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# Impact of food, gut-brain signals and metabolic status on brain activity in the pig model: 10 years of nutrition research using in vivo brain imaging

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Session 4: Metabolic health and brain activity modulated by the gut

**Review: Impact of food, gut-brain signals, and metabolic status on brain activity in the pig model – 10 years of nutrition research using *in vivo* brain imaging**

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Short title: Nutrition and brain imaging in the pig model

## **Abstract**

The purpose of this review is to offer a panorama on 10 years of nutrition research using *in vivo* brain imaging in the pig model. First, we will review some work describing the brain responses to food signals including basic tastants such as sweet and bitter at both oral and visceral levels, as well as conditioned preferred and aversive flavors. Second, we will have a look at the impact of weight gain and obesity on the brain metabolism and functional responses, drawing the parallel with obese human patients. Third, we will evoke the concept of the developmental origins of health and diseases, and how the pig model can shed light on the importance of maternal nutrition during gestation and lactation for the development of the gut-brain axis and adaptation abilities of the progeny to nutritional environments. Finally, three examples of preventive or therapeutic strategies will be introduced: the use of sensory food ingredients or pre-, pro-, and post-biotics to improve metabolic and cognitive functions; the implementation of chronic vagus nerve stimulation to prevent weight gain and glucose metabolism alterations; and the development of bariatric surgery in the pig model, for the understanding of its complex mechanisms at the gut-brain level. A critical conclusion will brush the limitations of neurocognitive studies in the pig model and put in perspective the rationale and ethical concern underlying the use of pig experimentation in nutrition and neurosciences.

**Keywords:** Nutrition, Microbiota, Cognition, Pleasure, Behavior

## **Implications**

The purpose of this review is to offer a panorama on 10 years of nutrition research using *in vivo* brain imaging in the pig model, and to improve the understanding of the gut-brain axis factors modulating brain activity. This research has implications in the context of animal and human nutrition and health, covering the scope of animal production as well as translational medicine. In addition to the original insight they provide in neurocognitive and developmental sciences, the scientific studies presented here are valuable for the development of new preventive, diagnosis, and therapeutics solutions to fight against nutritional and behavioral disorders.

## **Introduction**

The present review paper is dedicated to the exploration of brain activity *via in vivo* brain imaging in the pig model, in the context of nutrition and metabolic research, and to the understanding of the gut-brain axis factors modulating this brain activity. Brain activity is a rather imprecise phrasing encompassing several meanings and embracing different concepts. Throughout this review, the terms 'brain activity' will be used as an ellipsis referring to neurobiological outcomes, which can be visualized *via* brain-imaging techniques such as nuclear brain tomography or functional magnetic resonance imaging (fMRI). Using these brain-imaging techniques, we can investigate the cerebral blood flow, the brain glucose metabolism, or the blood-oxygen-level-dependent (BOLD) hemodynamic response, all of those being correlated with neural activity and integration processes. Brain activity or metabolism can be visualized in a resting state, or in response to specific stimulations and sensory-cognitive tasks.

Nutrition research in the conventional or miniature pig is justified by a two-way rationale: first, the (mini)pig is a farm (or pet) animal that is bred and raised to provide

human food resources (or company); second, the pig is probably the closest animal to human beings in terms of digestive physiology (Guilloteau *et al.*, 2010, Roura *et al.*, 2016), and it shares many other similarities with the human. Similarities include a relatively large gyrencephalic brain (Sauleau *et al.*, 2009, Roura *et al.*, 2016) associated with high cognitive abilities (Dilger and Johnson, 2010), which is why the pig is a very good preclinical research model in nutrition and neurobehavioral disorders (Lind *et al.*, 2007, Gieling *et al.*, 2011). Improving health and well-being of farm (and pet) animals is not only a legal and ethical responsibility, but also a prerequisite to food quality and human health. Understanding how nutrition can modify the pig's physiology, gut microbiota, metabolism, behavior and health, within the spectrum of comparative pathology, is highly valuable to gain knowledge in human nutrition and health, and fight against food-related metabolic and behavioral disorders in both species.

The role of the microbiota-gut-brain axis in processing information and nutrients, as well as in regulating digestive processes and eating behavior, has been well documented and still attracts interest (van de Wouw *et al.*, 2017). The brain is the conductor of almost all biological functions and behavior, including eating behavior and digestion. The way the brain responds to food/metabolic status and integrates information shapes the nutritional and emotional status of the individual, the satiety-hunger alternation, the perception of pleasure, and the emergence of goal-oriented behaviors. On this gut-brain communication depends the individual's ability to adapt to its nutritional environment, by making choices and decisions that impact health and well-being. Investigating brain activity in response to food or diet is not only a fascinating fundamental research topic, but also a pathway to applied and translational research to the benefit of animal and human health.

Different ways of tackling this topic in the pig will be presented. First, the implementation of functional and metabolic imaging in the pig model can be used to investigate the brain responses to food exteroceptive and interoceptive perception, in the context of hedonic, homeostatic, or cognitive processes. Second, investigating brain activity in swine opens the way to understanding how metabolic diseases and eating disorders are linked to diet-related neurocognitive anomalies. Many studies showed that the minipig model is a real asset to investigate obesity and metabolic syndrome (e.g. Johansen *et al.*, 2001). This question can be explored in adult animals with a declared pathology, but also before the onset of the disease, during sensitive periods such as the perinatal life. The relatively short developmental period and lifespan of pigs, compared to humans, is consequently a unique opportunity to decipher the nutritional events/status shaping brain functions from birth to the adult age. Finally, brain imaging can be used to assess the impact of preventive or therapeutic strategies in preclinical studies. In this review, the case of functional food ingredients, neurostimulation, and bariatric surgery will be evoked.

**Table 1** and **Figure 1** summarize 10 years of collective work in nutrition research using *in vivo* brain imaging in the pig model at the St Gilles INRA group. Specifically, the author would like to individually acknowledge Charles-Henri Malbert for his outstanding contribution to the development of nuclear brain imaging in the pig model, as well as Nicolas Coquery and Hervé Saint-Jalmes for the implementation of pig functional magnetic resonance imaging (fMRI) on the PRISM imaging platform. In addition to published references, a few unpublished data have been included in this review to illustrate the more recent questions and perspectives currently investigated by our group.

## Brain responses to food signals

### *Brain responses to basic tastes*

The brain integrates a huge amount of information coming from the environment and the inside of the organism, through different communication pathways. Exposure to food and their post-ingestive consequences generates both exteroceptive (e.g. smell, taste, vision) and interoceptive (*i.e.* gut perceptions) signals that the brain must integrate and associate to elaborate a multisensory mental representation of food items. This mental representation includes physical and hedonic dimensions depending on the individual experience and shaping the individual expectations. One of our first objectives was consequently to map the brain responses to basic tastants, such as sweet or bitter compounds, which are known to elicit strong innate appetitive or repulsive reactions (Steiner *et al.*, 2001). The role of gut nutrient sensing in modulating appetite and conditioning food preferences (notably *via* sugar gut infusions) has also been extensively described (Sclafani and Ackroff, 2012). For sweet/sugar perception, our first question was to elucidate whether the detection site might condition the brain responses elicited. Glucose portal infusion (Figure 1, e) and duodenal infusion (Figure 1, d) both reduced feed intake and ingestion speed in a similar way, but induced different systemic signals and different brain responses (Table 1, Boubaker *et al.*, 2012), which demonstrates that gut nutrient and/or taste perception can shape brain responses related to hedonic and homeostatic regulation of food intake in the pig. The next step was to compare exteroceptive (*i.e.* gustatory) and interoceptive (*i.e.* duodenal) perceptions of sweet taste/nutrient, and more precisely to compare the impact of combined vs. dissociated oral and intestinal

sucrose detection on the brain reward processes. Oral and/or duodenal sucrose sensing (Figure 1, c, d) induced differential cerebral blood flow changes in brain regions known to be involved in memory, reward processes and hedonic evaluation of sensory stimuli (Table 1, Clouard *et al.*, 2014). Such information are valuable to disentangle the respective roles of oral and visceral perception of sweet/sugar in modulating food intake and motivation, and for translational research using the pig model: *e.g.* to assess the impact of additives or artificial sweeteners, design new food products, or improve their nutritional quality with a benefits/risks approach in terms of palatability, health, gut-brain axis, and behavior.

