animal

The pig model in brain imaging and neurosurgery

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The pig model is increasingly used in the field of neuroscience because of the similarities of its brain with human. This review presents the peculiarities of the anatomy and functions of the pig brain with specific reference to its human counterpart. We propose an approximate mapping of the pig's cortical areas since a comprehensive description of the equivalent of Brodmann's areas is lacking. On the contrary, deep brain structures are received more consideration but a true three-dimensional (3D) atlas is still eagerly required. In the second section, we present an overview of former works describing the use of functional imaging and neuronavigation in the pig model. Recently, the pig has been increasingly used for molecular imaging studies using positron emission tomography (PET). Indeed, the large size of its brain is compatible with the limited spatial resolution of the PET scanner built to accommodate a human being. Similarly, neuronavigation is an absolute requirement to target deep brain areas in human and in pig since the surgeon cannot rely on external skull structures for zeroing the 3D reference frame. Therefore, a large body of methodological refinements has been dedicated to image guided surgery in the pig model. These refinements allow now a millimetre precision: an absolute requirement for basal nuclei targeting. In the third section, several examples of ongoing studies in our laboratory were presented to illustrate the intricacies of using the pig model. For both examples, after a brief description of the scientific context of the experiment, we present, in detail, the methodological steps required to achieve the experimental goals, which are specific to the porcine model. Finally, in the fourth section, the anatomical variations depending on the breed and age are discussed in relation with neuronavigation and brain surgery. The need for a digitized multimodality brain atlas is also highlighted.

Implications

The pig model is increasingly used in the field of neuro-science because of the similarities of its brain with human. Indeed, aside from the rodent model there is a critical need for a large animal model ethically acceptable, i.e. excluding non-human primates. In this review we present some experiments dealing with the various procedures achievable in the pig model ranging from image-guided brain surgery to functional brain imaging studies. The recent advances in functional imaging data processing pointed out our partial knowledge of pig brain neuro-anatomy and the requirement for a digitalized atlas matching MRI (magnetic resonance imaging) and histological resources.

Introduction

Swine have been used extensively as a model of human in biomedical researches such as cardiovascular, metabolic

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and transplantation (Phillips *et al.*, 1982; Larsen and Rolin, 2004; Imai *et al.*, 2006; Groth, 2007). In the last decade, an increasing number of studies in the field of neuroscience has been reported (Lind *et al.*, 2007). In addition to the study of the action or metabolism of various pharmacological drugs or neurotransmitters (Bhalla *et al.*, 2002; Rosa-Neto *et al.*, 2004a; Lind *et al.*, 2005b; Minuzzi *et al.*, 2005), pathological models dealing with traumatic brain injury (Grate *et al.*, 2003), stroke (Sakoh *et al.*, 2001) or neurodegenerative diseases such as Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) poisoning (Danielsen *et al.*, 2000; Cumming *et al.*, 2001; Dall *et al.*, 2002) have also been developed.

The major benefit of the swine for neuroscience research remains the size of its brain. It is large enough to allow evoked potential (EP) recordings, neurosurgery and conventional imaging in living animals. Furthermore, the pig has cerebral structures common to other mammalian species and, with relative well-defined cerebral circumvolutions, its brain appears to be comparable to human in terms of anatomy, histology and vascularization (for review: Lind *et al.* (2007)).
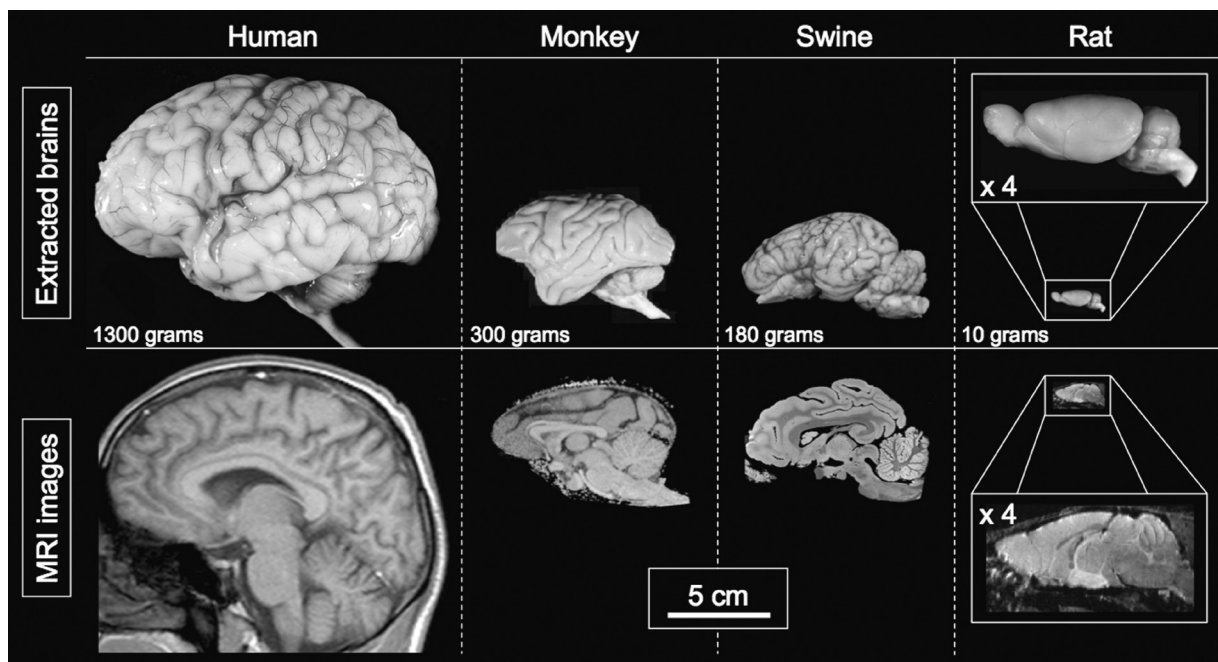
Figure 1 *Ex vivo* anatomical and magnetic resonance imaging (MRI) correlation between brains of four different species: human, rhesus monkey, swine and rat. Brains are displayed at the same scale. Enlarged pictures are provided for the rat. Human, monkey and swine brains are gyrencephalic, contrary to the rat brain, which is lissencephalic. The average brain weight is mentioned for each species. Images of the human brain, monkey brain, monkey MRI are obtained with permission from JC Fournet (University Hospital Sainte-Justine, Montreal, Canada, http://www.humpath.com), CA Pardo-Villamizar (Johns Hopkins University School of Medicine, Baltimore, USA) and C François (Inserm UMR975, Paris, France), respectively. Other images are from our institution.

This represents an undeniable advantage compared to the rodent model for multimodality imaging using magnetic resonance imaging (MRI), computed tomography (CT) or positron emission tomography (PET).

In this review, we will present the particularities of the pig brain in anatomical and functional terms with specific reference to primates and rodents. An overview of former works describing the use of functional imaging and neuro-navigation in the pig model will present the challenge in using the porcine model compared to other animal models. Finally, we will set in perspective work currently ongoing in our laboratory as examples of the capabilities of the pig model in the field of neurosciences.

Anatomical and functional considerations on the pig brain

Pig brain is comparable to that of human in gross anatomy (Felix *et al.*, 1999), myelination, growth and development (Dickerson and Dobbing, 1967; Flynn, 1984; Mayhew *et al.*, 1996). Furthermore, a considerable body of evidence indicates that it resembles primate more than rat when considering its cortical convolutions, shape and total number of neocortical neurons (Lind *et al.*, 2007). The rat has a lissencephalic, smooth-surfaced cerebral cortex, whereas the pig has a gyrencephalic, folded brain cortical surface with well-defined circumvolutions (Hofman, 1985) (Figure 1).

