PET Neuroimaging in Pigs

by ***Aage Kristian Olsen Alstrup[1,*] & Donald F. Smith[1,2]***
[1]PET Center, Aarhus University Hospital, Aarhus, Denmark
[2]Center for Psychiatric Research, Psychiatric Hospital of Aarhus University, Risskov, Denmark

Summary
Current interest in studying molecular processes as they occur in the living brain has accelerated the use of laboratory animals for neuroimaging of novel radiolabelled compounds. In particular, positron emission tomography (PET) has contributed to the development of radiolabelled compounds for assessing molecular processes in the living brain. The dynamics of PET typically require a relatively large organ size and blood supply in order to properly evaluate radioligand binding kinetics. To fulfil these requirements, pigs have often been used in such studies. Today, much is known about the metabolism, neurotransmission and molecular binding properties of the living porcine brain, and most findings support similarities between neuronal mechanisms in pigs and humans. Here, we review 10-years of PET findings on neuromolecular processes in the living porcine brain and, whenever possible, we relate PET findings in pigs to those obtained in humans.

Introduction
Positron emission tomography (PET) provides opportunities to study molecular processes as they occur in living body organs (*Bailey et al., 2005*). Of particular interest here is the use of PET for gaining information on molecular aspects of brain metabolism and neurotransmission. PET scanners made for human use are often used for basic neuroscience research. In such cases, a laboratory animal of suitable size and weight is required to correspond with the physical properties of the PET scanner. In our experience, young domestic pigs as well as adult minipigs are well suited for such studies. This review presents PET findings on brain metabolism and neurotransmission in the living pig brain published in peer-reviewed journals since 2000 and found via Pubmed.

At least six factors have contributed to the ever-growing interest in using pigs for PET neuroimaging. First, a wealth of information is already available concerning similarities of physiologic and pathologic processes in pigs and humans (*Benevenga, 1986; Tumbleson, 1986; Tumbleson and Schook, 1996*). Second, excellent atlases are available regarding the anatomy of the pig brain (*Félix et al., 1999; Watanabe et al., 2001; Yoshikawa, 1968*); the size of the pig brain (Figure 1) permits studies to be carried out in PET scanners otherwise designed for human use. Third, research groups have shown similarities in several aspects of brain regions such as brainstem, hippocampus, subcortical and diencephalic nuclei in pigs and humans (*Holm and Geneser, 1989; Holm et al., 1992; Ostergaard et al., 1992*). Fourth, the intelligence and versatility of pigs permits studies to explore possible relationships between particular behaviors and neurotransmission in specific brain regions (*Lind et al., 2005*). Fifth, multiple blood samples can be drawn from pigs to carry out accurate metabolite analyses in studies of new PET radioligands (*Cumming et al., 2003a*). Note, however, that no more than 10 % of total blood volume should be drawn from pigs in PET protocols that require their survival (*Diehl et*

**Correspondence:* Aage Kristian Olsen Alstrup, DVM, PhD
PET Center, Aarhus University Hospitals, Nørrebrogade 44, 10G, DK - 8000 Aarhus C, Denmark
Tel +45 8949 4396
Fax +45 8949 3020
E-mail aage@pet.auh.dk

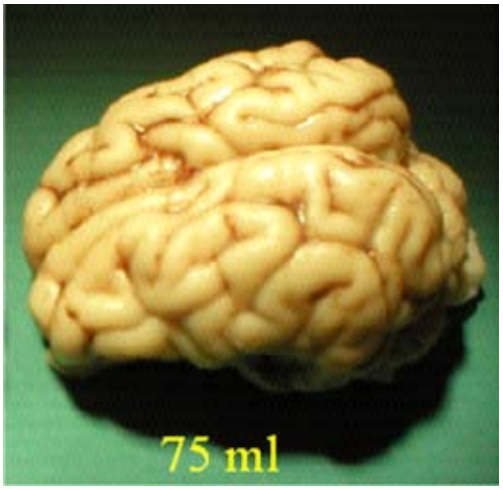

Figure 1. Brain of 40 kg female Danish Landrace pig.

al., 2001). Sixth, pigs can easily be maintained in anesthesia for long-term PET studies with multiple injections of radiotracers (see Table 1 for list of reviewed PET radiotracers) (*Alstrup, 2010; Alstrup and Winterdahl, 2009*). Clearly, pigs have much to offer PET studies of the living brain.

Breeds

The ancestor to all modern breeds of pigs is the wild boar (*Bollen et al., 2010*). Today many breeds of laboratory pigs exist, each with its own characteristics, and therefore it is essential in scientific publications to report which breed is used. However, a nomenclature system, as used in rats and mice, is not used for laboratory pigs. Furthermore, one major problem is that most breeds are not available for research all over the world, and the phenotype of the so-called "Landrace" pig may differ between countries (*Bollen et al., 2010*). Here, we give a brief description of pig breeds that are currently used most often in neuroscience.

Laboratory pig breeds are divided into domestic breeds and miniature breeds. Domestic pig breeds typically weigh 200-350 kg as adults and can, therefore, require special equipment for PET scanning (*Olsen et al., 2007; Bollen et al., 2010*). Landrace pigs are white, but are nevertheless variable in type and may have local characteristics such as drooping ears. Another white breed, the Yorkshire, is also often used as a laboratory pig, as well as coloured pig breeds such as Duroc and Hampshire. Very often, however, hybrid strains are used and can display various phenotypes, even when purchased from the same farmer (*Bollen et al., 2010*). While domestic pigs are primarily bred for meat production, miniature pigs are bred only for research purposes. The body weight of most adult miniature pigs used for brain imaging ranges between 35-70 kg, with the adult Yucatan minipig weighing approximately 70-90 kg. Due to the low adult weight of miniature breeds, they are often used for long-term PET studies, particularly when adult animals are required by the project. Most miniature breeds originate from the Minnesota minipig. Among them are the widely used Göttingen minipig and Sinclair minipig (*Bollen et al., 2010*). Adult Göttingen minipigs are often used in brain PET studies because they are easy to handle and weigh only 35-45 kg. Adult Göttingen minipigs may cost more than most other pig breeds, but their health status tends to be better and the breed is available in most parts of the world (*Bollen et al., 2010*). While brain weights are approximately the same in neonatal domestic and minipigs (27-90 gram), brain weight in adult Göttingen minipigs is lower than that of most adult domestic hybrids (*Jelsing et al., 2006*).