More recently, using fMRI, we aimed at comparing oral sweet and bitter gustatory stimulation (Table 1, Coquery *et al.*, 2018). The advantage of fMRI for comparing brain responses to different food stimuli is that multiple stimulations can be performed within the same imaging session, contrary to nuclear imaging (positron emission tomography, PET, and single-photon emission computed tomography, SPECT). The fMRI modality has already been implemented and used in the pig, notably to assess brain activity after deep brain stimulation (Knight *et al.*, 2013, Gibson *et al.*, 2016), but to our knowledge, the work performed by Coquery *et al.* (2018) is the first to describe blood-oxygen-level dependent (BOLD) brain responses to visual (Figure 1, a) and food stimuli in the pig. We showed that quinine and sucrose stimulations (Figure 1, c) induced different brain activation patterns in gustatory, hedonic, and cognitive areas (Table 1, Coquery *et al.*, 2018). This might contribute to the understanding of the effects of bitter compounds on gut physiology and behavior, for which significant effects were observed on gastric emptying, intestinal motility, gastrointestinal hormones, and food intake (Fu *et al.*, 2016, Roura *et al.*, 2016).



### *Brain responses to preferred or aversive food flavors*

Previous review papers explained why and how pig models could help disentangling the complex processes underlying food perception and preferences, and how such studies might benefit biomedical research (Clouard *et al.*, 2012b, Roura *et al.*, 2016). From 2011, we published the first studies using functional imaging to describe brain responses to conditioned food preferences and aversions in pigs, using 99mTc-HMPAO SPECT (Gaultier *et al.*, 2011, Boubaker *et al.*, 2012, Clouard *et al.*, 2014) and 18F-FDG PET (Clouard *et al.*, 2012c) to map cerebral blood flow and brain glucose metabolism, respectively. We validated the use of brain imaging in anesthetized pigs and confirmed that the brain is still able to integrate and react to food sensory stimuli (e.g. flavors) according to individual positive or negative experience and associated internal states (Table 1, Gaultier *et al.*, 2011, Clouard *et al.*, 2012c). Different brain responses were identified in a neural network including frontostriatal and limbic areas, which are known in the human and rodents to be involved in the regulation of food intake and pleasure (Figure 1, b, c). The associative conditioning between post-ingestive consequences and the meal's flavors shaped further hedonic valuation and food choices, which highlighted the gut-brain mechanisms influencing eating behavior in pigs. This model might be useful to investigate the onset of food aversion further invasive medical treatments, such as radiotherapy or chemotherapy, or exacerbated food preferences mediated by hedonic reinforcers such as added sugar (Clouard *et al.*, 2012b).

### *Impact of the metabolic or emotional status on brain responses to food signals*

Brain responses to food stimuli can be modulated by previous associative conditioning but also by the individual's metabolic or psychological state. In rodents, it has been shown that taste/olfactory perception capacity decreased with the visceral fat increase (Fernandez-Garcia *et al.*, 2017), and Thanos *et al.* (2008) documented significant changes in brain glucose metabolism in obese compared to lean rats exposed to appetitive food scent. Even though this question was not investigated in the pig model, we explored another relationship between the internal state and brain responses to odors. The emotional and physiological responses to stress are known to impact olfactory perception (Hoenen *et al.*, 2017). We recently aimed at describing the consequences of a psychosocial stress in pigs on the gut-brain axis, behavior and brain responses to olfactory food stimuli. We exposed stressed and control animals to low/high concentrations of novel food odors (Figure 1, b) and showed activations in the prepyriform, entorhinal, orbitofrontal, and anterior cingulate cortices, the dorsal striatum and hippocampus. We also showed that high-concentration olfactory stimuli induced a higher brain activity (Table 1, Coquery *et al.*, in revision). More interestingly, pigs with psychosocial stress had lower brain responses in the right hippocampus compared to unstressed control pigs, as well as lower magnetic-resonance-spectroscopy-estimated neuronal density in the hippocampus, which suggested an impact of the internal state on cognitive and limbic processes at the brain level (Table 1, Menneson *et al.*, in press).

## Impact of weight gain and obesity on brain functions

### *Brain anomalies in diet-induced obese minipigs*

The use of preclinical models is very important to decipher the determinants of obesity, eating disorders and metabolic diseases, but also to identify novel predictive biomarkers and innovative therapeutic strategies focused on the gut-brain axis (Val-Laillet *et al.*, 2015, Obrenovich *et al.*, 2016). The minipigs' limited size enables longitudinal studies at the adult age using the same imaging machines as in humans. The first studies using the minipig as a model of obesity date back to the 80's (e.g. Phillips *et al.*, 1982), but most of the publications appeared after the year 2000 (Johansen *et al.*, 2001, Larsen *et al.*, 2005, Christoffersen *et al.*, 2007, Lee *et al.*, 2009, Madsen *et al.*, 2010, Val-Laillet *et al.*, 2010a). Brain activity was described for the first time in obese minipigs in the Gottingen breed (Val-Laillet *et al.*, 2011b), and then in the Yucatan (Ochoa *et al.*, 2016a). The brain functional characteristics or anomalies associated with obesity and weight gain have been well described in the human, encompassing initial high-sensibility to highly palatable food stimuli in high-risk individuals (*i.e.* reward surfeit), but also decreased brain responses in the prefrontal cortex and striatum in obese patients (*i.e.* reward deficit), as well as a blunted dopaminergic system (Burger and Stice, 2011, Blum *et al.*, 2015).

Even though evidence suggests that chronic exposure to energy-dense palatable foods provokes a switch in the hedonic and motivational brain processes (Burger and Stice, 2011), and that successful body weight loss can reverse some obesity-related brain anomalies (Le *et al.*, 2007), there was no clear demonstration showing that these brain anomalies are induced by (or concomitant to) weight gain in obese people and not preexistent to it. Our brain imaging study in the minipig (Table 1, Val-Laillet *et al.*, 2011b), in association with physiological data (Val-Laillet *et al.*, 2010a,

Val-Laillet *et al.*, 2010c), was the first to demonstrate that diet-induced obesity could induce brain anomalies in comparison to lean subjects, including decreased basal metabolism in the prefrontal cortex and reward circuit (Figure 1, diet-induced obesity). These results suggested that some characteristics of the reward deficit phenotype observed in obese humans were probably an acquired feature of obesity, induced by regular excess food intake.

