclo-moiety resulted in novel ligands (Fig. 5).

The syntheses of EPB, HEPB, DCI-EPB, 2PABH, and 4PABH, were recent-

ly reported. With the exception of PABH and 4PABH, all compounds were separated into their enantiomers *via* chiral high-performance liquid chromatography as described recently. Optical purities higher than 98% ee were obtained. For *in vivo* studies and further investigations with PET EPB, DCI-EPB, 2PABH and HEPB were radiolabeled with ^{11}C , which was introduced by selective methylation of the 7-aza or 8-aza position.

Rats were investigated in a PET scanner in such a way that a dynamic data set was acquired. Experiments with rats gave excellent results. Using this method, the ^{11}C -methylated enantiomers of HEPB, DCI-EPB and 2PABH were compared with N- ^{11}C -methyl-(+)-EPB and (-)-EPB. Promising results were obtained for N- ^{11}C -methyl-(-)-DCI-EPB and N- ^{11}C -methyl-(-)-HEPB. Both radioligands showed high brain uptake with high levels of specific binding and thus revealed a similar *in vivo* behaviour as N- ^{11}C -methyl-(-)-EPB. In contrast, the labelled 2PABH derivatives did not display good brain uptake curves. As a result the 'DCI-EPB-' and the 'HEPB-pathway' represented two encouraging

concepts to develop a useful nAChR radio-ligand.

The N- ^{11}C -methyl compounds show impressive images of the thalamus: the site of highest nicotine receptor density; cytosine-blocked (-) and the (+)-isomer images show no specific brain uptake (Fig. 6). The eyes also accumulate radioligand, but this is only visible after cytosine block and with the (+)-isomer, where no specific brain uptake occurs.

To determine the concentration of the unchanged ^{11}C radioligand during the PET-study, a solid phase extraction was done and tissue tracer kinetics were analysed according to a two or three compartment model. As expected, the (-)-enantiomers revealed in contrast to the (+)-enantiomers high levels of specific binding. In addition, the *in vivo* kinetics revealed high affinity, high specificity and reversible transport. Thus, these ligands proved to be suitable ligands to study the central nAChRs *in vivo*. Despite these encouraging results, detailed toxicity studies prevented further clinical use: even at levels where no carrier was added to the ^{11}C compounds, the toxicity of the compounds was too high for routine human use.

In conclusion epibatidine and its analogues are excellent ligands for the nAChRs and the ^{11}C -methylated derivatives have ideal pharmacokinetic properties as PET ligands. However, their extreme toxicity prevents human application.

Collaborations exist outside the Center for Radiopharmaceutical Science among others with Prof. Gustav K. von Schulthess, University Hospital Zürich, Prof. Daniel Bertrand, University of Geneva, Associate Prof. Franz W. Vollweiler, Psychiatric Hospital Zürich, and pharmaceutical industries.

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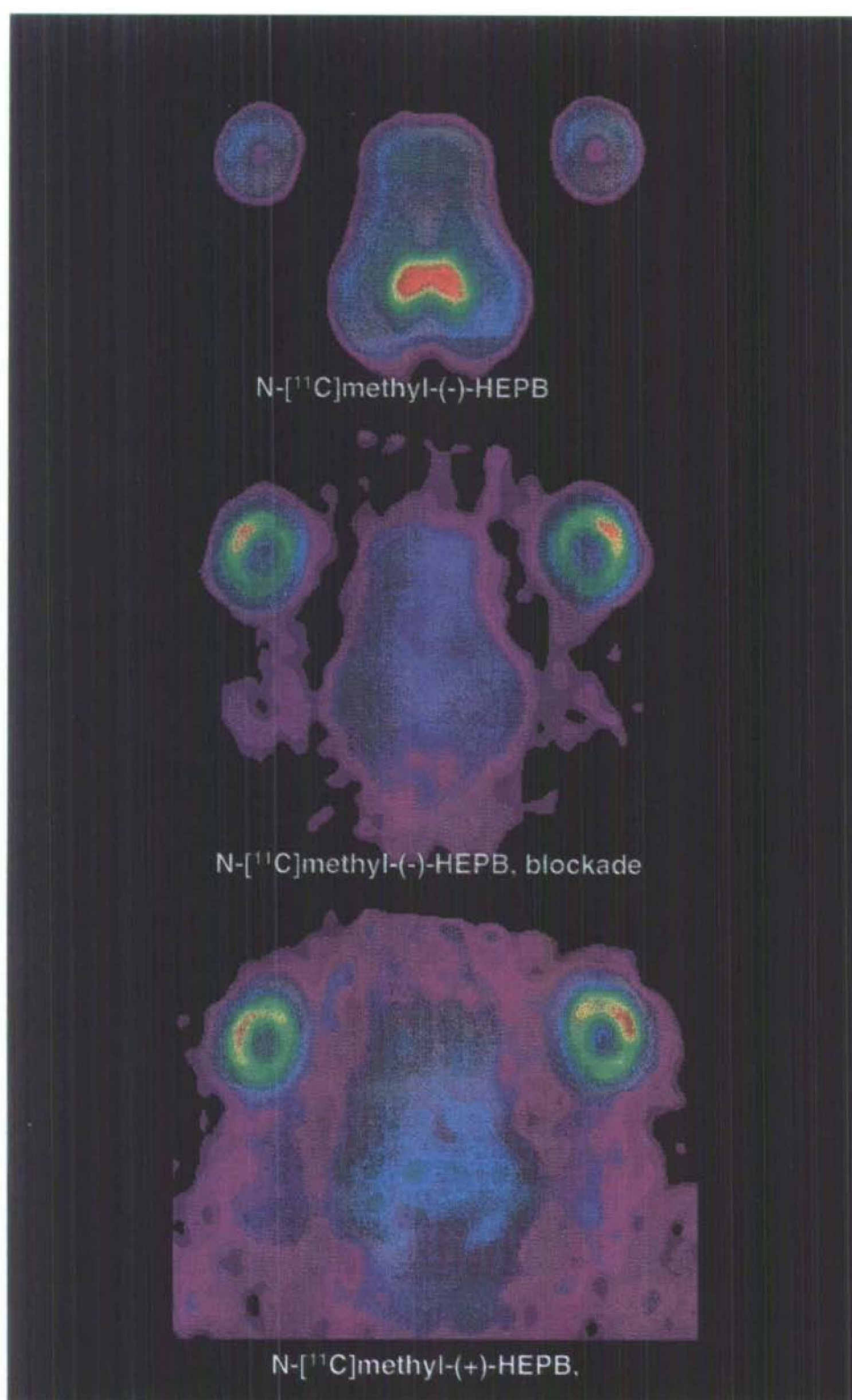


Fig. 6. Pig brain images of N-[^{11}C]methyl-(-)-HEPB (without and with blockade by cytosine) and of N-[^{11}C]methyl-(-)-HEPB; summation of all frames from 30–90 min. All pictures are coronal brain slices of the same position. Beside the brain the eyes are visible as two lateral activity spots. The eye uptake is the same in all cases, but the images of the eyes are clearer in the blocking experiment and with N-[^{11}C]methyl-(+)-HEPB because the intensity scale is normalised on the most active spots in the image.

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