Maternal Western diet during gestation and lactation modifies adult offspring's cognitive and hedonic brain processes, behavior, and metabolism in Yucatan minipigs

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A maternal Western diet during gestation and lactation modifies adult offspring's cognitive and hedonic brain processes, behavior and metabolism in Yucatan minipigs

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Abbreviations list

AIRi: Incremental Acute Insulin Response
APFC: Anterior Prefrontal Cortex
AUC: Area Under Curve
AUCG: Incremental AUC of Glucose
AUCG0-30: AUCG from 0 to 30 minutes after IV glucose injection
AUCI: Incremental AUC of Insulin
AUCI0-30: AUCI from 0 to 30 minutes after IV glucose injection
BDNFα: Brain Derived Neurotropic Factor
CAU: Caudate nucleus
Chol: Cholesterol
CNS: Central Nervous System
DA: Dopamine
DAT: Dopamine Transporter
DID'IT: Metaprogramme "Diet Impact and Determinants: interaction and Transition"
DOHaD: Developmental Origins of Health and Diseases
DA: Dopamine
EtCO2: End Tildal CO2 (maximal CO2 concentration at the exhalation end, measured by capnometry)
FDG: Fluro-Deoxy-Glucose (^{18}FDG: 18Fluor radioelement binding to glucose)
FDR: False Discovery Rate
FFA: Free Fatty Acid
FOV: Field Of View
FWE: Family Wise Error
FWHM: Full With a Half Maximum (Kernel FWHM)
Gb: Basal (fasting) glucose level
GLP1: Glucagon-like peptide 1
Hapto: Haptoglobin
HFF: High-Fat high-Fructose
HFS: High-Fat high-Sucrose
Ib: basal (fasting) insulin level
I-Ioflupane: Iode-Ioflupane (^{123}I: Iode 123 radioelement)
IVGTT: Intravenous Glucose Tolerance Test

KeV: Kilo-ElectronVolt

KG: Glucose disappearance constant (%/min), glucose tolerance index

LDL: Low Density Lipoprotein

BW: Body Weight

NAc: Nucleus accumbens

P2: Insulin action parameter

PFC: Prefrontal Cortex

PR: Progressive Ratio

PUT: Putamen

PYY: Peptide YY

QUICKY: Quantitative Insulin sensitivity Check Index

RIA: Radioimmuno Assay

RM: Reference Memory

ROIs: Regions of Interest

S2: Calculated index of insulin sensitivity

SBFT: Subcutaneous BackFat Thickness

SCFA: Short-Chain Fatty Acid

SD: Animals born of sows fed with STD chow

SG: Glucose Effectiveness, derived from the Minimal Model

SI: Insulin Sensitivity, derived from the Minimal Model

SPECT: Single Photon Emission Computed Tomography

SPM: Statistical Parametric Mapping

SVC: Small Volume Correction

TG: Triglycerides

Vd: Volume of distribution

WM: Working Memory

Abstract

This study explores the long-term effects of exposure to a maternal Western diet (WD *vs.* standard diet SD), in the Yucatan minipig, on the adult progeny at lean status (N=32), then at overweight status. We investigated eating behavior, cognitive abilities, brain basal glucose metabolism and dopamine transporter availability, microbiota activity, blood lipids and glucose tolerance. Although both groups showed similar cognitive abilities in a holeboard test, WD expressed a higher stress level than SD (immobility, $P<0.05$) and a lower performance in an alley maze ($P=0.06$). WD showed lower dopamine transporter binding potential in the hippocampus and parahippocampal cortex ($P<0.05$ for both), as well as a trend in putamen ($P=0.07$), associated with lower basal brain activity in the prefrontal cortex and nucleus accumbens ($P<0.05$) compared to lean SD. Lean WD displayed a lower glucose tolerance than SD animals (higher glucose peak, $P<0.05$ and tendency to a higher $AUCI_{0\text{-}30}$, $P<0.1$). Both groups developed glucose intolerance with overweight, but WD animals were less impacted than SD animals. These results demonstrate that maternal diet shaped offspring's brain functions and cognitive responses on the long term, even after being fed a balanced diet from weaning, but behavioral effects were only revealed in WD under anxiogenic situation. The WD animals seemed however to better cope with the obesogenic diet from a metabolic standpoint.