#### *Impact of specific nutrients on brain anomalies in obese minipigs*

Following the 2011 demonstration in obese minipigs, two questions arose: Are weight gain and excess calorie intake only liable for these brain anomalies described in obese subjects? And does food quality, independently from the quantity consumed, have a role in shaping brain activity in obese subjects? In two additional independent studies in obese minipigs, we decided to compare different isocaloric obesogenic diets differing in terms of lipids or carbohydrates quality, respectively. The first study compared a 5-week exposure to three obesogenic diets only differing in the nature of lipids used for their composition (*i.e.* fish oil / omega-3, sunflower oil / omega-6, or lard / saturated fatty acids). Omega-3 fatty acids inhibit the production of pro-inflammatory cytokines (Parker *et al.*, 2006), and omega-3 supplementation (DHA) can increase the activity of the prefrontal cortex (McNamara *et al.*, 2010), which is one of the brain areas that were found deactivated in obese humans and minipigs (Le *et al.*, 2006, Volkow *et al.*, 2009, Val-Laillet *et al.*, 2011b). We hypothesized that omega-3 might prevent the onset of central inflammation and protect brain areas involved in food intake control. We demonstrated significant changes in terms of blood-brain barrier (BBB) permeability and basal brain glucose metabolism according to the diets (Table 1, Val-Laillet *et al.*, 2011a and 2013). Contrary to what was

expected, we showed that a diet (overly) enriched with omega-3 did not protect the BBB nor the basal metabolism of brain areas that are dysfunctioning in the context of obesity (Figure 1, diet-induced obesity). Consistent results were obtained on intestinal permeability (Lalles *et al.*, 2011 and 2013), suggesting global effects of the diets on the gut-brain axis. Excessive dietary omega-3 might increase the fluidity of intestinal and BBB cell membranes, therefore increasing their permeability and facilitating the invasion of pro-inflammatory compounds towards blood and brain. Even though this hypothesis still needs validation, these results highlight the need for precise recommendations in terms of dietary omega-3, because excessive supplementation might be as deleterious as a deficiency for the gut and brain functions. The European Food Safety Authority stated that dietary recommendations for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for European adults are between 250 and 500 mg/day, and that supplemental intakes at doses up to 5 g/day do not raise safety concerns for adults in terms of cardiovascular disease risk, glucose homeostasis or immune function (Authority, 2012). The omega-3 supplementation in our minipig study was 1.9 g/day in average, which corresponded to 3.2 g of omega-3 per day in a normal-weight 63-kg adult woman. This was above the recommended dose and below the tolerable upper intake level of omega-3 according to the EFSA, but no other study in our knowledge ever investigated the brain metabolism outcomes at these doses.

The second study relating nutrient quality and brain activity compared a long-term exposure to three obesogenic isocaloric diets, only differing in the nature of their carbohydrates (*i.e.* starch as control, compared to glucose and fructose) (Ochoa *et al.*, 2014, Ochoa *et al.*, 2016a, Ochoa *et al.*, 2016b). Fructose, which is overly used in industrial food products, has been incriminated for its role in the onset of metabolic

syndrome and its impact on gut-brain metabolic signals in rodents (Dornas *et al.*, 2015, Ochoa *et al.*, 2015, Legeza *et al.*, 2017); but counter-arguments have been raised (Rizkalla, 2010), and the results obtained in the minipig model feed the controversy. Obesogenic diets altered fat accretion and metabolic profiles independently of the dietary carbohydrates, but only pigs chronically fed the fructose diet developed a strong preference for it (Ochoa *et al.*, 2014, Ochoa *et al.*, 2016b). After seven months of diet, all groups presented a similar weight gain and insulin sensitivity index, and compared to starch, chronic exposure to fructose and glucose induced bilateral activations in several reward-related brain regions including prefrontal and striatal areas (Figure 1, diet-induced obesity), with no significant differences between fructose and glucose groups (Table 1, Ochoa *et al.*, 2016a). Consistent to the dynamic vulnerability model (Stice *et al.*, 2013), it is plausible that our minipigs did not develop a blunted reward response yet, since they were food restricted for the entire experimental diet exposure and became only moderately obese. The brain metabolism differences observed here would rather be in favor of the reward surfeit theory and hypersensitization of the reward circuit, which might precede the reward deficit status, which was previously validated in the massively obese minipig model (Val-Laillet *et al.*, 2011b).

#### *Dynamic relationship between brain activity, body weight and diet changes*

Amongst the hot questions on the relationship between excessive consumption of palatable foods and the emergence of brain dysfunctions, is the onset dynamic of these anomalies and its relationship with the body weight status. It is still unknown whether regular consumption of highly palatable foods without weight gain, or different strategies to lose weight, can also influence neural networks and related

behavioral functions. Gautier *et al.* (unpublished data) aimed at following the neurocognitive processes in a cohort of adult minipigs, from a normal-weight status when exposed daily to high-fat-sugar (HFS) diet rationed not to exceed the metabolic needs, to the onset of obesity when *ad libitum* food intake was permitted, and finally to a third stage when animals were subjected either to a restrictive diet or bariatric surgery to make them lose weight. This is the first study describing longitudinally the impact of diet switch and/or body weight variations on metabolic and neurocognitive processes, using two *in vivo* brain-imaging modalities (PET and fMRI) in the same minipigs. Our preliminary and yet unpublished data indicated that brain responses to sucrose were dependent on both the metabolic/nutritional status and body weight, and that significant variations of brain activity were detected in the dorsal striatum, prefrontal cortex and insular cortex. Complete results are still pending, but we hope to identify brain-imaging markers that might predict weight gain or weight loss after nutritional interventions, in combination with metabolic markers (e.g. hormonal, transcriptomics, metabolomics, and metagenomics) to hypothesize the potential underlying microbiota-gut-brain mechanisms influencing eating behavior and disorders.

## **Perinatal nutrition and the gut-brain axis development**

### *The Developmental Origins of Health and Diseases*

The concept of the Developmental Origins of Health and Diseases (DOHaD), initially described by David Barker (Barker, 2001), postulates that early nutritional environment can modify the progeny's metabolism, physiology and behavior, and influence its susceptibility to declare further pathologies. In the human, it is admitted

that maternal obesity increases the prevalence of obesity and metabolic syndrome in the progeny (McGuire *et al.*, 2010). The effects of high-calorie and high-lipids diets on glucose metabolism, energy balance, cardiovascular function and adiposity of the progeny have been demonstrated in rodents, pigs and non-human primates (Albuquerque *et al.*, 2006, Williams *et al.*, 2014, Wang *et al.*, 2016), similarly to the susceptibility to declare cognitive, emotional, psychiatric, and behavioral disorders (Bale *et al.*, 2010). Maternal obesity and/or a plethoric high-fat diet during gestation and lactation also induce brain anomalies, with the possible onset of neuro-inflammation, decreased spatial memory, neurogenesis and brain plasticity alterations (*e.g.* in the hippocampus and reward circuit). The microbiota represents a major actor of the gut-brain communication (Bienenstock *et al.*, 2015, Carabotti *et al.*, 2015). In mice, maternal consumption of a high-fat diet during gestation and lactation impacts the intestinal immunity and microbiota of the progeny (Myles *et al.*, 2013), and a direct relationship exists between excessive food intake before weaning, the microbiota composition, and body adiposity accretion (Sefcikova *et al.*, 2011).