Anesthetics

Pigs can be anesthetized in several ways (*Bollen et al., 2010*). Good sedation and muscle relaxation are important to prevent head movement artefacts in the PET brain images. Furthermore, stable physiologic function is essential during PET scans to enable findings to be validated (*Olsen et al., 2007*). PET studies in awake and anesthetized humans and animals have shown, however, that anesthetics can influence the results of PET studies (*Tsukada et al., 1999; Blaizot et al., 2000*). Anesthesia in general reduces brain metabolism as well as the delivery

Table 1. Alphabetical list of PET radioligands used for imaging studies of pig brain since 2000.

Abbreviated name	Chemical name	Target
[^{18}F]-A85380	[^{18}F]-3-[2(*S*)-2-azetidinyl- methoxy]-pyridine	Nicotinic acetylcholine α4ß2 receptor
[^{11}C]-Butanol	[^{11}C]-Butan-1-0l	Cerebral blood flow
[^{15}O]-CO	[^{15}O]	Cerebran blood volume
[^{11}C]-CP643,051	[^{11}C]-(2*S*,3*S*)-3-(2-methoxy-5-trifluoromethoxy-benzyl)amino-1-methyl-2-phenyl -piperidine	Neurokinin type 1 receptor
[^{11}C]-DASB	[^{11}C]-*N*,*N*-dimethyl-2-(2-amino-4-cyanophenylthio) benzylamine	Serotonin reuptake site
[^{18}F]-DOPA	[^{18}F]-(*S*)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid	Dopamine synthesis
[^{18}F]-FBMV	[^{18}F]-4-(4-fluoro-benzoyl)-7-hydroxy-6-(4-phenyl-piperidin-1-yl)-octahydro-benzo[1,4]- oxazine	Vesicular acetylcholine transporter
[^{18}F]-FDG	[^{18}F]- 2-Deoxy-2-fluoro-D-glucose	Glucose metabolism
[^{18}F]-D-FET	[^{18}F]-*O*-(2-fluoroethyl)-D-tyrosine	Amino acid transport
[^{18}F]-L-FET	[^{18}F]-*O*-(2-fluoroethyl)-L-tyrosine	Amino acid transport
[^{11}C]-GSK189254	[^{11}C]-6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridine carboxamide	Histamine H_3 receptor
[^{11}C]-GSK931145	[^{11}C]-(+)-*N*-[[1-(dimethylamino) cyclopentyl] (phenyl)methyl]-2,6-dimethylbenzamide	Glycine type 1 transporter
[^{11}C]-Harmine	[^{11}C]-7-methoxy-1-methyl-9H-pyrido[3,4-ß]-indole	Monoamine oxidase type A
[^{15}O]-H_2O	Water	Cerebral blood flow
[^{11}C]-McN-5652	[^{11}C]-(6*R*,10b*S*)-rel-1,2,3,5,6,10b-hexahydro-6-[4-(methylthio) phenyl]-pyrrolo[2,1-a]-isoquinoline	Serotonin reuptake site
[^{18}F]-McN-5652	[^{18}F]-(6*R*,10b*S*)-rel-1,2,3,5,6,10b-hexahydro-6-[4-(methylthio) phenyl]-pyrrolo[2,1-a]-isoquinoline	Serotonin reuptake site
[^{11}C]-(*S*,*S*)-MeNER	[^{11}C]-(*S,S*)-2-(α-(2-methoxy phenoxy)benzyl) morpholine	Noradrenaline uptake site
[^{11}C]-Methyl-BIII277CL	[^{11}C]-methyl-[2R-[2-alpha, 3(R*),6 alpha]]-1,2,3,4,5,6-hexahydro-3-(2-methoxypropyl)-6,11,11-trimethyl-2,6-methano-3-benzazocin-9-ol hydrochloride	NMDA receptor
[^{11}C]-Mianserin	[^{11}C]-1,2,3,4,10,14b-Hexahydro-2-methyldibenzo[c,f]pyrazino[1 ,2-a]azepine hydrochloride	Multitarget antidepressant

[^{11}C]-Mirtazapine	[^{11}C]-1,2,3,4,10,14b-Hexahydro-2-methylpyrazino[2,1-a]pyrido[2,3-c][2] benzazepine	Multitarget antidepressant
[^{18}F]-NCFHEB	[^{18}F]-norchloro-fluoro-homoepibatidine	Nicotinic acetylcholine receptor
[^{11}C]-NMHE	[^{11}C]-*N*-methyl-homoepibatidine	Nicotinic acetylcholine receptor
[^{11}C]-NMSP	[^{11}C]-3-*N*-methyl-1,3,8-triazaspiro(4.5)decan-4-one,8-(4-(4-fluorophenyl)-4-oxobutyl)-1-phenyl	Dopamine D_2/D_3 receptor
[^{11}C]-NNC112	[^{11}C]-(+)-8-chloro-5-(7-benzofuranyl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine	Dopamine D_1 receptor
[^{11}C]-NPA	[^{11}C]-(*R*)-*N*-n-propyl- norapomorphine	Dopamine D_2/D_3 receptor
[^{11}C]-NS2214	[^{11}C]-(+)-(E)-1-[(1*R*,2*R*,3*S*)-3-(3,4-dichlorophenyl)-8-methyl-8-azabicyclo[3.2.1]-octane-2-carbaldehyde-*O*-methyloxime	Dopamine reuptake site
[^{11}C]-NS2456	[^{11}C]-(1*RS*,5*SR*)-8-methyl-3-[4-trifluoromethoxyphenyl]-8-azabicyclo [3.2.1]-oct-2-ene	Serotonin reuptake site
[^{11}C]-NS4194	[^{11}C]-(±)-3-(6-nitro-2-quinolinyl)-[9-methyl-11C]-3, 9-diazabicyclo-[4.2.1]-nonane	Serotonin reuptake site
[^{15}O]-O_2	Oxygen	Oxygen metabolism
[^{18}F]-OMFD	[^{18}F]-3-*O*-methyl-L-DOPA	Dopamine metabolite
[^{11}C]-PK11195	[^{11}C]-1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-iso-quinoline carboxamide	Peripheral benzodiazepine receptor
[^{11}C]-Raclopride	[^{11}C]-3,5-dichloro-*N*-[[(2*S*)-1-ethyl-2-pyrrolidinyl]-methyl]-2- hydroxy-6-methoxybenzamide	Dopamine D_2/D_3 receptor
[^{11}C]-RAL-01	[^{11}C]-cis-2-butyl-5-(4-hydroxy- phenyl)-5,6,11,11atetrahydro-1H-imidazo[1V,5V:1,6] pyrido- [3,4-b] indole-1,3(2H)-dione	Phosphodiesterase type 5
[^{11}C]-Rolipram	[^{11}C]-4-[3-(Cyclopentyloxy)-4-methoxy-phenyl]-2-pyrrolidinone	Phosphodiesterase type 4
[^{11}C]-ROMAO	[^{11}C]-(±)-1-(1-methyl-1*H*-pyrrol-2-yl)-2-phenyl-2-(1-pyrrolidinyl) ethanone	Monoamine oxidase type A