The global aspect of the pig brain differs somewhat from that of primate since the curvature of the telencephalon is less pronounced and the anterior pole is also less developed (Figure 2). Viewed from above, the pig brain has an elongated oval shape with the hemispheres being widest at the posterior third and the occipital pole being larger than the frontal pole. The olfactory system is also much more developed and occupies a large portion of the anterior part of the brain. Though the cerebral sulci and gyri of the pig have been described in a number of studies (Dellman and McClure, 1975; Craner and Ray, 1991a and 1991b; Jarvinen *et al.*, 1998; Okada *et al.*, 1999; Jelsing *et al.*, 2006; Lind *et al.*, 2007), there has been only a limited number of anatomical and histological studies describing the overall structure and cellular organization of the neocortex in pig. Furthermore, discrepancies between results exist (Campbell, 1905; Stephan, 1951). On the contrary, numerous functional studies dealing with specific regions of the cortex have been performed either in miniature or conventional domestic pig. According to the existing literature, we propose an approximate mapping of the pig's cortical areas in Figure 2.

Functional studies have successfully explored the visual cortex of the pig with EP recordings (Laube *et al.*, 2003; Sachs *et al.*, 2005; Strain *et al.*, 2006) or functional MRI (Fang *et al.*, 2006; Gizewski *et al.*, 2007). Exploration of the auditory cortices has also been addressed with EP recordings in miniature pig (Andrews *et al.*, 1990) or cytoarchitectonic techniques in domestic pigs (Plogmann and Kruska, 1990). Using EP recordings, the primary and secondary cortical somatosensory representations (SI and SII cortices) for the snout, the face, the forelimb and the hind limb have been described with varying precision among the studies (Woolsey and Fairman, 1946; Andrews *et al.*, 1990; Craner
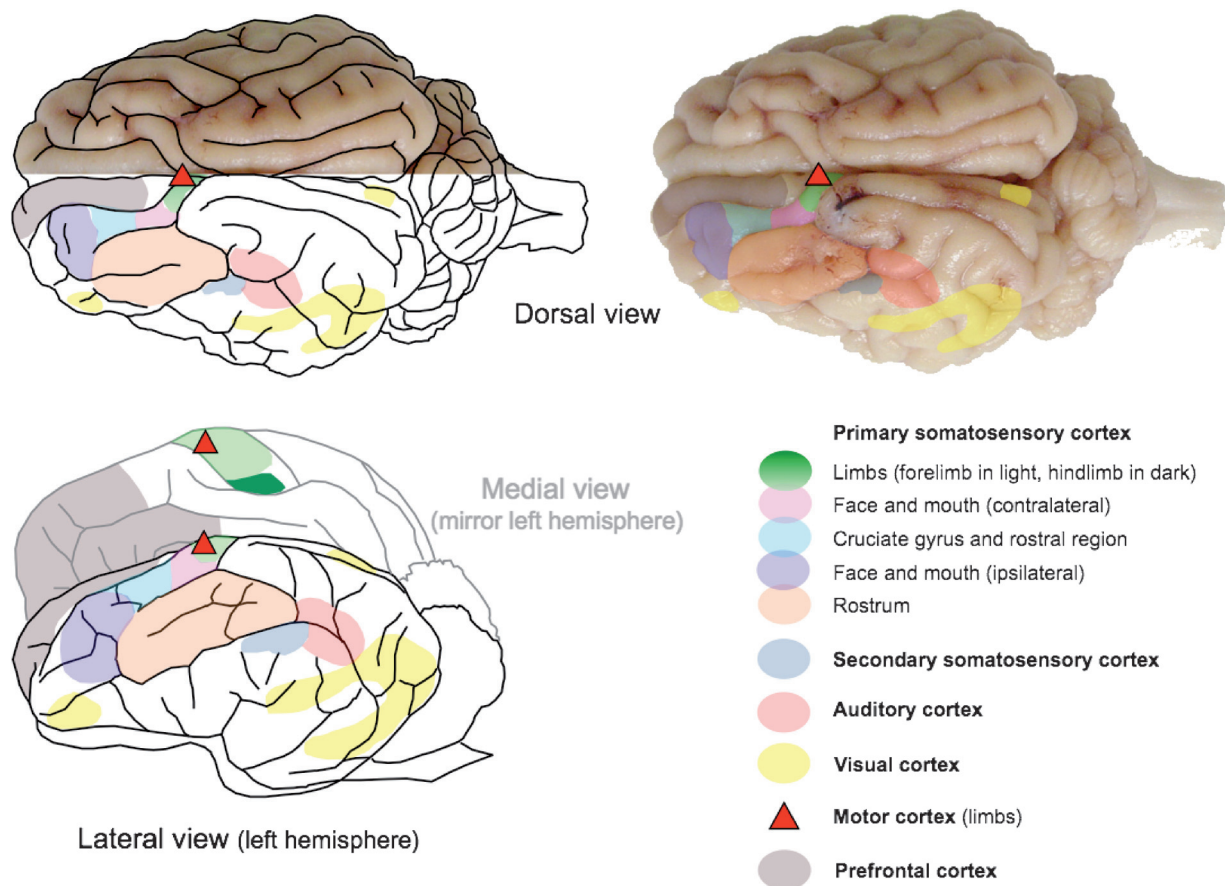
Figure 2 Dorsal, lateral and medial (left hemisphere) views of the brain of a Large White × Landrace pig weighing 30 kg, with schematic representations of the cortical areas. The somatosensory cortex (Woolsey and Fairman, 1946; Andrews *et al.*, 1990; Craner and Ray 1991a and 1991b; Okada *et al.*, 1999), auditory cortex (Andrews *et al.*, 1990), visual cortex (Laube *et al.*, 2003; Fang *et al.*, 2006; Gizewski *et al.*, 2007), motor cortex (Palmieri *et al.*, 1986) and prefrontal cortex (Jelsing *et al.*, 2006) have been described with varying accuracy among authors and some results are imprecise or conflicting. To date, no comprehensive functional mapping of the cortical areas has been described in swine.

and Ray, 1991a and 1991b; Okada *et al.*, 1999). With multiunit recordings, it has been shown that the overall organization of the primary somatosensory cortex (SI) was similar to that of other mammals (Craner and Ray, 1991a). Though it is important to specify that the most comprehensive description of the somatosensory cortex in pig currently available (Craner and Ray, 1991a and 1991b) has been made at the neonatal stage (7 days to 2 months) and may not strictly correspond to the adult stage. With anterograde tracing techniques, it has been shown that the somatosensory cortex had marked connectivity with the ventral posterior and the ventral posterior lateral nucleus of the thalamus (Jelsing *et al.*, 2006). SI contains a complete and somatotopically organized projection from the body surface progressing from the hind limb to the head with medial to lateral locations in the cortex. The large representation of the rostral snout in the fronto-lateral cortex (Figure 1) is probably related to the important use of the snout by the pig for tactile sensation in exploring its environment. The receptive fields for the rostrum are primarily contralateral (92%), although ipsilateral and bilateral receptive fields have been found for the face (Craner and Ray, 1991a). The motor cortex was found to be immediately caudal to the dorsofrontal prefrontal

cortex (PFC) and adjacent to SI without sulcal delineation with the latter (Breazile *et al.*, 1966; Palmieri *et al.*, 1986; Craner and Ray, 1991a; Jelsing *et al.*, 2006). Recently, the PFC has been fully described in miniature Göttingen pig in a comprehensive study based on the distribution of reciprocal thalamocortical projections investigated using anterograde and retrograde tracing techniques and cytoarchitectonic criteria (Jelsing *et al.*, 2006). On the observation that these areas were lacking a granular layer IV and had topographically organized reciprocal connections with the mediodorsal nucleus of the thalamus, the PFC was delineated and subdivided into four main regions: the frontopolar, the anterior cingulate, the anterior insular and the dorsofrontal region. The whole PFC in these young minipigs was evaluated to constitute about 24% of the total neocortex volume and 10% of the total brain volume. The hippocampal region (Holm *et al.*, 1993; Holm and West, 1994; Saito *et al.*, 1998) and the cerebellum (Larsell, 1954; Lange, 1974; Mwamengele *et al.*, 1993) have also been investigated in terms of histology, topology or immunohistochemistry.