#### *Early nutritional programming of neurocognitive functions in the minipig*

The pig provides a great opportunity to investigate the consequences of nutritional programming for the gastrointestinal tract (Guilloteau *et al.*, 2010) and neurodevelopment (Mudd and Dilger, 2017). Recent studies focused on the impact of perinatal nutrition on brain functions in pigs, showing for instance that perinatal choline deficiency and maternal dietary choline both influence brain development in young pigs (Mudd *et al.*, 2016, Mudd *et al.*, 2018). Though, most of the translational research on perinatal programming of obesity and associated diseases has been



performed in rodent models, whilst the use of large animals such as pigs is scarce (Gonzalez-Bulnes and Chavatte-Palmer, 2017).

To contribute to fill this gap, we performed an experiment in the Yucatan minipig model and constituted two groups of sows fed either a standard or HFS diet during the last third of gestation and all the lactation period. Maternal daily food rations were calculated to prevent obesity and body weight differences between control and HFS groups (contrary to most studies in rodents). All the piglets from both groups were fed a standard diet after weaning. The HFS maternal diet increased plasma levels of triglycerides and free fatty acids in weaned piglets, but more interestingly, it significantly decreased the fecal amount of short chain fatty acids (SCFA) 100 days after birth, even though all animals were fed a standard diet after weaning (Val-Laillet *et al.*, 2017). This indicates a decreased fermentation activity of the intestinal microbiota, already detected in HFS sows. An alternative hypothesis would be an increased intestinal absorption/use of SCFA, and further investigations in terms of intestinal metabolism or metagenomic sequencing are required to disentangle this question. In the holeboard test (spatial learning task), where the animals have to learn and remember the location of food rewards hidden in a complex environment, Yucatan piglets born from HFS sows had increased reference and working memory scores compared to control (Val-Laillet *et al.*, 2017), which corroborated previous data in conventional piglets (Clouard *et al.*, 2016). This might be explained by increased cognitive abilities and/or an increased motivation to perform/complete the cognitive task since it was reinforced by highly palatable food rewards (chocolate-sugar-coated peanuts). Any change in the motivation to obtain these food rewards can influence the animals' memory scores. This is where the exploration of brain functions and activity is fundamental to interpret such ambivalent results.

After the post-weaning behavioral tests, some piglets were euthanized to explore their brain plasticity and neuro-inflammation in the hippocampus and prefrontal cortex. The hippocampus is involved in spatial learning while the prefrontal cortex has a major role in hedonic valuation of food and goal-oriented behavior.

Interestingly, the piglets born from HFS mothers had a smaller hippocampal granular cell layer and a decreased neurogenesis (Val-Laillet *et al.*, 2017). An increased microglial cell density was also observed in the prefrontal cortex, and the activation of microglia was increased in the hippocampus and prefrontal cortex (unpublished data). These observations rather praise in favor of decreased cognitive abilities and a possible alteration of the prefrontal processes involved in food valuation and motivation, due to neuro-inflammation processes.

#### *Long-term impact of early nutritional programming*

Some piglets were kept alive until the adult age to perform new behavioral tests, metabolic measures and brain exploration with *in vivo* imaging techniques. The differences observed in the young age for the holeboard test were not found again, but during the maze test (another spatial cognitive test that is not reinforced by food rewards, contrary to the holeboard test), minipigs born from HFS mothers presented more stress and reduced cognitive abilities (Table 1, Gautier *et al.*, 2018). More interesting is the fact that these animals showed a reduced basal glucose metabolism in the prefrontal cortex and nucleus accumbens (Figure 1, maternal HFS diet), which are both parts of the brain reward circuit. We also demonstrated that these animals also presented a decreased dopamine and/or serotonin transporter availability in the striatum and hippocampus. These brain anomalies are usually observed in obese subjects, as previously demonstrated in humans (Wang *et al.*,

2001, Le *et al.*, 2006, Volkow *et al.*, 2008, Volkow *et al.*, 2009) and minipigs (Val-Laillet *et al.*, 2011b, Ochoa *et al.*, 2016a). It is consequently striking to find them in animals that were fed a standard diet from weaning without abnormal weight gain or metabolic disease until the adult age. This suggests that HFS diet might program the brain even in the absence of weight problem and possibly modulates adaptation mechanisms to further dietary challenge. Further research is needed in this model to demonstrate that the brain and the digestive system, including gut microbiota, can influence each other with causal relationship to modulate the susceptibility to health and diseases, and notably behavioral and metabolic disorders.

### **Impact of nutritional preventive or therapeutic strategies on brain activity**

As highlighted in the previous section, data are lacking to connect the microbiota and gut physiology to neurocognitive outcomes in the pig model. Through preventive or therapeutic interventional strategies aimed at influencing the susceptibility to health and diseases, it is possible to modulate metabolism, brain and behavior. In this last section, we will review three examples of interventions for which brain activity modulation was documented in the pig model.

#### *Impact of food functional ingredient/supplementation on brain activity*

Because many evidences indicate a crucial role for the microbiota in regulating different aspects of behavior and mental health, as well as comorbidities related to eating and metabolic disorders (van de Wouw *et al.*, 2017), pre- and probiotics open the way to novel biotherapeutic strategies. Pinto-Sanchez *et al.* (2017) recently demonstrated that a probiotic supplementation reduced depression scores and

increased the quality of life in patients with inflammatory bowel disease. Interestingly, they also showed changes in brain activity *via* fMRI, with reduced reactivity of the limbic system. Comparison data are lacking in the pig model, but the use of maternal prebiotics can impact the progeny's growth, intestinal microbiota, morphology and immunity in pigs (Le Bourgot *et al.*, 2014, Le Bourgot *et al.*, 2016, Le Bourgot *et al.*, 2017). The use of dietary prebiotics early in life in piglets also supported recognition memory and modulated the concentrations of SCFA in the colon, blood, and brain, as well as hippocampal serotonin (Fleming *et al.*, 2017). Interestingly, we recently showed that oral supplementation in butyrate might shape brain activity and plasticity (Val-Laillet *et al.*, 2018). Butyrate is a SCFA that is naturally produced in the gut through bacterial fermentation of dietary fibers playing an essential role in gastrointestinal functions (Guilloteau *et al.*, 2010). This microbiota metabolite (*i.e.* postbiotic) has a trophic role for colonocytes, modulatory properties on gut morphology and functional plasticity, and is part of the histone deacetylase (HDAC) inhibitors having positive effects on long-term memory processes (Guilloteau *et al.*, 2010, Val-Laillet *et al.*, 2018). For that matter, some authors suggested that butyrate could prevent memory impairment and improve cognition through the upregulation of neurotrophic factors (Moretti *et al.*, 2011, Valvassori *et al.*, 2014). To tackle this question in the pig model, we compared two groups of juvenile animals that were exposed during three weeks to either oral sodium butyrate or control treatment (Table 1, Val-Laillet *et al.*, 2018). We demonstrated that the butyrate oral supplementation triggered basal brain glucose metabolism changes in the hippocampus and nucleus accumbens, which are involved in cognitive and hedonic processes (Figure 1, g). Interestingly, *post-mortem* immunohistochemistry data revealed a higher granular cell layer volume, cell proliferation and neurogenesis with

the butyrate treatment, supporting a positive modulatory role of this postbiotic on hippocampal plasticity and neurocognitive processes (Val-Laillet *et al.*, 2018).