[^{11}C]-SB207145	[^{11}C]-8-amino-7-chloro-(*N*-methyl-4-piperidylmethyl)-1,4-benzodioxan-5-carboxylate	Serotonin type 4 receptor
[^{11}C]-Venlafaxine	[^{11}C]-(*R*/*S*)-1-[2-(dimethylamino)-1-(4-methoxy phenyl)ethyl]- cyclohexanol	Serotonin reuptake site
[^{11}C]-WAY-100635	[^{11}C]-*N*-[2-[4-(2-methoxy phenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridyl)cyclohexane- carboxamide	Serotonin type 1A receptor
[^{11}C]-Yohimbine	[^{11}C]-17α-Hydroxy-yohimban-16α-carboxylic acid methyl ester	Noradrenaline α_2 receptor

and elimination of radiotracers. However, published investigations of how anaesthesia and analgesics effect the pig brain are rare (*Kimme et al., 2007*), so great care is always needed if findings obtained in anesthetized pigs are to be generalized to awake humans (*Alstrup and Winterdahl, 2009*).

We have found the following procedure to be suitable for anaesthetizing pigs for PET brain imaging. First, the pig is given an intramuscular injection of midazolam and ketamine in the back of the neck. Second, after deep sedation is achieved, a catheter is inserted into an ear vein through which additional midazolam and ketamine are administered. Third, the pig is intubated with an endotracheal tube. Fourth, anaesthesia is maintained by either inhalation of isoflurane/O_2/N_2O provided from a respiratory or continuous intravenous infusion of an appropriate substance for long-term PET scanning (*Alstrup, 2010*). Fifth, supplementary analgesics are sometimes required in conjunction with potentially painful procedures, such as brain surgery (*Bollen et al., 2010*).

Recently, we evaluated isoflurane and propofol for their effects on binding of radiotracers to noradrenergic alpha$_2$ receptors and dopamine D_1 receptors in minipig brain (*Alstrup et al., 2011*). The binding potentials in several brain regions were lower for the noradrenergic α_2 tracer [^{11}C]yohimbine and higher for the dopamine D_1 tracer [^{11}C]SCH23390 during isoflurane anaesthesia than during propofol anaesthesia. Some of the observed differences seem, however, to be due mainly to changes in cerebral blood flow rather than to alterations in noradrenergic and dopaminergic neurotransmission (*Alstrup et al., 2011*). While some information on the effects of anaesthesia is already available, much more information and research are needed.

Materials and Methods

Instrumentation

Several types of PET scanners are available for studying the brain of pigs. Some PET scanners are combined with computed tomography (CT) to provide improved anatomic location of the biologic processes in merged images (*Alstrup and Winterdahl, 2009*). High-resolution research tomographs (HRRT) have been designed for brain research and have better spatial resolution than ordinary PET scanners. HRRT scanners are well-suited for brain imaging of minipigs and young domestic pigs. Because PET scanners are expensive, they are often used both for preclinical animal studies as well as clinical procedures in humans. PET scanners must, therefore, be cleaned and sanitized after use with pigs. We recommend the use of Standard Operation Procedures for cleaning of scanners and the scanning of pigs and humans on separate days. Pigs have zoonotic organisms, offensive smells and urine that are potential sources of contamination, so the scanner bed should be covered with plastic sheets and blankets to prevent contamination. Obnoxious smells can be prevented by cleaning pigs before they arrive at the PET facility. A bladder catheter can be installed readily in anesthetized female

pigs to prevent urine contamination as well as radiation noise arising from the bladder (*Olsen et al., 2007*). Nappies can be used to prevent urinary contamination by male pigs (*Alstrup and Winterdahl, 2009*). PET studies require intravenous injections of radiotracers that can be given via an ear vein of pigs, but the femoral vein is a better injection site for PET brain imaging in order to avoid potential "hot spots" in the field-of-view of the scanner.

Physiologic monitoring

Results of PET scanning depend on the physiologic condition of the pig. Pigs cannot completely autoregulate homeostasis during anaesthesia, especially not during long-term procedures (*Alstrup, 2010; Bollen et al., 2010*), so monitoring of physiologic processes is required. Basic parameters must be monitored in pigs to assure optimal animal welfare. It is advisable to monitor the following parameters during PET brain imaging of pigs: electrocardial activity, heart rate, respiration rate, body temperature, pulse oxymetry, and reflexes (corneal, palpebral and interdigital). To prevent hypothermia, pigs are placed on thermostatically-controlled electric blankets with monitoring of body temperature during anaesthesia (*Alstrup and Winterdahl, 2009*). Monitoring may also include blood pressure, blood gases (PaO_2 and $PaCO_2$), and blood glucose when arterial catheters are used (*Alstrup and Winterdahl, 2009*). The importance of blood gas monitoring is emphasized by the fact that changes in $PaCO_2$ affect cerebral blood flow and cerebral blood volume (*Olsen et al., 2006*). End tidal CO_2 ($ETCO_2$) can be measured as an alternative to $PaCO_2$ (*Alstrup, 2010*). We recommend continuous infusions of isotonic saline throughout PET-scanning sessions in order to prevent dehydration. Departure of physiologic parameters from normal porcine values requires appropriate adjustments to reinstate the pig in optimal condition.