Key words: perinatal programming, nutrition, neuro-ethology, physiology, pig

1. Introduction

The quality of early nutrition has long-term impacts on the offspring's phenotype, a phenomenon first described by Barker et al. (1989) as "fetal programming" (1,2). The nutritional environment during critical developmental stages can thus modulate the later individual susceptibility to diseases, with particular sensitivity of the nervous system in both human and animal models (3). The maternal diet can therefore have long-term effects on the offspring's development (4), including metabolism (5), brain functions and behavior of children. A maternal high-fat diet can result in altered metabolic and eating behavior regulation due to higher proliferation of orexigenic neurons in rats (6). The same behavioral and metabolic aspects are observed in children born from either obese or diabetic pregnant women, revealing a developmental programming of obesity (7). As a matter of fact, a maternal high-fat diet and / or post-natal over-nutrition can induce impairment of homeostatic control *via* hypothalamic functions in rodent (6,8–10) and non-human primates (11).

Some studies reported a deleterious impact of a maternal high-fat diet on the brain, with direct consequences on behavior and the onset of neurodevelopmental cognitive, affective and eating disorders (12). However, recent studies showed contradictory results: on one hand, a Western diet offered during late gestation and lactation to non-obese sows seemed to improve the memory functions in the progeny after weaning (13,14), although an increased motivation for the sweet and fatty food rewards during the cognitive tests could not be completely ruled out. Histological analysis, however, revealed a smaller hippocampal granular cell layer and decreased neurogenesis in WD piglets. On the other hand, numerous studies showed that maternal high-fat diet-induced obesity can impair cognition in rodent (15–18) and that obese and post-obese people (people who have experience a weight loss after obesity) have an altered posterior hippocampal function, linked to altered prefrontal activity (19).

Long-term consequences of an early hypercaloric diet on dopamine transporter (DAT) availability and dopamine (DA)-mediated behaviors (food choice and motivation) have also been described. South and Huang (20) demonstrated that a high-fat diet exposure decreased DAT density in the caudate-putamen. Interestingly, another study exploring a maternal high-fat diet effect on DA response to amphetamine (AMP) in the offspring showed altered regulation of DA transport in NAc (21,22). It was also shown that obese people presented lower density of DA receptor in striatum (23), associated with lower brain activity in prefrontal regions known to be involved in the inhibitory control of food intake, in humans and animal models (24,25).

As described before, most studies focused on maternal obesity and/or long-term exposure to Western diet on the offspring. The originality of the current study lies in the fact that neither sows nor progeny were obese (until the obesogenic challenge at the adult age), and the offspring was exposed to a balanced standard diet from weaning to the testing period at the adult age. Moreover, several studies assessed the effect of a high-fat diet, but not a high-fat and high-fructose/sucrose diet, that better represents human life conditions. Fructose is well known to present deleterious effect on health with an addictive potential (26–28). The pig model was chosen because of its potential to fill the gap between preclinical studies with rodents and clinical trials in humans. As a matter of fact, pigs are monogastric omnivorous, and their digestive characteristics are closer to those of humans than those of rodent and non-human primate models (29), including at early stages of life. In addition, they possess, like humans, a large gyrencephalic brain (*vs*. lissencephalic brain in rodent), which facilitates investigation *via* cerebral imaging. Furthermore, pigs show brain development closer to humans than rats or monkeys: brain develops perinatally in pigs and humans, while it develops post-natally in rats and prenatally in guinea-pigs and monkeys (30,31). Many other reasons for the choice of the pig as a model exist and are fully described in literature

(31,34,35). We hypothesized that a maternal high-fat and high-fructose diet during gestation and lactation would alter cognitive abilities in relation to functional abnormalities in brain areas involved in learning, reward and motivation, such as the hippocampus (33–35), prefrontal cortex (24,36), and striatum (37,38). In consequence, we also hypothesized that WD animals would have a higher motivation for sweet, accompanied by metabolic disorders and modification of the gut microbiota activity, assessing here by quantification of SCFA (39–41).