Apart from pre-, pro- and postbiotics, other kinds of food functional ingredients can be used to modulate behavior and brain activity. Stressful environments/events are known to alter well-being and eating behavior, sometimes leading to appetite loss and anorexia or to food cravings and hyperphagia. This has been described in humans (Guarda *et al.*, 2015, Geiker *et al.*, 2018) as in pigs (Clouard *et al.*, 2012b). In young pigs, we demonstrated positive effects of sensory feed functional ingredients based on natural plant extracts on eating behavior, with increased feed preference and intake after a feed transition for example (Clouard *et al.*, 2012a, Clouard and Val-Laillet, 2014). Then, we explored the brain responses to one of these feed functional ingredients and showed that the perception of the ingredient (Figure 1, b, c) in familiarized individuals induced different responses in reward and perception brain areas compared to familiar control feed flavor in naive animals (Table 1, Val-Laillet *et al.*, 2016). In addition to potential application in animal production to improve well-being and feed intake, translational applications might be assessed in fragile human populations suffering from appetite loss, decreased motivation to eat, and/or denutrition. These food functional ingredients might also be tested in the context of degraded/stressful environments, to assess their ability to counterbalance at the brain level the effects of stressors.

#### *Impact of chronic vagus nerve stimulation on brain activity*

Chronic vagus nerve stimulation (VNS) is used in clinical practice as a therapy for refractory epilepsy and some psychiatric disorders (Vonck *et al.*, 2009), but it is also

a promising potential strategy to fight against obesity and an original methodological approach to understand how the gut-brain signals transmitted *via* the vagus nerve can modulate brain activity, metabolism and eating behavior. The pig model was rapidly in good position amongst candidate animal models to assess VNS against obesity (for review, see Val-Laillet *et al.*, 2010b). The vagus nerves convey much information emanating from the gut, and notably information related to gastric distension, one of the first satiation signals. We showed in young pigs that the activity of several brain areas was found significantly associated with the increase of gastric distension pressure (Figure 1, f), such as the olfactory bulb, prefrontal cortex, and amygdala (Layec *et al.*, 2009, Sauleau *et al.*, 2009). But after bilateral vagotomy, only a limited number of brain areas still responded to gastric pressure (Table 1, Sauleau *et al.*, 2009). A pilot study also demonstrated that chronic gastric distension (*via* a gastric balloon, Figure 1, f) activated the nucleus accumbens (Layec *et al.*, 2009). With VNS, the aim is to mimic the nervous gut-brain signals emitted with stomach distension. The first pilot study investigating brain responses to VNS was performed in lean juvenile pigs (Table 1, Biraben *et al.*, 2008). At that time, nuclear functional brain imaging in the pig model was emerging, and our digital pig brain atlas (Saikali *et al.*, 2010) was not available, which explains why identification of activated brain structures was tricky and the interpretation very limited. Two years later, we reiterated a similar experiment in obese adult minipigs and demonstrated that chronic VNS during 14 weeks decreased feed intake, prevented the animals to gain further body weight contrary to sham animals, and modified the feed preferences with a decreased consumption of high-sucrose feed and increased consumption of standard feed (Val-Laillet *et al.*, 2010b).

With time, both VNS and brain imaging procedures were improved in the pig model, and two new studies were published (Malbert *et al.*, 2017a, Malbert *et al.*, 2017b). A first experiment compared three chronic VNS patterns in growing pigs (S1, S2, and S3, Table 1). Only S2 and S3 activated the dorsal vagal complex (DVC) and had an impact on the prefrontal cortex, contrary to S1 (Table 1, Malbert *et al.*, 2017b, Figure 1, h). Only S2 and S3 significantly reduced daily ingestion, the duration of eating bouts during the meal, as well as the consumption of the high-lipids diet during the three-choice feed test, where the animals had simultaneous access to control, high-lipids and high-glucose feeds provided by a three-through robotic feeder (Malbert *et al.*, 2017b). Taken together, these results demonstrate how specific nervous signals emanating from the gut can modulate the activity of the brain network involved in food intake regulation and goal-oriented behavior. This also highlights the importance of the prefrontal cortex in modulating food motivation and preferences. Another study in obese minipigs reused the same VNS procedure as that previously successfully tested (Val-Laillet *et al.*, 2010b) to investigate the VNS impact on metabolism and brain activity (Table 1, Malbert *et al.*, 2017a). Mean whole body insulin sensitivity and hepatic glucose uptake rate were significantly lower in non-stimulated obese minipigs compared to lean minipigs, and contrary to stimulated obese minipigs. Stimulated animals also had increased glucose metabolism in the anterior prefrontal cortex and cingulate cortex compared to non-stimulated animals (Table 1, Malbert *et al.*, 2017a, Figure 1, h). Once again, this study demonstrated that chronically mimicking satiation signals emanating from the gut could improve the activity of the prefrontal cortex, which is known to have inhibitory inputs toward the orexigenic brain areas and to be deactivated in obese individuals.

### *Impact of bariatric surgery on brain activity*

To conclude this section on interventional strategies, it is interesting to evoke what is still one of the most effective therapeutic compromises against morbid obesity: the Roux-en-Y gastric bypass (Kang and Le, 2017). A recent paper reviewed the evidences derived from neuroimaging and animal (mainly rodent) studies (Hankir *et al.*, 2018), explaining how a metabolic surgical procedure such as the Roux-en-Y gastric bypass (RYGB), which is drastically reorganizing gut, including peptide hormones and microbiota metabolites, is also reshaping homeostatic and hedonic brain processes. Different animal models of bariatric surgery have been described (Rao *et al.*, 2010) and the first demonstrations of RYGB in the pig model, in our knowledge, date back in the 90's (Frantzides *et al.*, 1995, Potvin *et al.*, 1997). Since then, the pig and minipig models have been widely used to describe the functional mechanisms of RYGB. Not being exhaustive, we can refer to studies having investigated the gastrointestinal histological morphology (Gentileschi *et al.*, 2006), long-term weight loss in super-obese minipigs (Birck *et al.*, 2013), changes in adipose tissue distribution (Chavez-Talavera *et al.*, 2017), beta-cell function, biliary function, and glucose metabolism (Lindqvist *et al.*, 2014, Sham *et al.*, 2014, Baud *et al.*, 2016, Chavez-Talavera *et al.*, 2017, Lindqvist *et al.*, 2017b), as well as incretins regulation (Lindqvist *et al.*, 2017a) for example. Despite all of this work, we found no study on the consequence of RYGB (or any other bariatric surgery) on brain activity and metabolism in pigs.

Identifying a way to reproduce the gut-brain effects of RYGB without the RYGB surgery *per se* would free the patients from possible deleterious consequences of invasive bariatric surgery (Lupoli *et al.*, 2017). That is why preclinical research in a relevant model such as the pig is necessary. In a longitudinal study (unpublished



data), we aimed at phenotyping diet-induced obese Yucatan minipigs subjected either to a RYGB (cf. Baud *et al.*, 2016 for surgery procedure), or a pair-fed restrictive dietary treatment. Peptides and hormones measurements, gastrointestinal histological morphology and immunohistochemistry, transcriptomics, metabolomics, and metagenomics analyses were performed to provide a systemic description of the intestinal (including microbiota) and metabolic phenotype. The obese minipigs were also subjected to fMRI and PET brain imaging before and three months after the surgery (RYGB or sham) to investigate the brain responses to sucrose (Figure 1, d, f). Our preliminary results (Table 1, Bergeat *et al.*, unpublished data) are in line with human brain imaging studies showing a modulation of the mesolimbic and frontostriatal pathway after RYGB (Ochner *et al.*, 2011, Scholtz *et al.*, 2014). To date, the minipig is the only preclinical model for which such an analogy has been described, which opens the way to further interventional studies aiming at unraveling the complex gut-brain interactions shaping eating behavior after bariatric surgery.