In addition to global description of the central structures available in stereotaxic atlases of the pig brain (Yoshikawa, 1968; Salinas-Zeballos *et al.*, 1986; Felix *et al.*, 1999),

numerous deep cerebral structures have also been described in details with standard histology and/or immunohistochemistry. Among the comprehensive and recently published list of these studies (for review: Lind *et al.* (2007)), we may report some studies dealing with portions of the brainstem (Gillian, 1943; Breazile, 1967), specific mesencephalic nuclei such as the red nucleus (Otabe and Horowitz, 1970), the ventral mesencephalon (Ostergaard *et al.*, 1992) or the raphe nucleus (Niblock *et al.*, 2004) as well as diencephalic structures such as the thalamus (Solnitzky, 1938), and hypothalamus (Solnitzky, 1939; van Eerdenburg *et al.*, 1992; Leshin *et al.*, 1995). Description of the basal ganglia in pig has brought some evidence that they are similar to that of primates. It seems that the pig brain has a PFC-basal ganglia-thalamo-cortical circuit comparable to that described in other animals (Jelsing *et al.*, 2006). The pig's striatum is well developed and, in contrast to rat, individualized into distinct caudate and putamen (Felix *et al.*, 1999). The distribution of dopamine in the brain is similar to primate (Rosa-Neto *et al.*, 2004a) and dopaminergic neurons of the substantia nigra project in a topographically organized manner to the dorsal and ventral compartments of the striatum as in primate (Agarwal *et al.*, 1993). In addition, the subthalamic nucleus (STN) in pig has similarities with the STN described in human and other species (rat, cat, non-human primate) when considering its shape, localization and cytoarchitecture (Yelnik and Percheron, 1979; Larsen *et al.*, 2004). Its orientation and dimensions (length 4.5 mm, width 1.9 mm, height 1.2 mm, volume 6.9 mm^3) have been evaluated in minipig (Larsen *et al.*, 2004). The porcine STN contains medium-sized neurons with uniform Golgi morphology. An immunohistological study revealed glutamate-positive neurons that receive large GABAergic, dopaminergic, and cholinergic inputs and a high number of acetylcholinesterase neurons comparable to findings in human (Larsen *et al.*, 2004). Due to the importance of this nucleus in surgical treatment for advanced Parkinson's disease (PD), it is highly probable that in the forthcoming years, pig might become an alternative model to rodent and primate for modelling PD (Mikkelsen *et al.*, 1999) and studying deep brain stimulation (Bjarkam *et al.*, 2004; Dalmose *et al.*, 2004).

Finally, the cerebrovascular anatomy of the pig shared similarities with human. Indeed, pig and human present a similar circle of Willis though the diameter of the posterior communicating artery is comparable to that of the internal carotid artery in pig. Thus, the connection between anterior and posterior circulation is well developed compared with that of human brain. In contrast to human, the pig presents two middle cerebral arteries originating from the internal carotid artery in each hemisphere, one coursing laterally and another rostrally over the olfactory tract (Imai *et al.*, 2006).

Brain imaging and neurosurgery in pig

Functional and molecular cerebral imaging
Recently, swine have been used as experimental models for brain imaging studies using PET (Smith *et al.*, 1998;

Danielsen *et al.*, 2000 and 2001b; Ishizu *et al.*, 2000; Sakoh *et al.*, 2000b; Cumming *et al.*, 2001; Dall *et al.*, 2002; Andersen *et al.*, 2005), MRI (Sakoh *et al.*, 2000a; Watanabe *et al.*, 2001; Jelsing *et al.*, 2005; Duhaime *et al.*, 2006) and functional MRI (Fang *et al.*, 2005 and 2006). Functional MRI has been used to evaluate the maturation of the brain in minipig from the early postnatal period to 6 months old. It has thus been possible to determine whether the sequence of temporal development of the visual cortex and its spatial localization are similar to primate (Fang *et al.*, 2006). In order to facilitate identification of activated cerebral structures in PET studies, a standard stereotaxic coordinate system in miniature Göttingen pig has been developed for voxel-by-voxel analyses across animals, with reference to a common standard MRI template for pig brain (Watanabe *et al.*, 2001; Andersen *et al.*, 2005). To date, functional imaging in pig has rarely been used to evaluate the effects of various physiological stimuli. It has been demonstrated *in vivo* with [^{11}C]raclopride that striatal dopamine neurotransmission was linked to exploratory behaviour (Lind *et al.*, 2005a).

On the contrary to functional imaging, several molecular imaging studies using PET have been performed in the pig (Bhalla *et al.*, 2002; Niblock *et al.*, 2005; Minuzzi *et al.*, 2006). These investigations have become available, thanks to the development of specific radioligand such as [^{11}C]WAY100635 and [^{11}C]DASB for serotonin transporters (Jensen *et al.*, 2003; Cumming *et al.*, 2007). A PET study with [^{11}C]WAY100635 radioligand has thus evaluated the effects of 3,4-methylenedioxy-methamphetamine (MDMA) ('ecstasy') on the serotonin system in Göttingen minipigs (Cumming *et al.*, 2007). The distribution of dopamine D1 and D2/3 binding sites within the basal ganglia of Göttingen miniature pig has been characterized with PET studies and compared to rhesus monkey (Ishizu *et al.*, 2000; Rosa-Neto *et al.*, 2004a). Similar dorsal-ventral distribution gradients were found in both species, with predominant D1 binding in the ventral striatum and more D2/3 binding in the dorsal-posterior striatum (Rosa-Neto *et al.*, 2004a). Thanks to the development of methods for measuring cerebral uptake and metabolism of [^{18}F]fluoro-L-DOPA (FDOPA) (Danielsen *et al.*, 1999 and 2001b), it is possible to analyse the activity of DOPA decarboxylase in living pig and apply this strategy to investigate the effect of neuroleptics (Danielsen *et al.*, 2001a). Similarly, PET studies with [^{11}C]raclopride, a radioligand binding in competition with the endogenous dopamine, have been conducted to analyse the effect of psychoactive drugs such as amphetamine (Lind *et al.*, 2005b), nicotine (Cumming *et al.*, 2003), MDMA (Rosa-Neto *et al.*, 2004b) and lysergic acid diethylamide (Minuzzi *et al.*, 2005). It has been shown that these psychoactive agents reduced the availability of [^{11}C]raclopride binding sites for dopamine D(2,3) receptors in the striatum of Göttingen minipigs. The variation of the regional cerebral blood flow (rCBF) has been used to analyse the effects of MPTP poisoning. As in primates, MPTP-treated pigs develop an experimental syndrome of Parkinsonism

(Mikkelsen *et al.*, 1999). This model has been associated with a 60% decline in the uptake of FDOPA, a tracer for the activity of DOPA decarboxylase in nigrostriatal fibers of the striatum (Danielsen *et al.*, 2000; Cumming *et al.*, 2001; Dall *et al.*, 2002). It is highly probable that this new model for induced Parkinsonism will challenge the animal models commonly used to date (i.e. MPTP-treated primate and 6-hydroxydopamine lesioned rat).

