EXPERIMENTAL SURGERY AND BRAIN IMAGING APPLIED TO NUTRITION STUDIES IN PIG AND MINIPIG MODELS

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ABSTRACT

The pig model is increasingly used in biomedical research and notably for nutrition studies. In addition to have numerous similarities with the human, in terms of anatomy and physiology, the pig has a brain that is structurally and functionally very close to that of non-human primates. The pig model consequently represents a unique opportunity to explore the neurophysiological determinants of the eating behavior and related pathologies. The aim of this conference is to present some examples of research using pigs and minipigs as models to study obesity and eating disorders, and benefiting from modern surgical and imaging techniques. The PRISM AniScans imaging platform and the associated experimental facilities will be presented. First, a pig model for the study of food preferences and aversions will be introduced, combining experimental surgery to modulate post-ingestive signals, behavioral tests to investigate food preferences and motivation, as well as brain imaging to assess the brain responses further to olfactogustatory and/or visceral food stimulations. Potential applications in the field of human and animal nutrition and health will be presented. Second, diet-induced obesity will be described in adult minipigs from different breeds (Göttingen, Pitman-Moore, Yucatan), with an emphasis on the behavioral and brain alterations induced by deleterious diets and weight gain. Comparing the respective effects of diets differing on their lipids and/or carbohydrates sources will highlight the importance of nutrient quality on eating behavior and health. Bariatric surgery alternatives will then be presented, such as the chronic vagus nerve stimulation and the deep brain stimulation, two promising therapeutic approaches to fight against obesity. A focus will be done on the state-of-the-art approaches used to explore these solutions (e.g. keyhole surgery, implantable devices, neuronavigation-assisted brain surgery, etc.), and research perspectives will be presented.

Key words: Brain Imaging, Eating Behavior, Experimental Surgery, Food Preference, Minipig, Obesity

INTRODUCTION

An extensive literature supports analogies at multiple levels between the pig and the human: general physiology and anatomy, sensory sensitivity, digestive function, cognitive abilities, cerebral features, etc. (Vodicka *et al.*, 2005; Lind *et al.*, 2007; Spurlock and Gabler, 2008; Sauleau *et al.*, 2009; Clouard *et al.*, 2012c). This proximity with the human makes the pig an interesting model to investigate the behavioral and neurophysiological determinants of nutritional pathologies related to eating disorders or deleterious food environments. The pig can develop some nutritional diseases described in humans (Jokinen *et al.*, 1985; Spurlock and Gabler, 2008). For example, several minipig strains, such as the Ossabaw, Yucatan or some Chinese pigs, have a natural predisposition to develop obesity, insulin resistance, type-2 diabetes and atherosclerosis in response to deleterious diets (Xi *et al.*, 2004; Bellinger *et al.*, 2006; Dyson *et al.*, 2006; Liu *et al.*, 2007; Neeb *et al.*, 2010; Clark *et al.*, 2011). The Göttingen minipig, currently the most popular minipig model in biomedical research and neurosciences, is also a very good model to investigate diet-induced obesity and metabolic syndrome (Johansen *et al.*, 2001). Conventional pigs can be used when a focus is made on the young age, but as soon as long-term studies or focus on adults is necessary, the minipig becomes an essential asset because its limited size is more adapted to laboratory purposes and medical exams. The originality of our research work is to combine the use of (mini)pig models and modern imaging tools to investigate the brain functions related to eating behavior and their relationships with health. The aim of this paper is to summarize some of our recent works, including the production of a pig model of food aversions and preferences, the description of brain anomalies related to diet-induced obesity, and the exploration of new therapeutic strategies alternative to bariatric surgery.

MATERIALS AND METHODS

This paper summarizes the main results of different studies performed in conventional piglets and adults from several minipig breeds. Detailed descriptions of the experimental designs are available in the original published papers and scientific communications.

Animals

Conventional piglets, Yucatan and Pitman-Moore adult minipigs were provided by the swine production experimental facilities of the INRA of St Gilles, France. Göttingen minipigs were purchased from Ellegaard (Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark). Table 1 summarizes the number and type of animals, as well as the methods and topics of recent brain imaging and nutrition studies performed in our lab in pig models.

Table 1. Recent nutrition studies using functional imaging and/or surgery in pig models.