Cerebral blood flow (CBF)

CBF is typically measured with either $[^{15}O]$-H_2O or $[^{11}C]$-butanol. Today, $[^{15}O]$-H_2O is the most commonly used PET tracer for CBF measurements in pigs (Figure 2). $[^{15}O]$-H_2O is injected intravenously as a single bolus, and dynamic PET scanning and blood sampling are performed for at least 3 minutes (*Olsen et al., 2006*). $[^{15}O]$-H_2O is also widely used as a PET radiotracer in stroke studies carried out in pigs (*Sakoh and Gjedde, 2003; Sakoh et al., 2000; 2001; 2003; Watanabe et al., 2007*). In stroke models that only affect one hemisphere, the brain map of CBF in the ischemic side of the pig can be superimposed on the map of CBF in the non-infarcted side to provide an index of how infarction alters CBF (*Watanabe et al., 2007*). Severe reductions in CBF during stroke are coincident with infarction in pig brains (*Watanabe et al., 2007*). The models for calculation of CBF assume diffusion equilibrium of the PET tracer between tissue and blood. For a diffusion-limited tracer like $[^{15}O]$-H_2O, the models may underestimate CBF. Alternatively, the freely-diffusible PET tracer $[^{11}C]$-butanol can assess CBF (*Herscovitch et al., 1987*). However, the longer half-life of $[^{11}C]$-butanol (20 minutes) than of $[^{15}O]$-H_2O (2 minutes) has made $[^{15}O]$-H_2O the preferred PET tracer for estimating CBF in pig studies.

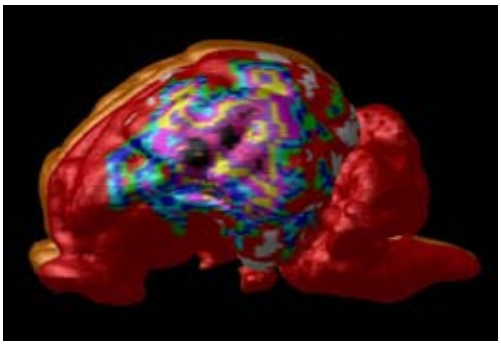

Figure 2. PET image of cerebral blood flow measured with $[^{15}O]$-H_2O in pig brain after middle cerebral artery occlusion (mean of three animals). Regions shown in black and magenta denote markedly reduced cerebral blood flow.

CBF can be used as a primary parameter in pig models of brain disorders, such as in studies of stroke (*Sakoh et al., 2000*), Parkinson´s disease (*Andersen*

et al., 2005) and drug addiction (*Rosa-Neto et al., 2004c*). CBF estimates can also evaluate drug effects (*Rasmussen et al., 2003*) or serve as a control parameter, because changes in CBF may affect the kinetics of PET tracers (*Olsen et al., 2006, Alstrup et al., 2010*). As in humans, the mean CBF in pigs is approximately 50 ml blood /100 cm^3/min (*Olsen et al., 2006*). Cerebral circulation is regulated in such a way that CBF is relatively constant under varying physiological conditions, both in awake and anesthetized subjects (*Dagal and Lam, 2009*). However, variations in arterial CO_2 levels ($PaCO_2$) dramatically affect CBF, with lowered values during hypocapnia and elevated values during hypercapnia (*Olsen et al., 2006*). As in humans, CBF changes approximately 4 % per mm Hg change in $PaCO_2$ (*Poulsen et al., 1997*). The increase in CBF during hypercapnia is an appropriate compensatory mechanism to prevent brain ischemia under conditions of insufficient ventilation (*Olsen et al., 2006*). Severe hypothermia may also decrease CBF in pigs (*Sakoh and Gjedde, 2003*).

Cerebral blood volume (CBV)

Cerebral blood volume (CBV) can also be estimated by PET in the living pig brain using [^{15}O]-CO (*Olsen at al., 2006*). [^{15}O]-CO can be administrated as a gas in a single-breath inhalation followed by 30 seconds of breath-holding (*Sakoh et al., 2000*). Hypercapnia increases CBV in pigs as measured by [^{15}O]-CO (*Olsen et al., 2006*). In addition, acute cerebral artery occlusion markedly reduces regional CBV in the pig brain (*Sakoh et al., 2000*).

Brain metabolism

Brain metabolism of oxygen ($CMRO_2$) in pigs can be measured by PET using [^{15}O]-O_2 (*Poulsen et al., 1997*). [^{15}O]-O_2 can be administrated as a single-breath inhalation followed by 10 seconds of breath-holding. As in humans, $CMRO_2$ in pigs is approximately 170 μmol/100 cm^3/min (*Poulsen et al., 1997*). Estimates of $CMRO_2$ are common in PET studies of disease models carried out in anesthetized pigs. Mörtberg and coworkers noted, for example, a marked decrease of $CMRO_2$ particularly in cerebral cortical regions of the pig brain after resuscitation from cardiac arrest (*Mörtberg et al., 2009*). Similarly, a porcine stroke model showed that unilateral infarction coincided with up to a 50% reduction of $CMRO_2$ compared with the non-infarcted, contralateral hemisphere (*Sakoh et al., 2001, Watanabe et al., 2007*). Also severe hypothermia has been shown to dramatically reduce $CMRO_2$ in pigs (*Sakoh & Gjedde, 2003*).

Brain metabolism of glucose (CMR_{glc}) can be estimated in pigs by PET using [^{18}F]-FDG (*Poulsen et al., 1997*). As in humans, CMR_{glc} in pigs is approximately 25 μmol/100 cm^3/min (*Poulsen et al., 1997*). [^{18}F]-FDG is given as an intravenous bolus and is taken up by brain cells. CMR_{glc} has been used in porcine stroke studies, showing marked decreases in glucose metabolism in ischemic brain regions (*Sakoh et al., 2001, 2003*).

Cholinergic Neurotransmission

Many PET studies of cholinergic neurotransmission have been motivated by interest in neuronal mechanisms of neurodegenerative diseases and cognitive disorders. Patt and coworkers explored the use of [^{11}C]-NMHE in pigs for assessing nicotinic acetylcholine receptors (nAChRs) (*Patt et al., 2001*). One advantage of using pigs instead of smaller laboratory animals for PET relates to the value of being able to draw a series of blood samples large enough for accurate metabolite analyses. They found marked differences in binding between the (+)- and (-)-stereoisomers of [^{11}C]-NMHE in pig brain, in favor of the (-)-form. The uptake of (-)-[^{11}C]-NMHE was greatest in the thalamus, and it was blocked by cytisine, a highly specific central nAChR ligand. Kinetic analyses indicated that regional values for distribution volumes of (-)-[^{11}C]-NMHE followed the known pattern of nAChRs with increasing densities from cerebellum to cortex to thalamus.