2. Materials and Methods

Experiments were conducted at the INRA St Gilles, France (January to April 2015), in accordance with the current ethical standards of the European Community (Directive 2010/63/EU), Agreement No. C35-275-32 and Authorization No. 35-88. The Regional Ethics Committee in Animal Experiment of Brittany has validated and approved the entire procedure described in this paper (project N°01299.01).

The present study is part of a longitudinal experiment where the progeny of sows fed a standard (SD) or Western (high-fat high-fructose, WD) diet during gestation and lactation were tested at 3 time periods during their life: 1) during the post-weaning stage, from postnatal day (PND) 25 to 3-month-old piglets (14), 2) at the adult age on non-obese animals, 3) and after a 6-week obesogenic challenge with a high-fat and high-sucrose diet (Figure 1A).

Breeding conditions

Diets. Pregnant sows (N=17) were fed during the last eleven weeks of gestation and the whole lactation (28 days) either a standard (N=8) or a Western diet (high-fat high-fructose HFF N=9) (Val-Laillet et al., 2017). A standard balanced diet ration was provided to their offspring from weaning to adulthood (10-14 months) (Tables 1a & b). The isocaloric ration

had to provide 0.3017 MJ/kg of metabolic weight (MW, calculated upon live weight: MW = $LW^{0.75}$). Then, animals of 1.5 year-old were then fed *ad libitum* an obesogenic high-fat and high-sucrose HFS diet for 5 weeks (Table 1, N=15, including 9 WD and 6 SD). The HFF, provided to pregnant sows, was replaced by HFSucrose feed for eating behavior tests performed in piglets to avoid the natural digestive discomfort cause by fructose in piglets (42,43), for the first step of this experiment available in Val-Laillet et al., 2017. For simplicity, we conserved this HFS feed for behavioral testing in adults and then for the obesogenic challenge. Daily feed intake was recorded during the obesogenic challenge.

Animals and housing. Thirty-two 10-month-old young adult Yucatan minipigs were used (16 born to SD sows and 16 born to WD sows) with a 50/50 sex ratio per group. They were housed in same-sex groups of 4 to 7 minipigs, in pens measuring 3 m x 2.4 m with *ad libitum* access to water and environmental enrichments (balls and chains). The animal room was provided with an 8/16 hours light/dark cycle (lights on from 8:00 am to 4:00 pm) as well as natural lighting from one window and filtered air at a temperature of 20°C ± 1°C. The study was carried out in three successive batches. One week before the start of obesogenic challenge, animals were placed in individual pens for the intravenous glucose tolerance test (IVGTT). The animals' weight and food intake was recorded weekly.

Spatial holeboard discrimination task

All the lean animals were subjected to this spatial discrimination test, involving palatable food rewards, and composed of three phases: a habituation phase of 5 days, which was followed by an acquisition phase of 10 days, and finally a reversal phase of 5 days (week-ends in-between). The holeboard task was not performed on overweight minipigs.

Test area and apparatus. The 5 x 5 m arena, confined by dark walls (height: 1 m), had four entrances with doors to prevent the pigs from developing a non-spatial, fixed pattern of visits that would reduce their working memory load (44,45). Sixteen plastic feeding bowls

211 (diameter 23cm) were placed in a grid pattern (4 x 4 bowls) with an equal amount of space
212 between adjacent bowls and walls. Each bowl was covered by a ball that could slide up and
213 down within a four-bar metallic and PVC mount. Minipigs had to lift the ball up with their
214 snout to gain access to the bowl falling again with gravity after the animal's withdrawal. To
215 prevent olfactory guidance, all the bowls contained a perforated false bottom under which
216 sweet chocolate peanuts (M&M's®) were placed before testing. Over four consecutive trials
217 for an individual pig, each entrance was used once. Order of testing between and within
218 groups of pen mates was alternated across days. Four of the sixteen bowls were baited with a
219 food reward (one sweet chocolate peanut) accessible to the minipig. The minipigs were
220 randomly assigned to one of eight configurations of four baited bowls, the patterns of reward
221 being evenly distributed between groups (Figure 1B). This configuration was changed during
222 the reversal phase. Minipigs were individually guided into the arena through one of the four
223 doors. The operator was located at a fixed position by the side of the arena invisible to the
224 minipigs. Similarly, an observer who recorded the performance of the minipigs stood by the
225 side of the arena. Visual cues (triangle, rectangle, circle, and vertical lines) were placed on the
226 doors inside the arena. Before the start of a trial, all minipigs of a pen were led into a waiting
227 room next to the testing arena, and the minipig to be tested was given access to the
228 appropriate entrance. The trial was terminated when the pig found all rewards or when 600s
229 had passed. After a trial, the pig was led back into the waiting area where its pen mates were
230 held (Figure 1B).