References	Pig breeds	Number and sex	Methods	Topics
Val-Laillet et al. 2010 Br J Nutr Val-Laillet et al. 2010 Physiol Behav	Göttingen minipig	19 male adults	CT-Scan, gastric scintigraphy, food tests, plasma measurements	Obesity phenotyping in the minipig
Val-Laillet et al. 2010 Appetite	Göttingen minipig	8 male adults	Food tests, bariatric surgery and vagus nerve stimulation	Vagus nerve stimulation in obese minipigs
Val-Laillet et al. 2011 Obesity	Göttingen minipig	17 male adults	Brain SPECT	Brain activity changes after diet-induced obesity
Gaultier et al. 2011 Eur J Neurosci Clouard et al. 2012 PLoS ONE	Large White x Piétrain	8 and 12 female piglets	Food conditioning and tests, brain SPECT and PET	Brain responses to food flavours with different hedonic values
Boubaker et al. 2012 J Neuroendocrinol Clouard et al. 2013 submitted	Large White x Piétrain	17 and 12 female piglets	Food tests, brain SPECT	Brain responses to oral and/or visceral glucose or sucrose
Val-Laillet et al. 2011, 2012, 2013 (international conferences proceedings)	Pitman-Moore minipig	15 female adults	CT-Scan, brain PET	Effects of diets with different fat sources on metabolic and brain functions
Ochoa et al. 2013 in prep.	Yucatan minipig	18 male and female adults	CT-Scan, brain PET, DaT-Scan, food tests, plasma measurements	Effects of diets with different carbohydrates sources on eating behaviour and brain functions

Behavioral Investigations

Animals were housed in individual cages equipped with computer-controlled systems recording eating behavior. Food-choice tests were performed using multi-compartment automated troughs; food motivation was explored *via* progressive ratio tests in which animals had to push a button to obtain a food reward; meal microstructure parameters (meal time, food ingested, ingestion speed, etc.) were recorded *via* strain gauges fitted to the troughs.

Imaging Tools and Techniques

All the studies were performed at the PRISM Ani-Scans imaging platform, which is housed at the INRA of St Gilles. CT-Scan was used for anatomical explorations (e.g. body adiposity distribution), to assess the blood-brain barrier permeability, and to perform image-assisted navigation during brain surgery. Brain metabolism was explored *via* Single Photon Emission Computed Tomography (SPECT, ^{99}Tc-HMPAO) or Positron Emission Tomography (PET, ^{18}F-fluorodeoxyglucose), two correlated methods estimating brain activity *via* blood perfusion and glucose consumption, respectively. SPECT is also used to assess the dopaminergic function (DaT-Scan, ^{123}I-ioflupane).

Anesthesia and Experimental Surgery

Animals were systematically anesthetized during the brain imaging sessions to prevent any motion during image acquisition. Anesthesia and analgesia were also required during surgery and all details are provided in the original publications. Classical surgery was performed to insert venous and/or duodenal catheters for blood sampling and/or nutrient infusion. Laparoscopic surgery was used to ovariectomise adult female minipigs when necessary, in order to prevent heats during the experiments. Surgery was also used to insert electrodes and stimulators in the study that assessed the effects of vagus nerve stimulation in obese minipigs.