Brust and coworkers used young pigs to assess some fluorinated epibatidine derivatives, (+)- and (-)-[^{18}F]-NCFHEB, compared with [^{18}F]-2-F-A85380, for PET brain imaging of nAChRs (*Brust*

et al., 2008). Previous studies had shown that both (+)- and (-)-NCFHEB have relatively high affinities to α4ß2 nAChRs but 20–60-fold lower affinities to ganglionic α3ß4 nAChRs (*Deuther-Conrad et al., 2004*), which would be expected to reduce the likelihood of adverse side effects. Brust and coworkers used distribution volumes to express binding of the radiotracers to nAChRs. (+)-[^{18}F]-NCFHEB reached the highest brain-to-blood ratios, with highest distribution volumes in thalamus, colliculi and hippocampus, and lowest values in cerebellum. There was little difference between (+)- and (-)-[^{18}F]-NCFHEB in their rate of metabolism and removal from the bloodstream. It is noteworthy that time-to-peak and maximum tracer accumulation of [^{18}F]-2-F-A85380 in pig brain closely resembled that found in humans (*Kimes et al., 2003*), thus confirming the relevance of such studies with respect to human brain pharmacokinetics. In addition, displacements studies with the nicotinic receptor inhibitor A81418 in the living pig brain confirmed the binding of the PET stereoisomers to the targeted receptors.

Acetylcholine has been linked with dementia, and its transport into synaptic storage vesicles is regulated by a macromolecule, the vesicular acetylcholine transporter (VAChT). Young pigs have been used for PET imaging of VAChT with radioligands derived from vesamicol (*Sorger et al., 2009*). Specifically, the study was carried out to determine the suitability of a radiolabeled conformationally-restrained vesamicol analogue, [^{18}F]-FBMV, for PET brain imaging of VAChT. The PET radiotracer accumulated in regions with cholinergic neurons and/or cholinergic terminals such as the striatum, the thalamus and the cortex (if you consult the original report, be aware that the legend of Figure 7 belongs to the PET images shown in Figure 8). In addition, displacement studies carried out by intravenous injection of (±)-vesamicol reduced specific binding of [^{18}F]-FBMV by 42 – 60%.

Dopaminergic neurotransmission

Dopamine synthesis

PET studies of dopaminergic neurotransmission in pigs have been particularly popular due to the early availability of suitable radiotracers (*Farde et al., 1985; Hartvig et al., 1991*) and to interest in the biological basis of neurologic and neuropsychiatric disorders (*Egerton et al., 2009; Volkow et al., 1998*).

Proper analysis of PET data often requires information on the metabolic fate of radioligands. To obtain that information for PET studies of dopamine synthesis in pigs, Brust and coworkers examined the dynamics of blood-brain transfer of the radiolabeled dopamine precursor [^{18}F]-DOPA and the radiolabeled dopamine metabolite [^{18}F]-OMFD in three age groups (*Brust et al., 2004a*). The influx of [^{18}F]-DOPA and [^{18}F]-OMFD from blood-to-brain in pigs declined with age, whereas the efflux of radioligand from brain-to-blood declined only for [^{18}F]-OMFD. As a result, the overall blood-brain transfer of [^{18}F]-DOPA showed a decline with age in young pigs, evidently due to maturation of mechanisms in the blood-brain-barrier for amino acid transport. In another PET study, Brust and coworkers examined additional factors affecting brain uptake of amino acids related to dopamine synthesis in pigs (*Makrides et al., 2007*). They used piglets and three PET radiotracers, [^{18}F]-D-FET, [^{18}F]-L-FET and [^{18}F]-L-OMFD, to further investigate the transport of amino acids across the blood-brain-barrier. The transport of radiotracer both into and out of the brain was much greater for [^{18}F]-D-FET than for either [^{18}F]-L-FET or [^{18}F]-L-OMFD in piglets. The marked difference between the passage of [^{18}F]-D-FET versus [^{18}F]-L-FET across the blood-brain-barrier of piglets reflects a stereoselective neurobiologic process (*Smith and Jakobsen, 2007*). Preliminary PET studies of amino acid transport in humans after resection of brain tumor failed, however, to show stereoselective transport of [^{18}F]-FET enantiomers (*Makrides et al., 2007*), which raises questions as to whether the findings in piglets are applicable to amino acid transport in the living human brain.

Bauer and coworkers used [^{18}F]-DOPA for PET in newborn piglets to examine relationships between low oxygen tension and elevated dopamine synthesis in brain (*Bauer et al., 2000*). They found that

asphyxia markedly enhanced the activity of aromatic amino acid decarboxylase, the main enzyme in dopamine synthesis. Next, Bauer and coworkers used newborn piglets to assess the degree to which reducing levels of oxygen and increasing levels of carbon dioxide in the bloodstream affect dopaminergic neurotransmission in brain (*Bauer et al., 2002*). In keeping with their previous findings (*Bauer et al., 2000*), hypoxia and hypercapnia markedly increased decarboxylation of [^{18}F]-DOPA as measured by PET in midbrain and striatum of newborn piglets. Their findings are consistent with the notion that increases in dopamine metabolism during hypoxic insult may cause an accumulation of acidic metabolites that may contribute to the pathogenesis of neuronal injury in newborn brain. Later, Brust and coworkers looked for maturational changes in dopamine synthesis in pig brain (*Brust et al., 2004b*). They compared the rate of decarboxylation of [^{18}F]-DOPA using brain PET in newborn and young pigs and found much higher values in frontal cortex, striatum and midbrain of young pigs than in newborn. Their findings show that brain PET in pigs can chart the differentiation of dopaminergic neuropathways. Walter and coworkers then used PET to determine whether acute traumatic brain injury affects the conversion of [^{18}F]-DOPA to dopamine in pig brain (*Walter et al., 2004*). An interesting aspect of the study concerns the comparison between newborn versus young pigs, in that traumatic brain injury markedly enhanced production of [^{18}F]-dopamine in brain regions of newborn pigs but failed to affect that parameter in young pigs.

Danielsen and coworkers also explored mechanisms involved in the regulation of aromatic amino acid decarboxylase in pigs (*Danielsen et al., 2001a; Danielsen et al., 2001b; Cumming et al., 2001*). It is noteworthy that the rate of decarboxylation of [^{18}F]-DOPA in striatum is similar in healthy pigs and humans (*Danielsen et al., 2001b; Egerton et al., 2010*). In one study, Danielsen and coworkers performed PET with [^{18}F]-DOPA and found that acute administration of haloperidol markedly elevates dopamine synthesis in pig brain (*Danielsen et al., 2001a*). In another study, they induced a Parkinson-like condition in Göttingen minipigs by injection of the neurotoxic MPTP (*Danielsen et al., 2000*); PET with [^{18}F]-DOPA showed reduced activity of aromatic amino acid decarboxylase in the striatum of MPTP-treated pigs. Subsequently, Dall and coworkers reported that xenografting of bilateral striatal implants of fetal mesencephalic brain tissue taken from ordinary piglets restored the decarboxylation of [^{18}F]-DOPA to normal levels in the striatum of MPTP-treated minipigs (*Dall et al., 2002*).