231 *Training and testing.* The day before the first day of habituation, the minipigs were pre-
232 exposed to the food reward *i.e.* crushed M&M's® in their daily food. Habituation phase had
233 three stages. First, M&M's® were scattered both around the floor of the arena and one in each
234 of the bowls with balls maintained in a high position to set the bowls freely accessible and
235 visible. Second, rewards were restricted to one M&M's® in each bowl with balls in a high

position. Third, the balls were lowered to make pigs learn how to lift the balls up. A visit to a bowl was recorded whenever the minipig lifted a ball with its snout. By the end of a trial, the door of the arena was opened. Between trials, the arena was cleaned and rewards were replaced and/or moved. The minipigs were tested in a random order and went through two daily trials, in the morning and in the afternoon, with an inter-trial interval of 2-4 hours. The entrances were alternated between trials and days. During testing period, animals received half of their daily food ration in the morning before testing, and the other half in the afternoon after testing.

Data recording. Trial duration, number of visits and revisits to baited bowls and non-baited bowls, total number of bowls visits, time between each visit of bowl (motivation cue) and number of rewards collected were scored. The reference memory (RM) score was calculated as the ratio between number of visits to the baited bowls and the total number of visits to all bowls, and the working memory WM score was calculated as the ratio between the number of baited visits and all visits to the baited bowls (44,46).

$$WM = \frac{\text{number of baited visits}}{\text{all visits to the baited bowls}}$$

$$RM = \frac{\text{number of visits to the baited bowls}}{\text{total number of visits to all bowls}}$$

Blocks of four trials were averaged when calculating WM scores and RM scores. Exploratory behavior (exploration of the area and the wall, number of visit of each virtual area - 1 to 16 illustrated in Figure 1B, configuration A) and emotional indicators (immobility, escape, vocalization) were recorded.

Alley maze test

Ten male Yucatan Minipigs (lean animals only, 5 WD and 5 SD), previously tested in the holeboard test, were subjected to a second spatial discrimination test with no food reward incentive to investigate spatial cognition independently from food motivation.

Testing area and paradigm. The test was conducted in the same 5x5m arena as for the holeboard discrimination task, but the setting was modified (Figure 1C). The alley maze was characterized by a fixed starting position (virtual area 1) and one correct route to a fixed goal position (virtual area 15), where incorrect alternatives such as visits to blind alleys (areas 16 to 25) or going back must be avoided (47). The test consisted in an acquisition phase of four consecutive days, followed by a reversal phase (changing of maze configuration) of equal duration. Subjects were tested once a day, in the afternoon. The morning of each day of testing, animals were subjected to water restriction. Individuals had to cross the maze, in five minutes maximum, to reach an environmental and social reward: access to water and straw-enriched pen, which contained familiar pen mates and enrichments (area with straw, plastic balls and metal chain), and where the animals could stay at least ten minutes. Note that access to the water at the exit of the test is simply a bucket filled with water: this device is a novelty for animals, used to drink from water dispenser, and therefore required learning.

The testing area ambience in the maze was degraded with the diffusion of aggressive sounds (72-82 Db, *e.g.* plane motor, chainsaw, jackhammer, *etc*. Nutolina Sound Effects – YouTube), wet floor and vinegar odor (1/3 diluted white vinegar) to induce anxiogenic conditions. If animals did not succeed in exiting the maze, they were guided out through the starting gate, to a dark "failure room" where individuals remained alone in individual pens, without water or any enrichment, during ten minutes at the maximum.