Stereotaxic and image guided surgery
Several stereotaxic atlases of the pig brain have been described (Yoshikawa, 1968; Szteyn *et al.*, 1980; Salinas-Zeballos *et al.*, 1986; Felix *et al.*, 1999; Watanabe *et al.*, 2001) as well as specific stereotaxic instruments either for piglets (Salinas-Zeballos *et al.*, 1986), pigs weighing between 30 and 40 kg (Poceta *et al.*, 1981; Saito *et al.*, 1998) or adults (Marcilloux *et al.*, 1989; van Eerdenburg and Dierx, 2002). The stereotaxic frame devoted to the pig developed by Marcilloux *et al.* (1989) was based on the fixation of the skull by the infraorbital ridges, the hard palate and the auditory canals. The ear-bars were carried by a swivelling system allowing adaptation to the orientation of the auditory canals, perpendicular to the sagittal plane in the new-born piglet but oblique in the weaned pig (Poceta *et al.*, 1981; Salinas-Zeballos *et al.*, 1986; Marcilloux *et al.*, 1989). Unfortunately, this system may have some adverse effects on the eardrums and cannot be used with Göttingen mini-pigs. Indeed, in this breed, the auditory canal is almost vertical, and hence the impossibility to fix the head in the Marcilloux stereotaxic frame. This peculiarity plus the need for a system compatible with MRI led Bjarkam *et al.* (2004) to use MRI-compatible localizers fixed on the skull and to develop a head-fixation box made of methyl methacrylate (Plexiglas®) with the head fixated by aluminium screws inserted bilaterally in the zygoma bone.

Stereotaxic surgery requires precise determination of a system of coordinates either based on bony landmarks or intracerebral structures easily identifiable on images. The choice of such a reference system varies between authors. Some of them have chosen to base their procedure on external skull structures as commonly performed in rodents (Paxinos and Watson, 2006). This strategy has been used in newborn pigs aged 1 to 12 days (Salinas-Zeballos *et al.*, 1986), for description of the PFC in miniature Göttingen pig aged of 3 months (Jelsing *et al.*, 2006) and for targeting the hippocampus of Landrace prepubertal pigs weighing less than 30 kg (Saito *et al.*, 1998). Saito *et al.* (1998) have reported the reliability of this method and minimal variation in distances between some external bony landmarks. Despite the fact that this strategy might be successful in young pig, there is a too large inter-individual variation in skull morphology in growing animals and these landmarks may vary considerably as the age of the animal increases (Marcilloux *et al.*, 1989; Bjarkam *et al.*, 2004). In addition, measures based on bone structures can hardly be applied generally since pig present considerable inter-individual and inter-breed skull variations in size and shape. This becomes even more of a problem in adult miniature Göttingen pig presenting a large pneumatized frontal sinus inducing considerable inter-individual variation on distances between bone and brain structures.

A more precise definition of a stereotaxic coordinates system has been achieved on the basis of stable intracerebral landmarks. Depending on the tools used, two stereotaxic systems have been proposed. Using ventriculography, the horizontal zero plane was defined on the basis of the plane connecting the recessus preopticus to the posterior commissure (CP) and CP as the origin of the system (Marcilloux *et al.*, 1989; Felix *et al.*, 1999). The recessus preopticus was found to correlate closely with the anterior commissure defining a system similar to that commonly used in human and primate (Felix *et al.*, 1999). Andersen *et al.* (2005) found difficult to identify the two commissures on MRI and defined the referenced plane on the basis of the line connecting the optic chiasma to the pineal gland. The origin of the stereotaxic system was then defined as the centre of the pineal gland. Fortunately, both coordinates systems remain interchangeable by a geometric transformation of rotation and translation. Whatever the procedure and the tools used, stereotaxic surgery has been successfully performed in swine. The hippocampus was targeted in prepubertal pigs weighing 10 kg with a technique based on external skull structures alone such as bregma, sutura coronalis and sagittalis (Saito *et al.*, 1998). Implantation of foetal dopaminergic grafts in the striatum (Danielsen *et al.*, 2000; Cumming *et al.*, 2001) or deep brain stimulation of the STN (Bjarkam *et al.*, 2004; Dalmose *et al.*, 2004) were performed in miniature pig on the basis of intracerebral landmarks (Figure 3).

Examples of studies on the porcine central nervous system

To illustrate the intricacies in using the pig model compared to more classical model such as the rodents, we have selected two examples of studies currently ongoing in our laboratory at UMR SENAH, Saint-Gilles, France. These experiments were performed in growing farm pig about 30 kg in weight.

Functional brain imaging during gastric distension
Signals arising from gastric distension have a critical role in the control of food intake (Smith, 1998; Ritter, 2004). These signals are likely being transmitted to the brain mainly by the vagal nerve (Phillips and Powley, 1998; Berthoud *et al.*, 2001). The contribution of spinal nerves is considered as insignificant (Sengupta and Gebhart, 1994). However a recent study performed in humans suggests that the situation is not so clear-cut (Vandenbergh *et al.*, 2005). The aim of this study was to identify brain areas and nervous pathways involved in the central processing of non-noxious gastric distension signals. Using functional brain imaging, we have investigated brain activity during gastric distension in pigs with intact vagal nerves or after a bilateral vagotomy. Animals were surgically fitted with a permanent access to the stomach through a gastric cannula. This cannula
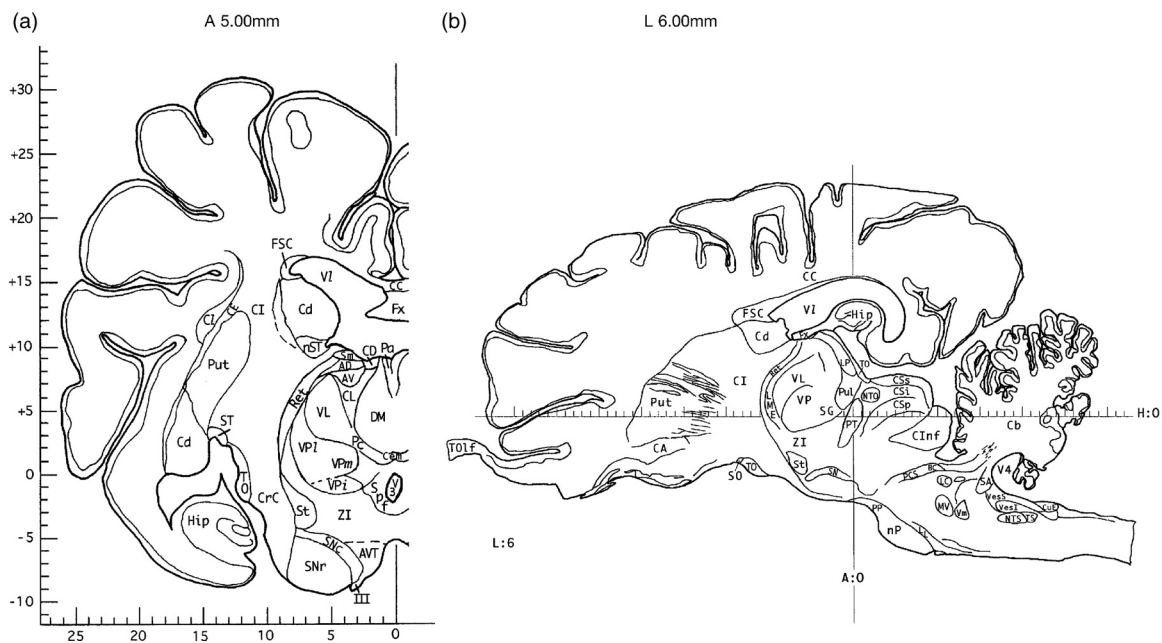
Figure 3 Location of the subthalamic nucleus (St) on coronal (a) and sagittal (b) sections. Its coordinates are 5.00 mm ahead the posterior commissure (CP) and 6.00 mm laterally from the midsagital plane respectively. The reference (zero) horizontal plane contains a virtual line connecting the recessus preopticus and the CP. Drawings from Felix *et al.* (1999) are with permission from Elsevier.

allowed insertion of an inflatable balloon into the stomach so as to achieve a pressure-controlled distension with preset pressure rise time. This was performed by a custom-made inflator using compressed air and controlled by dedicated software acting on high-speed electrovalves through a pressure-controlled loop. The pressures investigated were within the range of that observed after a medium- to large-size meal and were below the nociceptive range (25 mmHg above mean abdominal pressure for a pig).