### **Critical conclusion**

This overview of 10 years of nutrition research using *in vivo* brain imaging in the pig illustrates how relevant this preclinical model is to disentangle the respective roles of food, gut-brain signals, and metabolic status in modulating brain activity and eating behavior. The research topics exposed in this review cover the fields of food technology, digestive physiology, sensory sciences, metabolic adaptation, nutritional programming, experimental surgery, and behavioral neurosciences. Despite the intricacy of all sensory, physiological, and metabolic signals susceptible to modulate brain activity, several interventional targets were identified in these studies. Most of

this work remains rather descriptive and only superficially brushes mechanistic hypotheses. In the future, more applied and preclinical research investigating concrete strategies to improve health and well-being, both in animals and humans, are needed. Identifying brain activity and metabolism changes is a prerequisite to validate the existence of gut-brain processes that can be used as operational leverages. But the finality is clearly to operate actions: to identify at-risk metabolic and neurocognitive phenotypes, to improve individual adaptation abilities towards challenging diets or environments, to change individual eating habits in favor of healthy behaviors.

Coming back to the pig model, it is important to evoke its limitations/advantages and the questions arising from ethical concerns. Both these considerations should help identifying the added value of animal experimentation and provide a rationale for its reasonable use when no alternative exists, and as long as none has emerged. In terms of limitations of the pig model, the main constraints lie in the fact that all brain-imaging studies presented here were performed in anesthetized animals, which moderates the analogy with human neurobehavioral studies in the same thematic. In spite of that, we identified on a recurring basis some brain activity in highly integrative neural networks that are characteristic of complex cognitive processes. Of course, brain activity during general anesthesia is weakened and different from that in the awakened state, but all these studies demonstrated that the anesthetized brain can still integrate and interpret positive or negative food stimuli in relation to the internal state and individual experience. They open a small window on what might happen also in the awakened animals.

Although the pig is a very good model for nutritional and cognitive studies, there are dimensions of eating behavior that cannot be modeled with it, such as psychological

and cultural influences. For example, even if a pig model of *anorexia nervosa* can mimic metabolic and behavioral symptoms, it has nothing to do with the real disease (Danek *et al.*, 2017). Similarly, the individual history leading someone to becoming obese or food addict cannot be strictly reproduced in the animal. The emotional, motivational, and hedonic dimensions of eating can be investigated in pigs, but factors influencing them in the human are much more complex than in pigs. Cognitive control of eating is a very important leverage to change individual habits (Val-Laillet *et al.*, 2015), but it is specific to humans and does not apply to animals. What justifies the use of the pig model though, is the opportunity to investigate questions that cannot be solved by human studies only, as well as to control specific experimental conditions in order to disentangle the respective roles of many biological factors and influences. Animal studies are still legally mandatory before testing new medicines or medical treatments in the human; a precise control of the environment and diet is possible in animal models and completely illusory in humans; induction of a disease or disorder, or long-term interventions with possibly deleterious consequences in terms of health and well-being, cannot be justified in the human and are still tolerated in animals, when there is a clear rationale and strict ethical conduct. All these arguments praise in favor of a translational research combining studies in relevant animal models such as the pig, as well as studies in the human, with a constant and bilateral dialog between them, to achieve better public health outcomes.

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### **Declaration of interest**

The author declares no conflict of interest.

### **Ethics committee**

The experiments performed by our group and presented in this review paper were conducted in accordance with the current ethical standards of the European Community (Directive 2010/63/EU; Agreement No. A35-622) for experimental facilities and Authorization No. 35–88 or 01894 to experiment on live animals for the principal investigators of the studies. The Regional Ethics Committee for Animal Experimentation of Brittany and French Ministry of Research have validated and authorized the entire procedures described in these studies. Specific authorization numbers are mentioned in the original papers.

### **Software and data repository resources**

Most of the data presented in this review paper were not deposited in an official repository, except brain imaging data from two articles, which are available from the DRYAD public repository (<http://datadryad.org/>). For Val-Laillet et al. (2016), please refer to [doi.org/10.5061/dryad.ck3g3](https://doi.org/10.5061/dryad.ck3g3). For Ochoa et al. (2016a), please refer to

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**Table 1.** Summary of 10 years of nutrition research (2008-2018) using *in vivo* brain imaging in the conventional pig ([Large White x Landrace] x Piétrain) and minipig models. All these studies were performed by our INRA research group on the PRISM imaging platform of Rennes, France. The approximate age of the animals at the time of brain imaging is indicated in the second column. All references were ordered chronologically. Main imaging techniques used: 18F-fluorodeoxyglucose (18FDG) positron emission tomography (PET) to map brain glucose metabolism; 99mTc-hexamethylpropyleneamine oxime (99mTc-HMPAO) single-photon computed tomography (SPECT) to map cerebral blood flow; functional magnetic resonance imaging (fMRI) based on blood-oxygen-level dependent (BOLD).

N°	Pig models	Type of food-related sensory stimulation and/or dietary treatment	<i>In vivo</i> brain imaging modalities	Research highlights	References
1	3-month-old female growing conventional pigs	Chronic vagus nerve stimulation (VNS) during 5 weeks	99mTc-HMPAO SPECT	VNS triggered brain plasticity in subcortical structures of the reward circuit and activated the olfactory bulb	Biraben <i>et al.</i> 2008
2	3-month-old female growing conventional pigs	Gastric distension in animals with intact vagal nerves or bilateral vagotomy	99mTc-HMPAO SPECT	Before vagotomy, significant brain activity associated with increase of gastric distending pressure / After vagotomy, only a limited effect of increased gastric pressure on brain activity	Sauleau <i>et al.</i> 2009
3	3.5-month-old female growing conventional pigs	Olfactogustatory stimulation during brain imaging with different flavors previously associated with positive or negative post-ingestive consequences	99mTc-HMPAO SPECT	Higher activity in corticolimbic and reward-related areas with preferred flavour / deactivation of the basal nuclei and limbic thalamic nuclei with aversive flavor	Gaultier <i>et al.</i> 2011