Data recording. Trial duration, number of visits and revisits of correct areas and wrong areas, total number of areas visits, as well as exploratory behaviors and emotional indicators were recorded, in terms of occurrences and duration. Five indicators were calculated:

$$Index = \frac{\text{Number of correct areas visited once} - \text{number of wrong areas visited once}}{\text{Number of all visits of all areas}}$$

$$\%\ Exploration = \frac{\text{Number of area visited once}}{25}$$

$$\%\ Success = \frac{\text{Number of animals that succeeded in reaching the goal area (exit)}}{\text{Total number of animal tested}}\ X\ 100$$

$$Working\ memory = \frac{\text{Number of areas visited once}}{\text{Number of total visits}}\ X\ 100$$

$$Reference\ memory = \frac{\text{Number of correct areas visited once}}{\text{Number of total visits}}$$

Two-choice food test

These tests were carried out on 28 animals in individual cages with *ab libitum* water, a chain as environmental enrichment, and two automatic troughs with computer-controlled access (lean animals only, 14 WD and 14 SD, sex ratio=50/50).

Training and testing. After four days of habituation to the cage, the minipigs were subjected to a two-choice food test to assess their preferences between standard and high-fat high-fructose feeds. The two different feeds were presented in two different troughs containing 1 kg of feed each. They were presented at 09:00 a.m. to the animals during 60 minutes. Refusals were then weighed. Feed distribution in the troughs was interchanged over days and animals to avoid any laterality bias. The same two-choice food test was repeated for four days.

Operant conditioning with progressive ratio

Training and testing. The same twenty-eight lean minipigs having done the two-choice tests were subjected to operant conditioning with progressive ratio (PR) feeding tests in an individual cage. The rewarded feed was either standard or high-fat high-fructose. Minipigs were initially trained to press a button on a fixed ratio (FR) reinforcement whereby a single button press elicited the delivery of 10 g of standard feed in the trough. Each FR training session lasted 120 minutes, and was repeated once every day during four days. Then, all

minipigs were tested once every day during four days on the PR task. Each daily test session lasted 200 min and began by opening the trough door and releasing the press button. The individuals were tested using a PR 1 + 2 schedule every two rewards. Initially, one button press of any duration resulted in a reward delivery. The second reward necessitated one button press also but the two following 3, and the 2 next ones 5, and so on. Total number of rewards, total number of button presses, breakpoint (number of button presses to get the last reward) and Km (time where half of total obtained rewards was reached) were recorded.

Brain glucose metabolism imaging (TEP)

Brain glucose metabolism was investigated with Positron Emission Tomography (PET) after the IV administration of 18-fluoro-deoxy-glucose (18FDG), on 20 animals (lean animals only, 10 SD and 10 WD, sex ratio 50/50).

*Animal anesthesia and radiolabel administration***.** Pre-anesthesia was performed with an intramuscular injection of ketamine (5mg/kg – Imalgene 1000, Merial, Lyon, France) in overnight-fasted animals. Isoflurane inhalation (Aerane 100 ml, Baxter SAS, France) was used to suppress the pharyngotracheal reflex and then establish a surgical level of anesthesia, 3-5% v/v and 2-3% v/v respectively. Respiratory frequency was adjusted at 15 breathing/minute, tidal volume between 420-470ml to maintain $EtCO_2$ maintained between 3.8 and 4.5%. A venous catheter was inserted into the left ear of the animal in order to inject the radiolabeled molecule compound. Surgical tape and cotton wool were used to conceal the animal's eyes and ears, respectively, and the animal was covered with a warming blanket (38°C), to limit sensory stimulation and thermoregulatory reaction. The radiolabel was injected at 250 MBq activity, when animal's anesthesia concentration was stabilized at 2.0. Minimum Alveolar Concentration (MAC).