Brain functional imaging was performed using single photon emission tomography (SPECT). Briefly, this method used the property of ^{99}Tc-HMPAO (D-L hexamethylpropylene amine oxime) to enter into the neuron because it is lipophilic. Though ^{99}Tc-HMPAO is not an ideal tracer of CBF since it underestimates high flow areas, it has the unique capability to allow CBF imaging in conscious animal. Indeed, upon its injection in the bloodstream, it will enter neurons and stay within the cell body because it undergoes a biochemical transformation that changes its lipophilic properties into a hydrophilic one, probably as a result of an action with gluthathione. The kinetic of this transformation is within the order of the minute. Afterwards, it is possible to image the brain radioactivity in anaesthetized animal while the recorded radioactivity distribution reflects the brain perfusion at the time of radiolabel injection. Furthermore, anaesthesia has no adverse affect on the nuclear imaging procedure. As for all functional brain imaging method, the relationship between brain activity and regional vascularization of the brain structures allows from the rCBF to map brain function.

The experimental protocol consisted in two imaging sessions before and after truncal vagotmy. Five Large White × Landrace female pigs aged of 3 months and weighing 30 kg were used in this study. An initial imaging session was performed on animals at different pressures in a randomized order (0, 10 and 20 mmHg). After vagotomy (5 cm above the diaphragm) of the same animals, a second imaging session was made at different pressures as previously described. A 7-day washout was allowed between experiments on the same animal. Gastric distension and SPECT were achieved on halothane-anaesthetized pigs under artificial ventilation after a 20-min resting period without external solicitations. Thirty minutes after its insertion in the stomach through a gastric cannula, the barostat bag was inflated to the test pressure. Concomitant to inflation, an intravenous injection of ^{99}Tc-HMPAO (555 to 740 MBq, Ceretec™, GE Healthcare, Amersham, UK) was performed. Fifteen minutes after radiolabel injection, brain images were acquired in three dimensions by SPECT on an Elscint gamma camera (Elscint Ltd, Haifa, Israel) fitted with a low energy-high resolution collimator. Each image was acquired for 90 s with a step shoot angle of 6° allowing to record about 2 to 5 kcounts/s, which is the formal requirement for three-dimensional (3D) reconstruction from SPECT images.

Axial brain slices were reconstructed offline using a filtered back projection with a dedicated Sheep–Logan filter to remove diffusion artefacts. Back projections of brain images were normalized and co-registered using Automated Image Registration algorithm to a SPECT pig template built in our laboratory (Figure 4). This SPECT pig template corresponds to the mean of 16 SPECT obtained in anaesthetized animals of the same breed, age and weight. Briefly, each of the individual SPECT volumes used
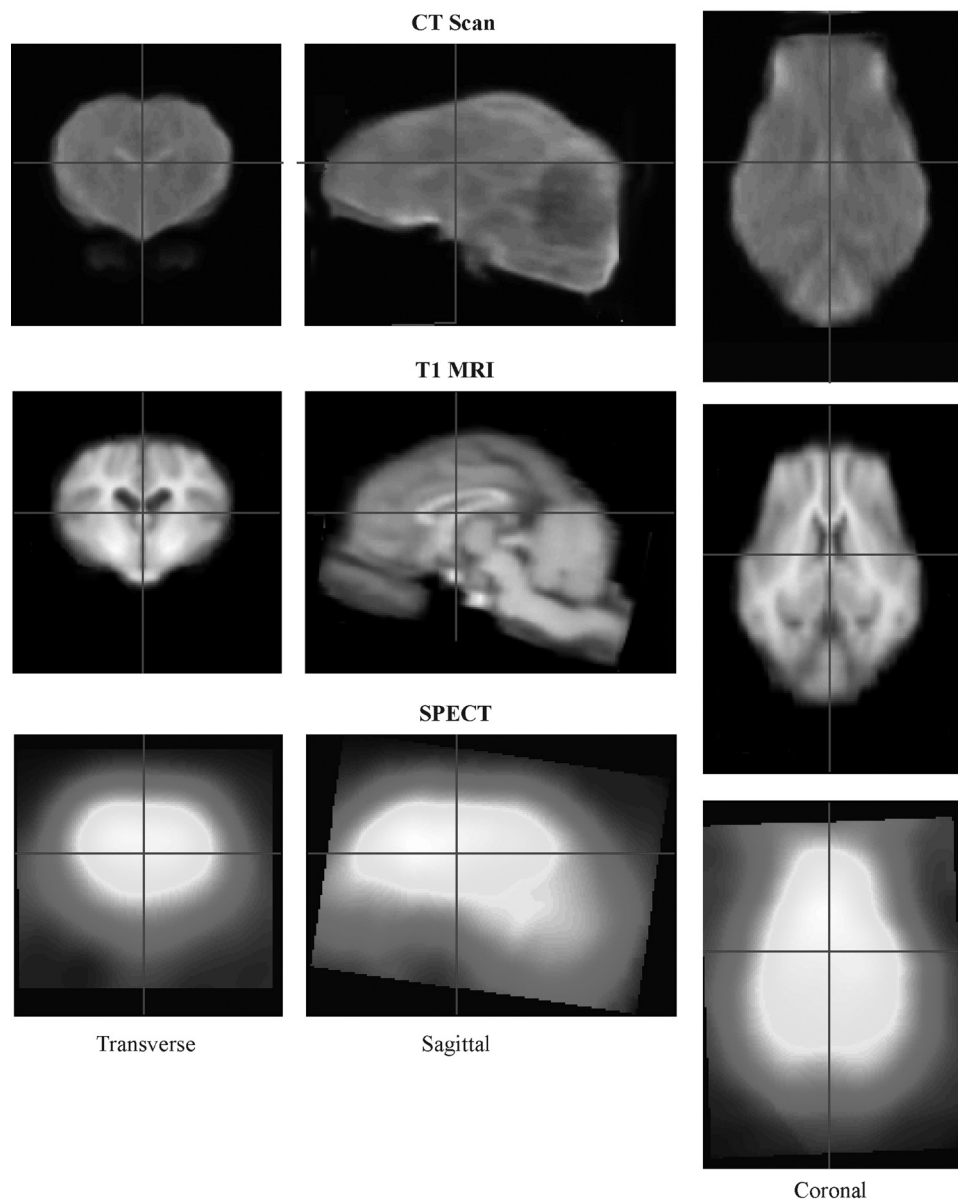
Figure 4 Intermodality co-registered templates of the pig brain used to reset each functional volume of individual pigs into the same reference volume. The MRI T1 (magnetic resonance imaging) template was based on 22 pigs (Andersen *et al.*, 2005). The CT (computed tomography) scan and SPECT (single photon emission tomography) templates were based on 16 animals. The fuzzy aspect of the images was due to the intersubject averaging procedure. Co-registration was achieved using the Automated Image Registration algorithm (University of California, Los Angeles, USA) and involved affine and elastic transformations in the three dimensions.

for the template was initially co-registered to an anatomical segmented image of the brain of the same animal obtained with a CT scanner. This CT volume was, prior its use for SPECT co-registration, co-registered with a T1 MRI pig template calculated by Andersen *et al.* (2005). This T1 MRI template was obtained as a mean of 22 MRI's brain imaging. The intermediate step using CT images is required because the error in co-registering the low resolution SPECT image onto the high resolution MRI image is too large. Using this procedure, it is possible to register the SPECT volume into a referenced volume mimicking the Talairach space. For simplicity and interoperability, we chose to use the stereotaxic space defined by Felix *et al.* (1999).