4	Normal-weight or obese adult 2.5-year-old male Göttingen minipigs	5-month exposure to standard diet (102 kcal/kg <sup>0.75</sup> ) or <i>ad libitum</i> Western diet	99mTc-HMPAO SPECT	Deactivation of the prefrontal cortex, ventral tegmental area, and nucleus accumbens in obese compared to lean subjects / Prefrontal cortex and insular cortex activity negatively associated with body weight	Val-Laillet <i>et al.</i> 2011b
5	Obese adult Pitman-Moore minipigs	5-week exposure to isocaloric high-energy high-fat diets enriched either with fish oil (omega 3, O3), sunflower oil (omega 6, O6), or lard (saturated fat, S)	18FDG PET / Dynamic injected CT-scan to map BBB permeability	Anterior prefrontal cortex metabolism lesser for O3, intermediary for S, and higher for O6 / Decreased activity in the nucleus accumbens for O3 compared to O6 / BBB the most permeable in O3, intermediary in S, and the least permeable in O6 in cortical areas / Correlation between BBB permeability and prefrontal cortex activity	Val-Laillet <i>et al.</i> 2011a, 2013 (conference proceedings)
6	3-month-old female growing conventional pigs	Duodenal or portal glucose 20% (120 ml in 10 min) infusion after an overnight fasting	99mTc-HMPAO SPECT	Both duodenal and portal glucose activated dorsolateral prefrontal cortex and primary somatosensory cortex / Duodenal glucose activated pyriform area, orbitofrontal cortex, caudate and putamen / Portal glucose activated insular cortex	Boubaker <i>et al.</i> 2012
7	3.5-month-old female growing conventional pigs	Olfactogustatory stimulation during brain imaging with different flavors previously associated with positive or negative post-ingestive consequences	18FDG PET	Aversive or less preferred flavors deactivated prefrontal cortex, activated posterior cingulate cortex, and induced asymmetric brain responses in basal nuclei and temporal gyrus	Clouard <i>et al.</i> 2012c
8	3.5-month-old female growing conventional pigs	Oral (5% sucrose or artificial saliva) and/or duodenal (16% sucrose or saline, 300 ml) sucrose stimulation after an overnight fasting	99mTc-HMPAO SPECT	Duodenal sucrose and combined oral/duodenal sucrose induced similar activity patterns in the putamen, ventral anterior cingulate cortex and hippocampus / Oral sucrose deactivated some brain areas in the prefrontal and insular cortices / Combined oral/duodenal sucrose activated right insular cortex and oral sucrose dissociated from caloric load induced specific activity patterns in hippocampus and parahippocampal cortex	Clouard <i>et al.</i> 2014

9	Obese adult 20-month-old male and female Yucatan minipigs	7-month exposure to a high-energy high-fat diet enriched either with starch, glucose or fructose - all diets were isocaloric (1 kg/day)	18FDG PET	Compared to starch, chronic exposure to fructose and glucose induced bilateral brain activations in anterior and dorsolateral prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, caudate and putamen	Ochoa <i>et al.</i> 2016a
10	2-month-old female growing conventional pigs	Olfactogustatory stimulation with food flavor with or without functional sensory additive in animals that were habituated or not to this additive since weaning	18FDG PET	Familiar feed flavors activated the prefrontal cortex / Amygdala, insular cortex, and prepyriform area were only activated in familiarized animals exposed to the feed flavor with additive / Perception of feed flavor with additive in the familiarized animals activated the dorsal striatum differently than perception of control feed flavor in naive animals	Val-Laillet <i>et al.</i> 2016
11	3-month-old female growing conventional pigs	15-day chronic abdominal vagal electrical stimulation with three different procedures: a long-lasting 1000- $\mu$ s pulse (S1), a series of constant-amplitude pulsons lasting 1000 $\mu$ s (S2), and a series of pulsons with rising amplitude also lasting 1000 $\mu$ s (S3)	18FDG PET	Both S2 and S3 activated the dorsal vagal complex and increased the metabolism of different afferent cortical structures, notably the insular, prefrontal, and cingulate cortices	Malbert <i>et al.</i> 2017b
12	Normal-weight and obese adult 18-month-old Yucatan minipigs	12-week chronic abdominal vagal electrical stimulation in obese minipigs compared to sham obese and sham normal-weight	18FDG PET	Vagal stimulation was associated with increased glucose metabolism in the cingulate and prefrontal cortices	Malbert <i>et al.</i> 2017a
13	3.5-month-old female growing conventional pigs	3-week exposure to water beverage with or without butyrate (110 mg/kg body weight/day)	18FDG PET	Sodium butyrate compared with control treatment triggered basal brain glucose metabolism changes in nucleus accumbens and hippocampus, increased hippocampal granular cell layer volume and neurogenesis	Val-Laillet <i>et al.</i> 2018

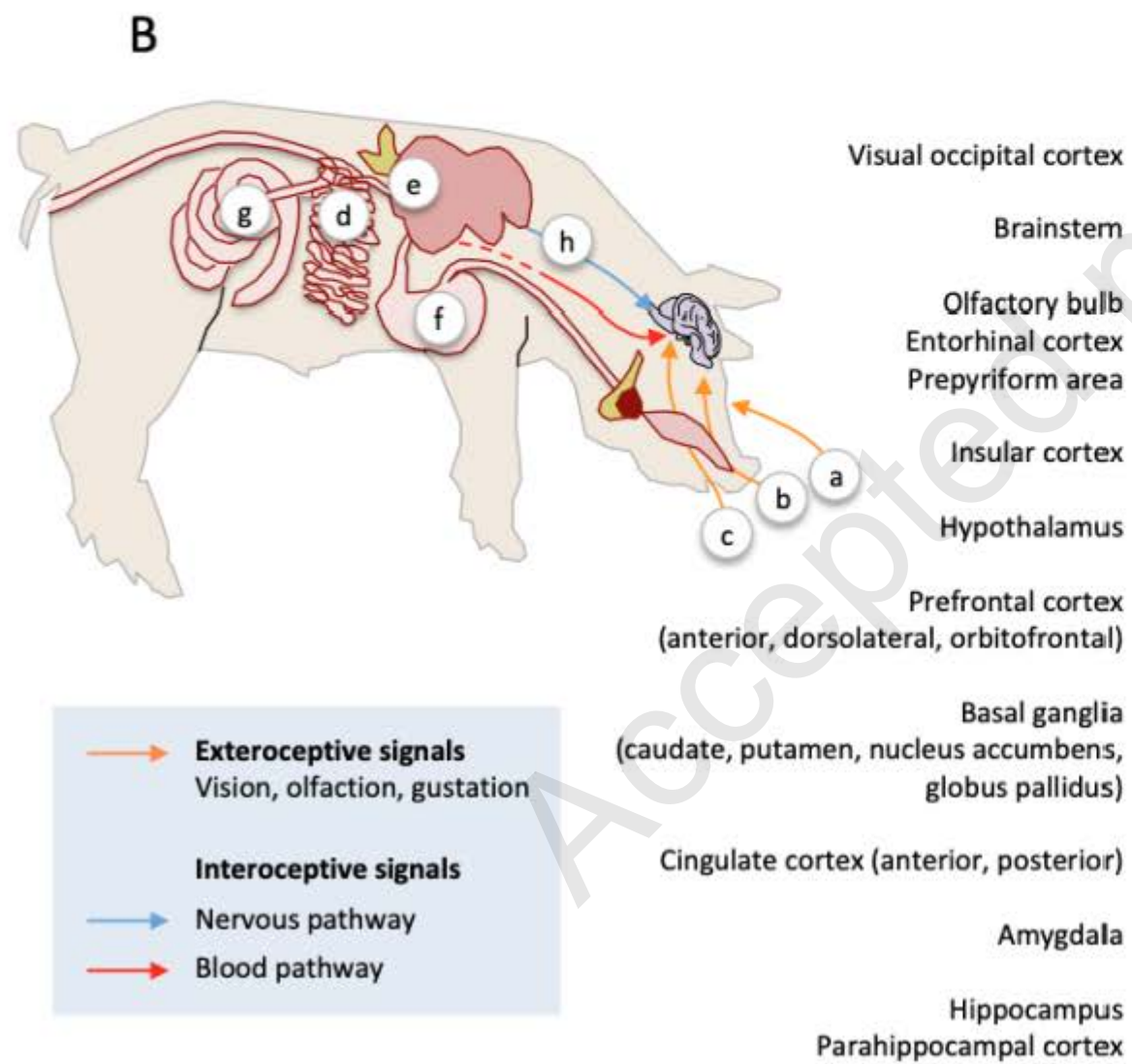
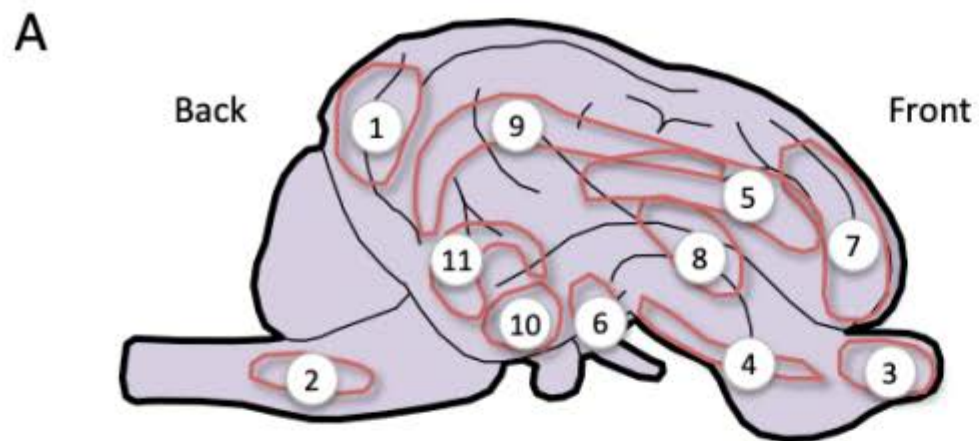