RESULTS AND DISCUSSION

Food flavor and nutrients: what drives pleasure and motivation in the brain

The use of pig models can help to understand the behavioral and neurophysiological mechanisms underlying the onset of food preferences and aversions, and consequently lead to the development of recommendations, clinical methods and/or pharmacological solutions aimed at improving health and nutrition in high-risk populations (Clouard *et al.*, 2012c). In a set of complementary papers (Gaultier *et al.*, 2011; Clouard *et al.*, 2012b), we managed to condition piglets to develop a preference or aversion towards specific food flavors, using Pavlovian conditioning. A duodenal catheter was surgically implanted in piglets, which allowed us to modify the post-ingestive consequences of a flavored meal by infusing solutions with negative (lithium chloride, LiCl), neutral (saline), or putatively positive effects (glucose). The piglets rapidly developed specific food preferences and aversions. Further conditioning, piglets were exposed again to the specific food flavors previously associated with negative, neutral or positive consequences, and their brain activity was recorded. Exposure to flavors with different hedonic values induced metabolism differences in neural circuits known to be involved in humans in the characterization of food palatability, feeding motivation, reward expectation, and more generally in the regulation of food intake. In Gaultier *et al*. (2011), exposure to a preferred flavor induced a higher activity in corticolimbic and reward-related areas, while an aversive flavor induced a deactivation of the basal nuclei and limbic thalamic nuclei. In Clouard *et al*. (2012b), aversive or less preferred flavors triggered global deactivation of the prefrontal cortex, specific activation of the posterior cingulate cortex, as well as asymmetric brain responses in the basal nuclei. Glucose duodenal infusions were not potent enough to induce clear food preferences though. We further confirmed that food preference is more difficult to condition than food aversion, and that nutrient signals probably act in synergy with food flavor (Clouard *et al*., 2012a). In another set of papers, we explored the brain activity further glucose (Boubaker *et al*., 2012) and sucrose sensing (Clouard *et al*., 2013a; Clouard *et al*., 2013b) at the visceral level, and showed that sugar sensing could modulate the meal microstructure as well as the activity of brain areas involved in the regulation of eating behavior. Interestingly, we demonstrated that paired but not uncoupled oral and duodenal sucrose sensing induced differential activation in the brain reward circuit, suggesting that oral signals might potentiate visceral signals during cognitive processes related to food pleasure (Clouard *et al*., 2013a; Clouard *et al*., 2013b). Even though these results were obtained in conventional piglets, the same techniques can be applied to miniature piglets, and ongoing research projects in minipigs will aim at unraveling the long-term mechanisms shaping eating behavior from early life to the adult age.

Diet-induced obesity: when food quantity and quality alter brain functions and health

Neuroimaging studies in humans suggest that obesity might be associated with brain anomalies, including a decreased activity of the prefrontal cortex (Le *et al*., 2006; Volkow *et al*., 2009) and a decreased availability of dopamine D2 receptors in the striatum (Volkow et al., 2008). Though, it was not possible to state whether these features were pre-existent or an acquired feature of obesity. Thanks to the minipig model, we managed to answer this important question. In a preliminary set of papers (Val-Laillet *et al*., 2010b; Val-Laillet *et al*., 2010c), we described the diet-induced obesity in adult Göttingen minipigs fed a Western diet, and demonstrated that many behavioral, metabolic and anatomophysiological features of obesity in this model are similar to what is described in humans (hyperphagia, food craving, weight grain and adiposity accumulation, insulin resistance, cardiovascular problems, etc.). To complete this phenotyping, we decided to compare the brain activity of obese and lean minipigs, and demonstrated that obese subjects had a decreased metabolism in the prefrontal cortex and reward circuit, including the nucleus accumbens and ventral tegmental area (Val-Laillet *et al*., 2011a). The prefrontal cortex activity was found negatively associated to body weight, similarly to what Volkow *et al*. (2009) described in obese humans. Therefore we demonstrated that less prefrontal cortex basal metabolism was an acquired feature of obesity, dependent on weight gain and/or the nutritional environment. The next question was to know whether the quality of the diet, independently to the amount of calories ingested, could alter eating behavior, brain functions and health. In a first experiment performed in Pitman-Moore minipigs, we compared the effects of three high-fat isocaloric diets differing on their fat sources (lard, sunflower oil, or fish oil). All diets led to similar body weight and adiposity but had contrasted and surprising effects on the blood-brain barrier permeability and brain metabolism in the prefrontal cortex and nucleus accumbens (Val-Laillet *et al*., 2011b; Val-Laillet *et al*., 2012; Val-Laillet *et al*., 2013). This study demonstrated that the quality of fat sources could modulate brain functions independently from the quantity of food ingested, but raised new questions concerning the nature of the mechanisms involved. In a second experiment that is not yet finished, we compared in Yucatan minipigs three other obesogenic isocaloric diets differing on their carbohydrates sources (starch, glucose, or fructose). Our data indicate that chronic intake of all diets induced obesity and physiological alterations, regardless of carbohydrate type. Minipigs showed an initial preference and higher motivation for the glucose diet, but after 8 weeks, only minipigs chronically fed the fructose diet developed a significant preference for this diet (Ochoa *et al*. 2013, in preparation). Further analyses will show whether related effects can be found for brain activity and dopaminergic availability in the prefrontal and reward brain regions. We are expecting that the fructose-specific habituation process might shape some brain circuits in addition to food preference.