Image Acquisition. PET imaging was performed with a Siemens HR+ (Siemens Ecat, 962, HR+) in 3D mode. Subjects rested in prone position (ventral decubitus). The PET procedure included a Scout scan 30 minutes after injection to control animal position, then, image acquisition was performed 45 minutes after radiolabel injection for 30 minutes, using a FOV (field of view) of 15.52 cm. A transmission scan using three ^{68}Ge rod rotating sources was performed to correct for tissue attenuation. Images were reconstructed with a filtered backprojection using a Ramp filter (Kernel FWHM, 6mm) to obtain a 3D image with a 0.64-mm spatial resolution per voxel, on x and y axis, and 2.42 mm on z direction. This image was corrected for attenuation, scatter and uniformity.

Image processing. SPM12 software (Welcome Trust Center of Neuroimaging, London, UK) implemented in MATLAB 7.1 (The Mathworks Inc., Natick MA, USA) was used for spatial pre-processing and statistical analysis. First, images were manually segmented to remove extracerebral matter on OsiriX 6.1 software (48). SPM12 software was adapted to the characteristics of the pig's brain. Template images were based on 16 female pigs used as reference images, called *template* (24,49). Second, images were manually reoriented to center reference coordinates on a reference point set at the *posterior commissura* (CP) according to the stereotaxic reference defined by Saikali et al. (50). A first masking step was performed by thresholding the proportional normalized images from this study. Spatial normalization was restricted to linear 12-parameters affine transformation, and then, images were smoothed using a 5-mm Gaussian kernel.

DAT Imaging

Dopamine transporter imaging was carried out to assess brain dopaminergic function with single-photon emission computed tomography (SPECT) (lean animals only, N=16: 8 WS

and 8 SD, sex ratio: 50/50). ^{123}I-FP-CIT SPECT allowed for the determination of the pre-synaptic dopamine transporter (DAT) density in central neurons.

Animal anesthesia and radiolabel administration. Animals were anesthetized similarly to the procedure used for PET imaging. A venous catheter was inserted into the left ear of the animal in order to inject the radiolabel compound. The radioactive molecule was injected at a 185 MBq *in toto* irrespective of the body weight.

Image Acquisition. DAT imaging was performed with a double head gamma camera (VG Millenium, GE) fitted with low energy-high resolution collimators. Imaging procedure included two consecutive 3D image acquisitions of 45 min each (3.5° Step and Shoot acquisition with 45 sec duration for each projection). The first image data set was used to register the acquisition with the pig 3D brain atlas (50). The last image data set was the Dat imaging *per se* (4h30 after injection). Images data set were reconstructed from the sinogram with a filtered backprojection with Hann filter at 0.5 Hz (eNTEGRA , GE). Images were also corrected for attenuation with a Chang correction of order 0 (Entegra, GE).

During brain imaging investigation, animals were housed at the AniScan imaging platform (US 1395, AniScan, INRA, Saint-Gilles, FRANCE) from the morning of the first acquisition, and stayed for 9 days in individual pens on the platform (animals did not return in their daily collective pens between both PET and DAT scan, but stayed on the platform). The imaging was performed exactly at the same time for all animals to avoid any bias due to the nycthemeral cycle of DAT density. After imaging, animals did not go back to collective pens but were housed in individual pens, with *ad libitum* water access, to perform biological sampling as describe above.