Dopamine reuptake

Danielsen and coworkers used Göttingen minipigs and PET to explore possible relationships between disturbances in dopaminergic neurotransmission induced by MPTP and presynaptic dopamine reuptake (*Danielsen et al., 2000*). As PET radiotracer, they chose [^{11}C]-NS2214 and found a pronounced reduction in radioligand binding. However, subsequent work based on quantitative autoradiography failed to detect dopamine reuptake sites in the brain of Göttingen minipigs (*Minuzzi et al., 2006*). The lack of demonstrable dopamine reuptake sites in minipig brain is a relatively rare example of a notable difference in a specific neurobiological process between the brain of pigs and the brain of humans and rodents (*Chalon et al., 2006; Hall et al., 1999*).

Dopamine D_1 receptor

Cumming and coworkers presented findings on dopamine D_1 receptors from their study of MPTP-induced Parkinsonism in Göttingen minipigs (*Cumming et al., 2001*). They performed PET with [^{11}C]-NNC112 and found that the binding potential of dopamine D_1 receptors in the striatum of healthy pigs resembled that of human striatum (*Abi-Dargham et al., 2000*). MPTP intoxication failed, however, to affect the binding of both [^{11}C]-NNC112 and [^{11}C]-PK11195 in the pig brain, perhaps due to the relatively mild degree of neuropathology caused by the treatment (*Cumming et al., 2001*). Subsequently, Rosa-Neto and coworkers explored the anatomical distribution of dopamine D_1 receptors in

the minipig by PET with [^{11}C]-NNC112 (*Rosa-Neto et al., 2004a*). They found a relative abundance of radioligand binding in the ventral, anterior part of the minipig striatum with similar PET-findings for dopamine D_1 receptors in monkey brain.

Dopamine D_2/D_3 receptor

Pigs have often been used in PET studies of dopaminergic D_2/D_3 neuroreceptors. Danielsen and coworkers used the dopamine D_2/D_3 receptor antagonist [^{11}C]-raclopride for PET in minipigs to determine whether MPTP-treatment affected receptor binding, finding no reliable effect (*Danielsen et al., 2000*), while Rosa-Neto and coworkers described the anatomical distribution of dopamine D_2/D_3 receptors in the dorsal, posterior striatum of minipigs with [^{11}C]-raclopride-PET (*Rosa-Neto et al., 2004a*). Cumming and coworkers used [^{11}C]-raclopride-PET to probe the role of dopamine D_2/D_3 receptors in pigs given nicotine, MDMA ("Ecstacy"), and LSD (*Cumming et al., 2003b; Rosa-Neto et al., 2004b; Minuzzi et al., 2005*). In such studies, reductions in [^{11}C]-raclopride binding are assumed to reflect either drug-induced release of dopamine from presynaptic terminals or competition between the drug and the PET radioligand for receptor binding (*Laruelle, 2000*). They found that each of the treatments reduced dopamine D_2/D_3 receptor binding in pig striatum. Clearly, PET neuroimaging with [^{11}C]-raclopride has demonstrated the suitability of pigs for studying cerebral dopamine D_2/D_3 receptors.

Ishizu and coworkers examined effects of haloperidol on binding of [^{11}C]-NMSP in pig brain and noted a relationship between the concentration of haloperidol in the bloodstream and the degree of inhibition of dopamine D_2/D_3 receptors in pig striatum (*Ishizu et al., 2000*).

Agonists of dopamine D_2/D_3 receptors have also been used for PET studies in pigs. Cumming and coworkers examined properties of the dopamine D_2/D_3 receptor agonist [^{11}C]-NPA in PET studies of minipig brain (*Cumming et al., 2003a*). They found the binding of [^{11}C]-NPA to take place mainly in striatum and thalamus, with low levels in cerebellum. Surprisingly, neither MPTP-treatment nor subthalamic electrical stimulation reliably affected the results.

Noradrenergic neurotransmission

Noradrenaline uptake

Minuzzi and coworkers examined briefly the distribution of noradrenaline uptake sites in pig brain using PET with [^{11}C]-(*S*,*S*)-MeNER (*Minuzzi et al., 2006*). Their findings show binding of the PET radioligand mainly in the midbrain and thalamus of the pig brain, with sensitivity to blockade by desimpramine.

Noradrenaline α_2 receptor

Jakobsen and coworkers used pigs to search for an appropriate PET procedure for studying noradrenaline α_2 receptors in living brain (*Jakobsen et al., 2006b*). They determined the time-course and anatomic distribution of [^{11}C]yohimbine by PET and found higher levels in regions of the cerebral cortex and diencephalon than in the medulla of living pig brain. Displacement studies with either yohimbine or the noradrenaline α_2 antagonist RX821002 (*Hudson et al., 1999*) revealed a reduction of [^{11}C]yohimbine binding primarily in cerebral cortical and diencephalic regions. Thus, the findings show that noradrenaline α_2 receptors can be studied by PET imaging in living pig brain.

Serotoninergic neurotransmission

Serotonin reuptake

The use of selective serotonin reuptake inhibitors (SSRIs) as antidepressant drugs has stimulated interest in developing PET procedures for studying the molecular mechanisms in the living brain. Numerous SSRIs are available for radiolabeling with positron-emitting radionuclides, but few compounds have proved suitable for PET due to rapid metabolism in the bloodstream, slow passage into brain, and high nonspecific binding. Smith and coworkers used pigs to explore the possibility that a preclinical compound, NS2456, radiolabeled with C-11 for PET could serve to quantify serotonin re-

uptake sites in the living brain (*Smith et al., 2001a*). Kinetic analyses indicated that radioligand binding was greater in the thalamus of the pig brain than in cerebellum, a region with relatively few serotonin uptake sites. The overall level of binding in pig brain regions was, however, too low to view [^{11}C]-NS2456 as a suitable compound for quantifying serotonin uptake sites.

