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

Val-Laïlet, D.

INRA, UR1341 ADNC, F-35590 Saint-Gilles, France
E-mail: david.val-laïlet@rennes.inra.fr

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.

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**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.

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