New therapeutic solutions against obesity: how neuromodulation regulates eating behavior

The fact that diet-induced obesity in minipigs provokes eating disorders and brain anomalies similar to what is described in humans justifies the use of this model to validate new therapeutic solutions. Two strategies are currently being explored in our lab: the chronic vagus nerve stimulation (VNS) and the deep-brain stimulation (DBS). VNS consists in inserting electrodes on both vagus nerves near the stomach to modify the signals that are emitted towards the brain, with the aim of mimicking satiety. We tested a novel VNS strategy (International Patent: Malbert *et al*., 2008) in obese Göttingen minipigs and showed that this treatment successfully decreased weight gain, food consumption and sweet craving (Val-Laillet *et al*., 2010a). Interestingly, VNS can also modify the activity of some brain regions in pigs (Biraben *et al*., 2008), which suggests the onset of neuronal plasticity in brain circuits regulating eating behavior. Using the minipig to test electrodes and stimulators dedicated to the treatment of obesity, as well as to develop laparoscopic approaches to implant them, will pave the way to human clinical studies in the near future. The other strategy currently explored in our lab consists in stimulating directly some brain regions involved in the regulation of food motivation and pleasure. This strategy requires the use of image-guided brain surgery for the implantation of deep-brain electrodes. This method has already been tested in pigs (Sauleau *et al*., 2009) and will be used in further experiments to investigate the DBS effects on eating behavior and brain metabolism of obese minipigs.

REFERENCES

Bellinger, D. A., E. P. Merricks, and T. C. Nichols. 2006. Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications. ILAR journal / National Research Council, Institute of Laboratory Animal Resources 47: 243-258.

Biraben, A., S. Guérin, E. Bobillier, D. Val-Laillet, C. H. Malbert. 2008. Central activation after chronic vagus nerve stimulation in pigs: contribution of functional imaging. Bull Acad Vet France 161: 441-448.

Boubaker, J., D. Val-Laillet, S. Guerin, and C. H. Malbert. 2012. Brain processing of duodenal and portal glucose sensing. Journal of neuroendocrinology 24: 1096-1105.

Clark, B. A., M. Alloosh, J. W. Wenzel, M. Sturek, and T. Y. Kostrominova. 2011. Effect of diet-induced obesity and metabolic syndrome on skeletal muscles of Ossabaw miniature swine. Am J Physiol Endocrinol Metab.

Clouard, C., M. Chataignier, M.-C. Meunier-Salaün, and D. Val-Laillet. 2012a. Flavour preference acquired via a beverage-induced conditioning and its transposition to solid food: Sucrose but not maltodextrin or saccharin induced significant flavour preferences in pigs. Applied Animal Behaviour Science 136: 26-36.

Clouard, C., M. Chataignier, M.-C. Meunier-Salaün, and D. Val-Laillet. 2012b. Exposures to conditioned flavours with different hedonic values induce contrasted behavioural and brain responses in pigs. PLoS One 7: e37968.

Clouard, C., M. C. Meunier-Salaun, C. H. Malbert, P. Meurice, and D. Val-Laillet. 2013a. Motivational and brain responses triggered by oral and/or duodenal sucrose sensing in pigs. In revision.

Clouard, C., M. C. Meunier-Salaun, and D. Val-Laillet. 2012c. Food preferences and aversions in human health and nutrition: how can pigs help the biomedical research? Animal 6: 118-136.

Clouard, C., M. C. Meunier-Salaun, and D. Val-Laillet. 2013b. Importance des stimulations orales et viscérales dans la mise en place des choix alimentaires chez le porc et réponses cérébrales sous-jacentes, Colloque Annuel de la Société Française d'Etude du Comportement Animal, Dijon, France

Dyson, M. C., M. Alloosh, J. P. Vuchetich, E. A. Mokelke, and M. Sturek.2006. Components of metabolic syndrome and coronary artery disease in female Ossabaw swine fed excess atherogenic diet. Comp. Med. 56: 35-45.

Gaultier, A., M. C. Meunier-Salaün, C. H. Malbert, and D.Val-Laillet. 2011. Flavours exposures after conditioned aversion or preference trigger different brain processes in anaesthetised pigs. European Journal of Neuroscience 34: 1500-1511.

Johansen, T., H. S. Hansen, B. Richelsen, and R. Malmlöf. 2001. The obese Göttingen minipig as a model of the metabolic syndrome: dietary effects on obesity, insulin sensitivity, and growth hormone profile. Comp Med 51: 150-155.

Jokinen, M. P., T. B. Clarkson, and R. W. Prichard. 1985. Animal models in atherosclerosis research. Experimental and molecular pathology 42: 1-28.