Later, Brust and coworkers carried out a series of thorough studies in which young pigs were used to explore the value of McN-5652 for assessing serotonin reuptake sites by PET (*Brust et al., 2003a; Brust et al., 2003b; Kretzschmar et al., 2003*). They radiolabeled the compound with either C-11 or F-18; the latter radionuclide has the advantage of prolonging the lifespan of the radioligand so that it can be sent from a radiochemistry laboratory to a research site. The (+)-enantiomer of McN-5652 showed specific binding to serotonin reuptake sites in porcine brain, whereas the (-)-form provided an index of nonspecific binding. The PET findings obtained in young pigs confirmed and extended results from studies on the pharmacokinetics of McN-5652 in the brain of baboons and humans (*Szabo et al., 1999; Szabo et al., 1995a; Szabo et al., 1995b; Buck et al., 2000*).

Further PET studies of serotonin uptake sites were carried out in pigs as new radiotracers came along. Human studies had shown [^{11}C]-DASB to be suitable for assessing serotonin reuptake sites by PET particularly in the midbrain (*Ginovart et al., 2001; Meyer et al., 2001*), but other radiolabeled compounds deserved attention. Jensen and coworkers compared the properties of [^{11}C]-NS4194 and [^{11}C]-DASB for PET brain imaging in pigs (*Jensen et al., 2003*). They confirmed the value of [^{11}C]-DASB for exploring serotonin reuptake sites by PET in regions of pig brain as in humans, whereas the cerebral distribution of [^{11}C]-NS4194 in pigs was clouded by nonspecific binding. Thereafter, Cumming and coworkers applied [^{11}C]-DASB in a PET study of minipigs to see whether MDMA affected serotonin uptake sites (*Cumming et al., 2007*). Daily high doses of MDMA reduced [^{11}C]-DASB binding throughout the minipig brain, with most marked decreases in cerebral cortical regions.

Serotonin type 1A receptor

Cumming and coworkers determined whether daily doses of MDMA affected serotonin type 1A receptors in minipig brain (*Cumming et al., 2007*). They performed PET with [^{11}C]-WAY-100635 and detected no reliable effect of MDMA on binding by serotonin type 1A receptors in any brain region.

Serotonin type 4 receptor

Serotonin type 4 (5-HT$_4$) receptors have been implicated in cognitive disorders (*King et al., 2008*) and are, therefore, a target for PET neuroimaging. Kornum and coworkers used [^{11}C]-SB207145 to explore 5- HT$_4$ receptors in the brain of adult Göttingen minipigs (*Kornum et al., 2009*). They noted similar distributions of 5- HT$_4$ receptors in pig brain and human brain, with highest levels in striatum and pyramidal cell layer of hippocampus, lowest levels in cerebral cortex, and no detectable specific binding in cerebellum.

Histaminergic neurotransmission

Histamine H_3 receptor

Histamine H_3 receptors in the central nervous system are implicated in certain neurologic and neuropsychiatric disorders (*Ito, 2000*), but they have been relatively difficult to study by PET. Plisson and coworkers managed, nonetheless, to image histamine H_3 receptors in the pig brain with [^{11}C]-GSK189254 (*Plisson et al., 2009*). The regional distribution of [^{11}C]-GSK189254 in pig brain was highest in striatum, moderate in cerebral cortices, and low in cerebellum, which resembles the distribution of histamine H_3 receptors in human brain (*Anichtchik et al., 2001*).

Monoamine oxidase

Monoamine oxidase type A

The intraneuronal metabolism of monoaminergic neurotransmitters by monoamine oxidase (MAO) affects mental processes (*Stahl and Felker, 2008;*

Meyer et al., 2006) and is, therefore, a target for PET neuroimaging. Jensen and coworkers used PET to determine the regional distribution of type A MAO in brain regions of Göttingen minipigs using [^{11}C]-harmine as radiotracer (*Jensen et al., 2006*). Binding of [^{11}C]-harmine in pig brain regions was highest in thalamus, hypothalamus, and ventral forebrain (i.e. cingulate cortex), while lowest binding was in occipital cortex and cerebellum. The findings in pig brain regions resemble the distribution of [^{11}C]-harmine noted in human brain regions (*Ginovart et al., 2006*). Intravenous injection of the potent MAO inhibitor pargyline markedly reduced binding of [^{11}C]-harmine, confirming the value of PET for assessing the enzyme in pig brain.

Later, Jensen and coworkers used pigs to compare the PET kinetics of two novel radiotracers, the enantiomers of [^{11}C]-ROMAO, for brain mapping of type A MAO (*Jensen et al., 2008*). Prior studies had indicated that inhibition of type A MAO was much greater for [^{11}C]-(*R*)-ROMAO than for the antipode. An unexpected finding was, however, that the level achieved in the brain by the (*S*)-enantiomer far exceeded that of the (*R*)-form during the PET scanning interval, with highest values in striatum. Blockage of MAO by parygline showed, nonetheless, a general reduction in binding of [^{11}C]-(*R*)-ROMAO throughout the pig brain, in keeping with the widespread distribution pattern of the enzyme.

Phosphodiesterase

Phosphodiesterase type 4

Phosphodiesterase inactivates second messenger compounds, cyclic AMP and cyclic GMP, in the brain and elsewhere in the body (*Kleppisch, 2009*). Parker and coworkers carried out PET imaging of phosphodiesterase type 4 in the brain of pig with the enantiomers of [^{11}C]-rolipram (*Parker et al., 2005*). Their work showed that binding of the (*R*)-form of [^{11}C]-rolipram exceeded that of the (*S*)-form throughout the pig brain. Competition studies indicated that specific binding of [^{11}C]-(*R*)-rolipram in the pig brain exceeded that of the (*S*)-form by a factor of 10 – 20, making [^{11}C]-(*R*)-rolipram eligible for PET imaging of phosphodiesterase type 4 in the living pig brain.

Phosphodiesterase type 5

The presence of phosphodiesterase type 5 (PDE5) in certain brain cells (*Shimizu-Albergine et al., 2003; Bender and Beavo, 2004*) induced Jakobsen and coworkers to explore the possibility of assessing that enzyme by PET imaging in pigs (*Jakobsen et al., 2006a*). Accumulation of the PET radiotracer [^{11}C]RAL-01 was noted mainly in diencephalon and occipital cortex of pig brain, but was only slightly displaceable by a relatively high dose of unlabelled RAL-01. Based on their findings in pigs, Jakobsen and coworkers concluded that [^{11}C]RAL-01 may have insufficient specific binding for sensitive detection of PDE5 in living human brain.