Voxel-based analyses were performed using parametric tests via Statistical Parametric Mapping (SPM2) software adapted to the characteristics of the pig brain, size of which being about 1/3 of that of a human. Regression analyses were performed to identify brain areas of which the activity was associated/correlated with the increase of distending pressure. This brain activity was compared before and after vagotomy. Voxels significantly associated to the increase of the distending pressure were displayed on the MRI template of pig brain.

Before vagotomy, the activity of several brain areas was found significantly associated with the increase of gastric distending pressure, only (Figure 5). After vagotomy, only a
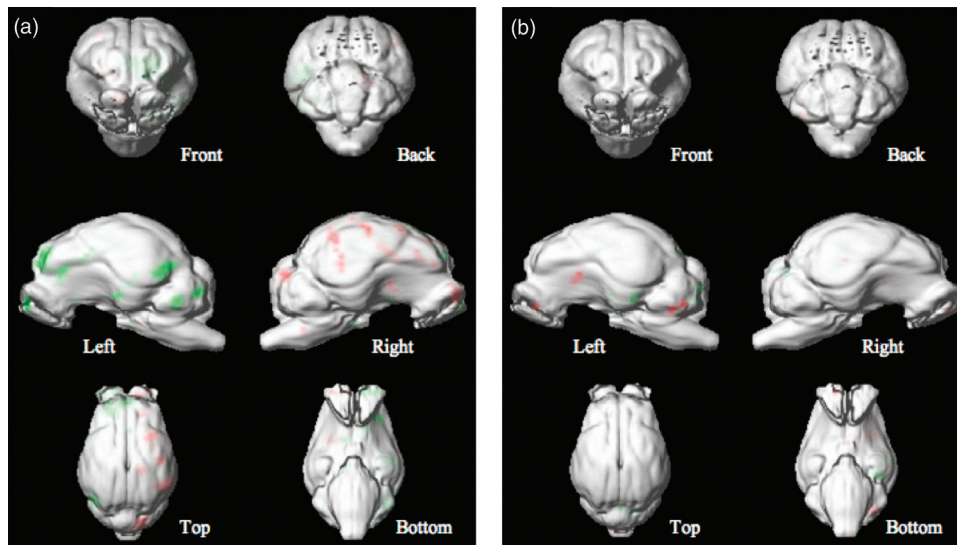
Figure 5 The 3D-rendered images of the front, back, right, left, top and bottom brain views of: (a) intact and (b) vagotomized pigs with activated (red) and deactivated (green) voxels correlated ($P < 0.02$) with the increase of the fundic distending pressure. Voxels were projected onto the cortical surface of the rendered magnetic resonance imaging template built from 22 animals.

limited number of brain areas were associated with the increase of gastric pressure. This study demonstrates that spinal afferences encode non-painful gastric distension signals. Furthermore, in intact animal, vagal and non-vagal afferences originating from gastric wall and activated by non-painful gastric distension are involved in the brain representation of post-prandial gastric fullness.

Deep brain stimulation and neuronavigation
Deep brain stimulation (DBS) is widely used as a functional treatment for various movement disorders such as PD, dystonia and essential tremor. New indications are emerging in neuropsychiatry such as obsessive–compulsive disorders or depression (Wichmann and Delong, 2006). This therapy is based on continuous high frequency stimulation of a deep structure: STN, globus pallidus internalis or thalamus. Usually, a quadripolar electrode is implanted in the nucleus and high frequency stimulation is provided through a pulse generator implanted subcutaneously in the subclavicular area. This functional therapy has proved its efficacy in severe PD with improvement of motor symptoms, reduction of motor fluctuation and dyskinesia. However, the exact mechanism of action of DBS is still unknown as well as the influence of electrical stimulation on behaviour. Since stimulation of a nucleus affects complex cortico-subcortical loops involved in motor, cognitive and behavioural processes, studies on living organisms remain essential to assess the collateral effects of such a therapy. Among those, excessive weight gain has been observed in patients with PD after stimulation of the STN. Whether this effect is exclusively due to the decrease in energy expenditure consecutive to the improvement of motor symptoms is still unknown. An additional effect on energy metabolism by current diffusion to the hypothalamus or motivational change by activation of the limbic circuit of the basal

ganglia may be hypothesized (Perlemoine *et al.*, 2005; Montaurier *et al.*, 2007).

With the aim to study feeding behaviour in implanted animals, we have developed a surgical procedure for electrode implantation in the STN based on intraoperative microrecording and CT images based on neuronavigation. Six Large White × Landrace female pigs aged of 3 months and weighing 30 kg were used in this study. Neuronavigation was based on images acquired with a CT scanner while pigs were maintained in a custom-made stereotaxic frame similar to that described by Marcilloux *et al.* (1989). The CT images were transferred to a DICOM PACS Workstation running OsiriX, an Open Source Dicom viewer (Rosset *et al.*, 2004) allowing 3D reconstruction, and direct length and angle measurements (Figure 6). The stereotaxic coordinates of the target were calculated on the basis of identification of intracerebral stable landmarks, i.e. the anterior and posterior white commissures, outlined by injection of contrast liquid. In order to define precisely the position of the STN, we further performed microrecordings with three parallel microelectrodes (FHC, Bowdoinham, ME, USA) distant of 2.5 mm from each other (Figure 7). The microelectrode demonstrating the highest neuronal activity was replaced by a chronic electrode (model 3389; Medtronic, Minneapolis, MN, USA) fixed to the skull with dental cement. The electrode's wire and the extension lead were fixed to the skull (Figure 8). The extension emerged on the midline between the scapulas and was connected to a pulse generator (Kinetra; Medtronic, Minneapolis, MN, USA) fixed on the back of the animal in a harness designed in our laboratory. Pigs recovered quickly from the surgery and continuous high stimulation was maintained for 3 months.