Le, D. S., N. Pannacciulli, K. Chen, A. Del Parigi, A. D. Salbe, E. M. Reiman, and J. Krakoff. 2006. Less activation of the left dorsolateral prefrontal cortex in response to a meal: a feature of obesity. Am. J. Clin. Nutr. 84: 725-731.

Lind, N. M., A. Moustgaard, J. Jelsing, G. Vajta, P. Cumming, and A. K. Hansen. 2007. The use of pigs in neuroscience: modeling brain disorders. Neuroscience and Biobehavioral Reviews 31: 728-751.

Liu, Y., Z. Wang, W. Yin, Q. Li, M., Cai, C. Zhang, J. Xiao, H. Hou, H. Li, and X. Zu. 2007. Severe insulin resistance and moderate glomerulosclerosis in a minipig model induced by high-fat/ high-sucrose/ high-cholesterol diet. Experimental animals / Japanese Association for Laboratory Animal Science 56: 11-20.

Malbert, C. H., A. Biraben, E. Bobillier, and D. Val-Laillet., 2008. Device and method for reducing weight, In: INRA (Ed.), PCT/EP2008/061204.

Neeb, Z. P., J. M. Edwards, M. Alloosh, X. Long, E. A. Mokelke, and M. Sturek. 2010. Metabolic syndrome and coronary artery disease in Ossabaw compared with Yucatan swine. Comp. Med. 60: 300-315.

Sauleau, P., E. Lapouble, D. Val-Laillet, and C. H. Malbert. 2009. The pig model in brain imaging and neurosurgery. Animal 3: 1138-1151.

Spurlock, M.E. and N. K. Gabler. 2008. The development of porcine models of obesity and the metabolic syndrome. J. Nutr. 138: 397-402.

Val-Laillet, D., A. Biraben, G. Randuineau, and C H. Malbert. 2010a. Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipig. Appetite 55: 245-252.

Val-Laillet, D., S. Blat, I. Louveau, and C. H. Malbert. 2010b. A computed tomography scan application to evaluate adiposity in a minipig model of human obesity. Brit. J. Nutr. 104: 1719-1728.

Val-Laillet, D., S. Guérin, and C. H. Malbert. 2010c. Slower eating rate is independent to gastric emptying in obese minipigs. Physiol. Behav. 101: 462-468.

Val-Laillet, D., S. Layec, S. Guérin, P. Meurice, and C. Malbert. 2011a. Changes in brain activity after a diet-induced obesity. Obesity (Silver Spring) 19: 749-756.

Val-Laillet, D., P. Meurice, J. P. Lallès, and C. H. Malbert. 2011b. Central functions altered by chronic high-lipids diets enriched with omega 3, omega 6 or saturated fat, Nutrition Society Winter Meeting, London, United Kingdom.

Val-Laillet, D., P. Meurice, J. P. Lallès, and C. H. Malbert. 2013. Central functions altered by chronic high-lipids enriched with omega 3, omega 6 or saturated fat, Digestive Disease Week, Orlando, Florida, USA

Val-Laillet, D., P. Meurice, J. Mourot, J. P. Lallès, and C. H. Malbert. 2012. Impact of different dietary fat sources on the blood-brain barrier and brain metabolism of obese minipigs, European Congress on Obesity, Lyon, France

Vodicka, P., K. Smetana, Jr., B. Dvorankova, T. Emerick, Y. Z. Xu, J. Ourednik, V. Ourednik, and J. Motlik. 2005. The miniature pig as an animal model in biomedical research. Annals of the New York Academy of Sciences 1049: 161-171.

Volkow, N. D., G. J. Wang, F. Telang, J. S. Fowler, R. Z. Goldstein, N. Alia-Klein, J. Logan, C. Wong, P. K. Thanos, Y. Ma, and K. Pradhan. 2009. Inverse association between BMI and prefrontal metabolic activity in healthy adults. Obesity (Silver Spring) 17: 60-65.

Volkow, N. D., G. J. Wang, F. Telang, J. S. Fowler, P. K. Thanos, J. Logan, D. Alexoff, Y. S. Ding, C. Wong, Y. Ma, and K. Pradhan. 2008. Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. Neuroimage 42: 1537-1543.

Xi, S., W. Yin, Z. Wang, M. Kusunoki, X. Lian, T. Koike, J. Fan, and Q. Zhang. 2004. A minipig model of high-fat/high-sucrose diet-induced diabetes and atherosclerosis. International Journal of Experimental Pathology 85L 223-231.