Multi-target antidepressants

In a series of studies, Smith and coworkers determined in pigs whether some clinically-active antidepressant drugs could serve as PET radioligands for brain imaging. In one study, the dual-action antidepressant venlafaxine was radiolabelled and used for PET in pigs (*Smith et al., 2001b*). Concentrations of [^{11}C]-venlafaxine were only slightly higher in midbrain regions than in cerebral cortex and cerebellum and were dependent on cerebral blood flow (*Smith et al., 1997*). As a result, the pig studies indicated that [^{11}C]-venlafaxine was not well-suited for PET neuroimaging. A similar conclusion was reached for [^{11}C]-mianserin, based on PET imaging of living pig brain (*Marthi et al., 2002b*). Then, Smith and coworkers turned their attention to [^{11}C]-mirtazapine for PET brain imaging in pigs (*Marthi et al., 2002a; Marthi et al., 2003; Smith et al., 2006a; Smith et al., 2006b*). They found [^{11}C]-mirtazapine to have suitable properties for PET brain imaging, with greater accumulation in frontal cortex and thalamus than in cerebellar regions (Figure 3). Competition studies confirmed the presence of receptor bindings by [^{11}C]-mirtazapine (*Marthi et al., 2002a; Smith et al., 2006a*), although the exact types of receptors involved were uncertain due to the multitarget profile

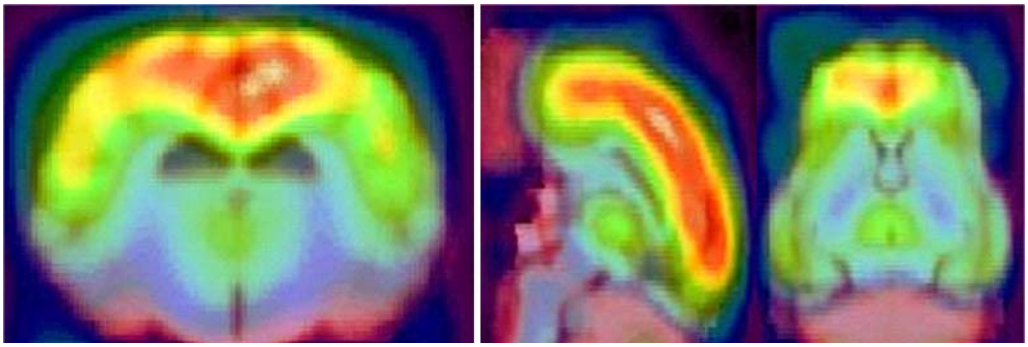

Figure 3. PET image of [^{11}C]mirtazapine in pig brain. Coronal view on left, sagittal view in middle, and transaxial view on right. Low binding is shown in blue and green, and high binding is shown in yellow and red (corresponds to a binding potential of approximately 2). We thank P. Cumming for having prepared the illustration.

of the antidepressant (*Millan, 2006*). Subsequent PET brain imaging with [^{11}C]-mirtazapine and its enantiomer has revealed similar regional distributions in pigs and humans (*Smith et al., 2009; Marthi et al., 2002a; Marthi et al., 2004; Smith et al., 2008; Smith et al., 2006b; Smith et al., 2007*).

Neurokinin (NK)
Neurokinin type 1 receptor
Neurokinin type 1 receptors mediate actions of Substance P, which is a neuropeptide involved in learning and memory, mood and anxiety, stress mechanisms, and emotion-processing (*Vink and Heuvel, 2010*). Bender and coworkers used pigs for PET to determine whether [^{11}C]-CP643,051 can provide a means of imaging neurokinin type 1 receptors in the living brain (*Bender et al., 2004*). They noted most binding of the PET radioligand in the striatum of the pig brain, although the amount of binding was relatively low. Administration of another antagonist reduced the level of [^{11}C]-CP643,051 in pig striatum, although also that effect was relatively small. Their findings showed that [^{11}C]-CP643,051 is suitable in some respects for studying central neurokinin type 1 receptors by PET in living pig brain.

Glutamatergic neurotransmission
NMDA receptor
NMDA (*N*-methyl-D-asparate) receptors contribute to glutamatergic neurotransmission involved with neuroprotection and neurodegeneration (*Hardingham, 2009; Hardingham and Bading, 2003*). Kokic and coworkers were interested in obtaining an appropriate PET radioligand for brain imaging of NMDA receptors (*Kokic et al., 2002*). They chose methyl-BIII277CL for PET radiolabeling, based on its potency and specificity for the NMDA receptor (*Carter et al., 1995*). However, [^{11}C]-methyl-BIII-277CL lacked receptor specificity in the pig brain and was therefore judged to be poorly suited for PET imaging of NMDA receptors.

Glycinergic neurotransmission
Glycine type 1 transporter
Glycine is an inhibitory neurotransmitter involved with glutamatergic neurotransmission (*Betz et al., 2006*). Passchier and coworkers used pigs in order to evaluate several compounds as PET radiotracers for imaging the glycine type 1 transporter (*Passchier et al., 2010*). Detailed studies including PET neuroimaging in pigs provided a sound empirical basis for concluding that [^{11}C]-GSK931145 has suitable properties for assessing glycine type 1 transporter in the living brain (*Passchier et al., 2010*). Their work is an excellent example of how PET neuroimaging in pigs can contribute to discovery and development

of procedures for imaging as yet unexplored aspects of neurotransmission in the living human brain.

Peripheral benzodiazepine receptor

Changes in the population of peripheral benzodiazepine receptors provide an index of neuroinflammation in humans (*Doorduin et al., 2008*). Cumming and coworkers mapped the anatomical distribution of peripheral benzodiazepine receptors in the brain of pigs by PET with [^{11}C]-PK11195 to serve as a basis for animal models of neurologic disorders (*Cumming et al., 2006*). They noted no difference between brain regions in the distribution of [^{11}C]-PK11195 in either Landrace pigs or Göttingen minipigs. However, neuropathology caused by MPTP was associated with the expected increase in [^{11}C]-PK11195 levels in pig brain regions.

Conclusion

PET neuroimaging provides opportunities for studying molecular processes that cannot be studied by any other technology in the living brain. The value of PET brain imaging in pigs rests to some extent on whether the findings can be generalized to humans (*Bjarkam et al., 2008*). The studies reviewed here show, we believe, how PET neuroimaging in pigs informs our understanding of neuromolecular processes and serves as a "proving ground" for testing new radioligands, disease models, pharmacokinetic methods, equipment and imaging procedures. In our view, PET neuroimaging in pigs has provided ample evidence that the outcome of most studies pertains also to humans. As new neurotransmitter systems come-to-light, PET neuroimaging in the living porcine brain can be expected to contribute strongly in the development of appropriate procedures for exploring additional, as yet uncharted, central molecular events in humans.

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