The feeding behaviour of the implanted animals was compared to that of control animals with specific reference
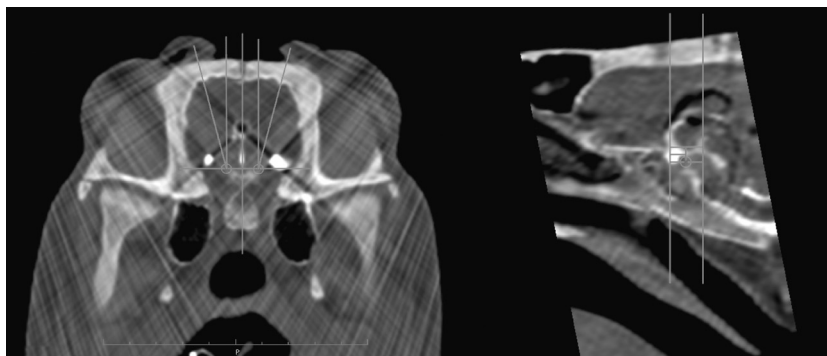
Figure 6 Coronal (left) and sagittal (right) computed tomography scan images of the brain of a Landrace × Large White pig aged of 3 months and weighing 30 kg. The stereotaxic coordinates of the target were defined with ventriculography allowing visualization of stable intracerebral landmarks that were the interhemispheric line and the bicommissural line. This animal underwent implantation of bilateral electrode in the subthalamic nucleus.
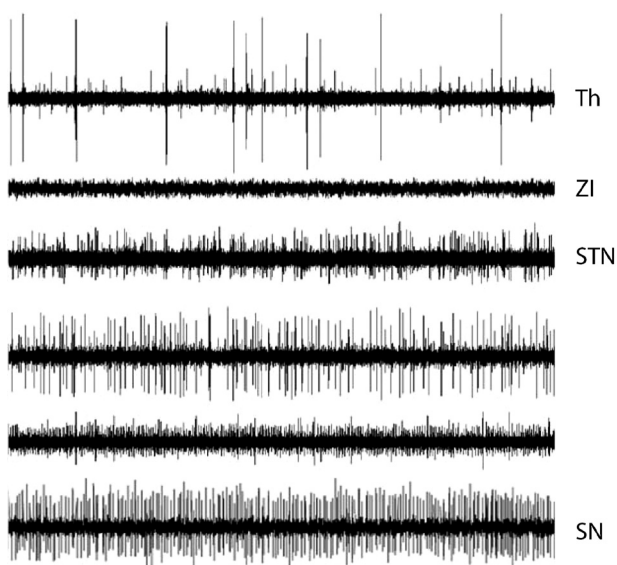

Figure 7 Electrophysiological identification of the subthalamic nucleus (STN) with microrecording (Trace duration: 10 s). Thalamic cells were recorded about 5 mm above the STN Thalamus (Th), then after a silent area of about 2 mm that might correspond to the zona incerta (ZI), a noticeable increase in background activity and spontaneous firing discharge were observed. These corresponded to penetration of the electrode tip into the STN. Sustained irregular activity was recorded over 1.4 ± 0.4 mm (mean $\pm$ s.d.). The lower border of the nucleus was more difficult to differentiate from activity in the substantia nigra (SN). The latter presented however a more tonic pattern of activity than the STN.

to the motivational aspect of feeding. At the end of the experiment, animals were euthanized and the placement of the electrodes was checked using histological examination. It has thus been shown that continuous subthalamic stimulation induced change in the feeding behaviour of the animals with different aspects on the motivation of the animals to feed.

Discussion

Pigs used in research originate either from common agricultural or research-dedicated breeds, the most common of the latter being the Yucatan and the Göttingen minipigs.

Figure 8 Coronal computed tomography image acquired at the level of the subthalamic nucleus in a pig implanted with bilateral electrodes for 100 days and aged 6 months. The four contacts of the electrode (model 3389 from Medtronic) are clearly viewed on the right side. The radio-opaque artefacts on the skull correspond to the extension lead of the electrodes that are rolled under the skin.

Since animals from different breeds present physiological and anatomical variations, they may offer advantages and/or disadvantages depending on the purpose of the study (Bollen *et al.*, 2000). For example, anatomical considerations are crucial for brain surgical intervention. In pig, the brain is protected by a massive skull which changes in size and shape during the growth, in addition to individual variability in skull structure (Poceta *et al.*, 1981; van Eerdenburg and Dierx, 2002). Moreover, there is a dramatic increase in thickness with the animal growing rendering the surgical intervention difficult (Figure 9). For example, the thickness of the skull is about 6 to 7 mm in its middle portion with a maximum of 30 mm at the occipital crest in Landrace pig aged only of 3 months, i.e., before the emergence of the frontal sinus. Since immature pigs grow quickly and mature adults overweight 250 kg, studies
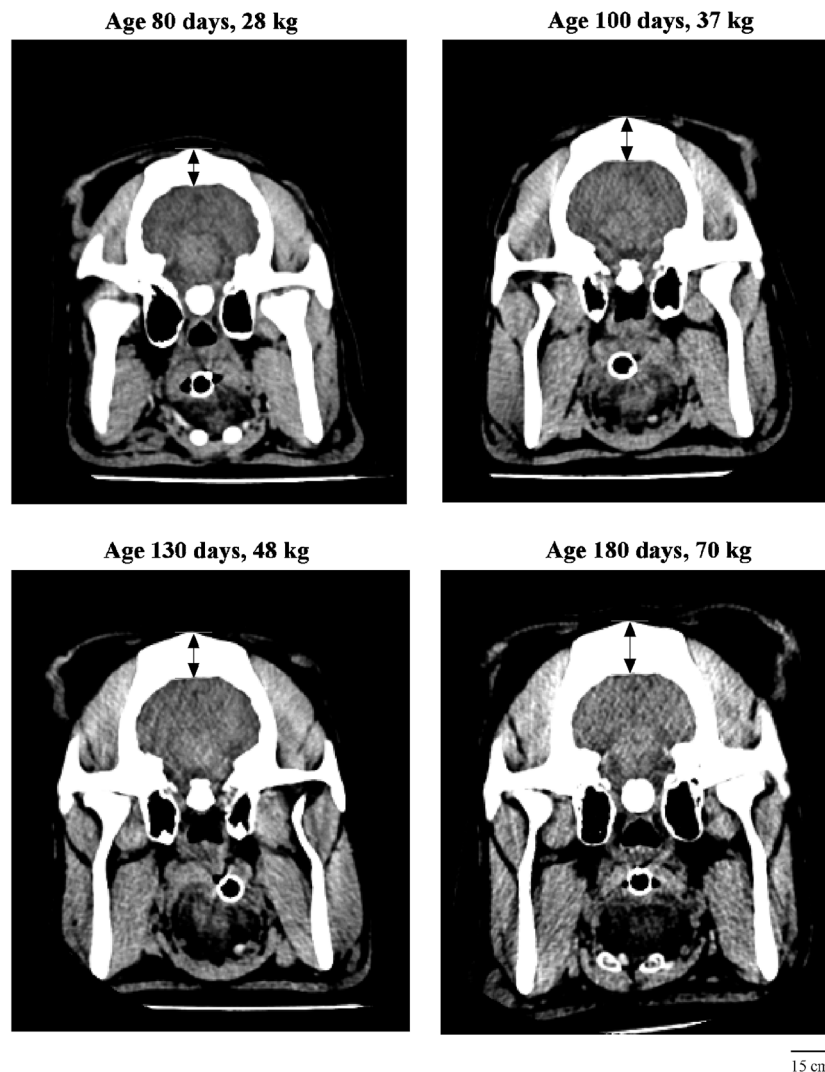
Figure 9 Computed tomography scan images of the head of a conventional pig at various age and weight. Irrespective of the age/weight, the size of the brain is unchanged (51, 52, 52 and 53 mm in width). On the contrary, the thickness of the skull (black arrows) is increasing with age/weight (15, 19, 21 and 22 mm). This thickening results in a larger amount of diffused photons for functional imaging studies and an increased difficulty to maintain electrodes in deep brain structures for a large time period. All images shared a similar scale.

duration is limited to 3 or 4 months. Furthermore, they often necessitate restricted caloric intake to limit the weight of the animal. Alternatively, adult minipigs are easily manipulated since they weight less than 60 kg (for Göttingen minipigs). However, these mature minipigs have a large frontal sinus that pneumatizes the skull, which makes the access to the brain tricky (Figure 10). It necessitates oblique trajectories and a wider scalp incision. The development of this frontal sinus starts around puberty and grows rapidly thereafter (van Eerdenburg and Dierx, 2002). Thus, a number of studies have been conducted in agricultural pig but miniature pig might be preferred for chronic or long-term studies for which a significant growth is an issue (Panepinto *et al.*, 1978; Imai *et al.*, 2006).