14	Normal-weight adult 18-month-old male and female Yucatan minipigs	Animals that were born from mothers fed either a standard diet (SD) or a Western diet (WD) during gestation and lactation	[123I]-FP-CIT SPECT (aka DaTscan) to map brain availability of presynaptic dopamine (and serotonin) transporter / 18FDG PET	At the adult age, WD progeny showed lower dopamine/serotonin transporter binding potential in hippocampus and parahippocampal cortex, as well as a trend in putamen, associated with lower basal brain activity in prefrontal cortex and nucleus accumbens compared to SD progeny	Gautier <i>et al.</i> 2018
15	Adult 18-month-old Yucatan minipigs	Gustatory stimulation with artificial saliva, quinine (10 mM) or sucrose (5%) after an overnight fasting	fMRI	Quinine and sucrose stimulation promoted different cerebral activation patterns / Insular cortex was activated with both sucrose and quinine, although some other regions were specifically activated by sucrose or quinine	Coquery <i>et al.</i> 2018
16	3.5-month-old female growing conventional pigs	Gustatory stimulation with unknown odors (citrus) at low or high (10x higher) concentration in pigs with or without psychosocial stress (social isolation, environmental impoverishment, and stressful visual and auditory stimuli)	fMRI / MRS (neuronal density)	Citrus odors activated prepyriform, entorhinal, orbitofrontal, and anterior cingulate cortices, dorsal striatum and hippocampus / Increased odor concentration induced higher brain activity in the reward circuit / Contrasted brain responses in stressed vs. non-stressed animals, and notably decreased hippocampal activity and neuronal density in stressed animals	Coquery <i>et al.</i> (in revision) / Menneson <i>et al.</i> (in revision)
17	Normal-weight and obese adult male and female Yucatan minipigs (from 1 to 2 years old)	Gustatory stimulation with sucrose (5% or 16%) in the same animals at three stages: normal-weight, after 3-month of <i>ad libitum</i> exposure to Western diet, and 3 months after gastric bypass or restrictive diet	18FDG PET / fMRI	Variations of brain activity are observed depending on the weight/metabolic status of the animals in the dorsal striatum, prefrontal cortex and insular cortex	Gautier <i>et al.</i> (unpublished data)
18	Obese adult 2-year-old male and female Yucatan minipigs	Gustatory stimulation with sucrose (5% or 16%) after an overnight fasting in obese minipigs 3 months after gastric bypass (GB) or restrictive diet (sham, pair-feeding design)	fMRI	Compared to sham, GB animals had higher brain activity in orbitofrontal cortex, anterior cingulate cortex, caudate nucleus and hippocampus / Compared to GB, sham animals had higher brain activity in insular cortex, somatosensory cortex and putamen	Bergeat / Coquery <i>et al.</i> (unpublished data)

[<sup>123</sup>I]-FP-CIT, *N*-(3-Fluoropropyl)-2 $\beta$ -carbomethoxy-3 $\beta$ -(4-[<sup>123</sup>I]iodophenyl)nortropine; BBB, blood-brain barrier; CT-scan, computed-tomography scanner; DatScan, dopamine transporter scanner; MRS, magnetic resonance spectroscopy; VNS, vagus nerve stimulation.

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**Figure 1.** Meta-representation summarizing 10 years (2008-2018) of nutrition-related functional brain imaging data in the pig model. A) Schematic representation of a pig's brain with the main regions of interest (ROI, 1-11) explored during the studies described in Table 1. B) Schematic representation of a pig with the gut-brain axis and different levels (a-h) of sensory/nutrient stimulation or treatment. C) Synthetic table showing the brain ROI that were modulated by different types of sensory/nutrient stimulation (in green) or treatment (high-fat-sugar diet in red, possible therapies in purple). For detailed explanations, please refer to the reference numbers presented in Table 1. Visual stimulation: 15; Complex odor/flavor stimulus: 3, 7, 10, 18; Basic taste oral stimulus: 8, 15, 16; Duodenal sugar infusion: 6, 8; Portal sugar infusion: 6; Gastric distension: 2; Maternal high-fat-sugar (HFS) diet: 14; Diet-induced obesity: 4, 5, 9, 16; Butyrate treatment: 11; Vagus nerve stimulation: 1, 12, 13; Roux-en-Y gastric bypass (RYGB): 16, 17. An asterisk indicates a significant modulation of the brain ROI with the type of stimulation or treatment. Absence of an asterisk for a specific ROI and stimulation/treatment corresponds either to an absence of modulation or to a missing data. The two sets of ROI represented by the frontostriatal network are highlighted (darkest blue) because they were almost systematically modulated in our studies and are part of the reward circuit described in the rodents and humans.



**C**

	a	b	c	d	e	f	g	h			
	Visual stimulus	Complex odor/flavor stimulus	Basic taste oral stimulus	Duodenal sugar infusion	Portal sugar infusion	Gastric distension	Maternal HFS diet	Diet-induced obesity	Butyrate treatment	Vagus nerve stimulation	RYGB
1	*						*				
2						*	*		*		
3						*	*		*		
4	*		*								
5	*	*	*			*	*		*	*	*
6						*	*		*		
7	*	*	*	*	*	*	*		*	*	*
8	*	*	*		*	*	*	*	*	*	*
9	*	*	*			*	*		*	*	*
10	*	*			*						
11	*	*	*				*	*	*	*	*

**Frontostriatal reward network**