Ancillary measurements and biological sampling

373 *Food intake, weight and adiposity*. The animals were weighed weekly without having
374 eaten. The HFS diet intake was measured weekly. The adiposity was measured once after all
375 behavioral testing and brain imaging, *via* the assessment of subcutaneous backfat thickness
376 (SBFT) by ultrasonography (51). To assess the overweight/obese status after the obesogenic
377 challenge, we defined a threshold according to human classification of obesity based on BMI,
378 meaning that obesity was defined by a weight gain over 49% of normal body weight.
379 Overweight was defined as a weight gain comprised between 26% and 49%.
380 *Lipid profile and glucose tolerance test (IVGTT).* Blood sampling was assessed with a
381 long catheter inserted (Vygon, ref 1219.13 leadercath) into the jugular vein, under light
382 anesthesia (intramuscular injection of ketamine (5mg/kg)) the day before the IVGTT. For
383 IVGTT (at lean status: 8 females WD and 8 females SD; at overweight status: 6 WD -3
384 females and 3 males - and 4 SD - 1 female and 3 males), overnight fasted animals received
385 0.3 g glucose per kilogram of body weight by intravenous injection, before proceeding to
386 serial blood sampling, from 30 minutes before injection to 75 minutes after injection (52).
387 The end of the injection time corresponded to T0. Lipid profile (free fatty acids (FFA),
388 triglycerides (TG), cholesterol, Chol), inflammation status (haptoglobin, Hapto), QUICKY
389 index (1/[log(basal insulin)+log(basal glucose)]) Chol/TG ratio (as indicator of Low Density
390 Lipoprotein, LDL, level), FFA/Chol ratio (hepatic dysfunction) were evaluated at the basal
391 state before glucose injection. After glucose injection, incremental total (over 75 min) and 0-
392 30 min (over the first 30 min after IV glucose injection) Area Under Curve (AUC) for
393 Glucose (AUCG and $AUCG_{0\text{-}30}$ respectively), and Insulin (AUCI and $AUCI_{0\text{-}30}$ respectively),
394 glucose peak, incremental Acute Insulin Response (AIR), the constant of glucose
395 disappearance (KG), and insulin sensitivity (S2, S2= ((30 x KG)/ AUC0-30min) × Vd. It is
396 expressed in mL/min/(IU/mL)/kg) (53) were calculated and the glucose effectiveness (SG)
397 and the insulin sensitivity (SI) indexes were derived from the minimal model (54).

Insulin concentration was measured by RIA (INSULIN-CT, CisbioBioassays, France). The intra- and inter-essay CV were 15 and 11% respectively at 35µUI/mL. Plasma glucose, triglycerides, FFA, total cholesterol and haptoglobin were measured using automated spectrophotometric method. The intra-essay CV was <5%.

Microbiota activity. Feces were sampled to assess microbiota fermentation activity *via* the quantitative analysis of short-chain fatty acids (SCFA). The collection was made directly on the animals, and stabilized with 0.5% ortho-phosphoric acid at a rate of 1 mL of acid per gram of feces. After 4000 rpm centrifugation for 15 min at 4°C, 1 mL supernatant by sample was stored at -20°C until SCFA assay by gas chromatography (lean status: N=14, 8 WD and 6 SD; overweight status: N=15, 9 WD and 6 SD). Collection was made once before obesogenic challenge, and twice during obesogenic diet, after 2 and 4 weeks.

Statistical analysis.

Data were analyzed with the R 3.1 software (University of Aalborg, Denmark).

Holeboard discrimination task and alley-maze. Differences between SD and WD groups in working memory, reference memory, each visit of bowl and trial duration, within and between phases were assessed with a non-parametric Mann-Whitney test because of general non Gaussian distribution of data. Batch effect was controlled and sex effects were tested by sex-separate non-parametric analysis. The labyrinth results were analyzed by descriptive statistics, using PCA, to assess maternal diet effect. Friedman test was used to test trials effect within maternal diet. Maternal diet effect was analyzed using Mann-Whitney test.

Eating behavior. The food consumption data are not Gaussian and were analyzed using a non-parametric Friedman test to assess the time effect (successive trials) and Mann-Whitney test to assess the group effect.

PET imaging. Statistical analysis was performed with SPM 12. Statistical analysis was based on a General Linear Model (GLM) using a t-test (« Two-Sample t-test »). Both groups, WD and SD PET brain images, were compared together using a two-sample t-test, applying *Whole Brain Method* with FWE (*Family Wise Error*) then FDR (*False Discovery Rate*) corrections. This approach was followed by an *a priori* analysis, by SVC (Small Volume Correction) on ROIs: hippocampus, striatum (including nucleus accumbens – Nac, caudate nucleus – CAU, and putamen – PUT) and prefrontal cortex (PFC). A minimum cluster size was fixed at 20 voxels (1 mm), with a significance threshold set at $P<0.005$.