Recent researches using the pig as a model in neuroscience are mainly imaging studies. This results from the increasing relevance of recent imaging tools to explore brain functions, together with the opportunity to use an animal model with a large developed brain. The pig model appears to be obviously superior to rodent for this purpose especially, because of its compatibility with imaging platform used in human. To date, stereotaxic surgery in pig has undergone a less notable development. As for imaging, pig presents a major advantage that is the size of its brain and the similarities with human brain, but pig is considerably disfavoured by the thickness of the skull bone and the presence of a large median sinus in mature animal. Nevertheless, stereotaxic procedures have been successfully performed in pig, thanks to the development of specific stereotaxic apparatus and atlases.

Paradoxically, though studies with advanced technology have been conducted in pig for years, one has to recognize that fundamental description of the pig brain is still lacking. Precise identification of cerebral structures involved in any specific process requires complete and valid data in conventional and functional neuroanatomy. Though deep
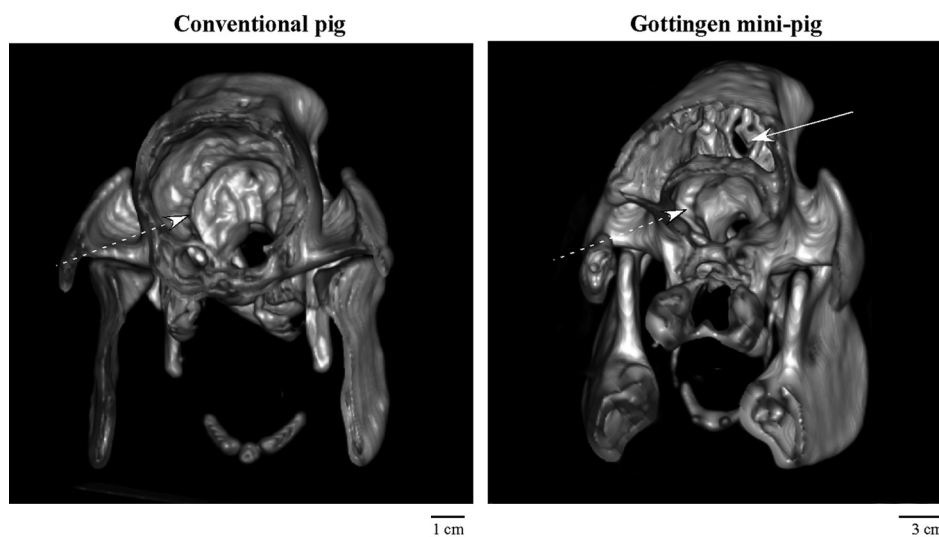
Conventional pig **Gottingen mini-pig**

1 cm   3 cm

Figure 10 Maximal intensity projections of the skull of a conventional pig (left panel) *v.* a Göttingen minipig of the same weight (26 kg). Note the presence of a large pneumatization of the skull (white arrow) above the brain (doted white arrow) in the Göttingen minipig. This pneumatization makes the access to the brain extremely tricky and requires oblique trajectories whereas vertical ones can be used in the conventional pig.

structures have been relatively well described in pig (Felix *et al.*, 1999), the cortex of the pig has been poorly described and, to date, there is no functional neither structural maps equivalent to Brodmann's description in primate and human. Nomenclature of cortical sulci and fissures as well the localization of functional cortical areas vary from one author to another (Craner and Ray, 1991a and 1991b; Okada *et al.*, 1999; Lind *et al.*, 2007). Moreover, a number of studies have been restricted to the postnatal ages and only sparse data are available for piglet aged of more than 3 months. When available, these studies have mainly been conducted in miniature Göttingen pig and some discrepancies may occur when one tries to transpose to conventional pig. As a result, precise localization of any activated signal identified by functional imaging remains somewhat imprecise. Another difficulty arises from the wide range of pig breeds that have been used in the different studies inducing considerable anatomical variability and the lack of normative values, e.g. for EP recordings (Strain *et al.*, 2006).

In comparison with the other available experimental models (sheep, pig and rat), the non-human primates represent an optimal choice. However, a European directive that was introduced in December 2008 strictly limited the use of non-human primates as animal model (http://europa. eu/rapid/pressReleasesAction.do?reference=IP/08/1632&format =HTML&aged=0&language=FR&guiLanguage=en). In this context, alternative to the non-human primates must be considered. Murine models might be of significant interest for neurological research. However, their brain sizes limit the possibility for functional imaging. The spatial resolution of the best micro PET available today is 2.5 times more than a standard PET scanner able to scan large animals (Suk *et al.*, 2008) whereas the ratio between large animal *v.* murine brain is ranging between 1 and 3 or less. This limitation is even more important with the wider availability of 3T MRI scanner capable to image large animal brain with a relative spatial resolution comparable to a 7T miniature MRI machine imaging a murine brain. Furthermore, murine brain lacks the development of the cortex in comparison to primates or larger animals. All of these reasons suggest that larger animals such as ruminants (goat and cattle) and pigs might provide an attractive alternative to rodents. For example, the sheep has been successfully used to develop a model for PD using MPTP poisoning (Beale *et al.*, 1989; Baskin *et al.*, 1994). It has been involved in brain microdialysis studies (Westerink, 1995) or intracranial electroencephalography (Opdam *et al.*, 2002). However, neuroscience studies using mature sheep remain infrequent primarily because of the ruminant-related differences in physiology. For instance, peripheral vagal nutrient sensing has been adapted to forage ingestion and as a consequence the key player is a potassium-sensitive vagal receptor that is absent in non-ruminant species (Cottrell and Iggo, 1984). Furthermore, as a seasonal animal, leptin secretion and sensitivity in the adult sheep is altered during the year, unlike in pigs or humans (Zieba *et al.*, 2008). Finally, there are remaining questions regarding similarities with human in the role of ghrelin in the control of food intake in sheep (Sugino *et al.*, 2004; Grouselle *et al.*, 2008). Consequences of differences in digestive physiology overwhelmed the context of digestive behaviour. Indeed, nutrient source and glucose metabolism of the brain, especially the utilization of ketones, differ in sheep in comparison to human and rodent (Lindsay and Setchell, 1976). This may account for the lack of PET studies in sheep with the notable exception of foetal and neonatal lamb (Thorngren-Jerneck *et al.*, 2001). Additionally, despite studies describing (partially) the functional mapping of the cortex of the sheep (Kirk *et al.*, 1987), from our knowledge, this model has never been used for functional neurosurgery or chronic DBS.

Conclusions

Numerous studies with fMRI or PET have proved that the pig is a suitable model for studying various neuro-transmitters and radioligands. Stereotaxic surgery is possible despite the thickness of the skull and the presence of a large frontal sinus in some adult animals. Nevertheless, specific points require further improvements to refine the usability of the pig model. A better description of the cortex and its functional areas as well as a better stereotaxic localization of deep structures are absolutely mandatory for brain imaging and stereotaxic surgery. We look forward for high quality atlases for both cortex and deep structures allowing determination of stereotaxic coordinates and localization of activated areas in imaging studies. Especially, there is a need for a digitalized atlas matching MRI and histological resources built using a large number of animals. This atlas should additionally benefit from associated electrophysiological cortical mapping studies.

Acknowledgements

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