Identification of brain regions. The statistical analysis with SPM produced a listing of clusters of which the activation differed between treatments or according to covariates. Each voxel was associated with a set of coordinates (x, y, z) corresponding to its peak location in the *commissura anterior-commissura posterior* line with the origin set at *commissura posterior*. The brain regions of differential activity were identified with a three-dimensional digital pig brain atlas developed in our laboratory (50). Sex-separate t-test analysis was performed to assess maternal diet effect in males in one hand, and in females in another hand.

DAT imaging. The amounts of radioactivity in the volumes of interest (VOI) were compared between the SD and WD groups using type I ANOVA after normality checking with the R 3.1 software (University of Aalborg, Denmark). The calculation registration matrix and its further use on the late DAT image was done using Pmod 3.506 software (PMOD Technologies, Zurich) adapted to our pig brain atlas. DAT binding potential was calculated in ROIS related to the amount of radioactivity in a low density DAT region (55), *i.e*. the occipital cortex (56):

$$BP_{ND}=(BP_{ROI}-BP_{Occipital})/BP_{Occipital}$$

First, exclusive DAT region without SERT contribution to non-specific radioactivity uptake were investigated, *i.e*. the striatum. Second, non-specific ROIs were investigated, *i.e.* DAT

448 and SERT regions: hippocampus, parahippocampal cortex (PHC) and orbitofrontal cortex
449 (OFC).

450 *Lipid profile and glucose tolerance test (IVGTT)*. Analysis was performed using type III
451 two-was Anova to test the effects of maternal dietary treatment (WD *vs.* SD), status effect
452 (Lean *vs*. Overweight) and interaction. Normality of data and associated residuals were
453 checked, and non-Gaussian data were transformed by the logarithmic approach.

454 *Feces sampling*. SCFA concentrations were analyzed using type III ANOVA to test the
455 perinatal diet × time interaction. Pair comparisons of the perinatal diet effect were performed
456 with type I ANOVA.

457 *Other measures*. Weight gain, dorsal fat and ingestion were analyzed using ANOVA for
458 repeated measures, followed by post-hoc Tukey tests.

459 Data and figures are presented as mean ± SEM, with *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
460 and $^{\#}$: $0.5 \leq P < 0.1$.

461

462 **3. Results**

463 *Holeboard discrimination task*. No difference was found in cognitive performance
464 between SD and WD animals for both working and reference memories during the holeboard
465 discrimination task (W=131 & W=142 respectively, $P>0.05$, Figure 2A). Overall, there was
466 no effect of the perinatal nutritional environment neither for the other parameters recorded
467 during the holeboard test. Interestingly, a separate sex analysis revealed that WD females
468 tended to visit more bowls (31.8 ± 2.0) during the habituation phase compared to SD females
469 (27.4 ± 2.1, W=50, P=0.065). During the acquisition phase, WD males tended to visit fewer
470 baited bowls than SD males (number of baited bowl visited once/number of baited bowl
471 available = 3.97 ± 0.02 *vs*. 4.0 ± 0.04 respectively, W=20, P=0.076).

Alley-maze test. During the acquisition phase, SD pigs improved their working memory (Friedman chi-squared=9.963, df=3, *P*=0.019), reduced test duration (Friedman chi-squared=8.4783, df=3, *P*=0.037) compared to WD pigs that did not. In acquisition phase, SD pigs showed a trend for higher exploration behavior (number of total visited areas: W=21.5, *P*=0.094), and this tendency was confirmed during the reversal phase (number of areas visited once: W=21.5, *P*=0.095). During reversal, a trend for immobility was found in WD group compare to SD (immobility duration/ test duration: W=5, *P*=0.072). Multivariate PCA analysis revealed a higher variability in WD group compared to SD, in both phases of test, where WD showed higher test duration, immobility and exploration of the wall, whereas SD group had a trend for better WM, RM, index and higher exploration rate (exploration duration/test duration) (Figure 2B, 2C).

Eating behavior. Both WD and SD animals preferred the standard feed compared to the HFS feed (W=18, *P*<0.001) during the two-choice tests (Figures 2A, 2B). SD animals significantly increased their HFS feed consumption whereas WD animals did not (Friedman chi-sq=11.9, df=3, P<0.01, Figure 2B). During the operant conditioning test with progressive ratio, no difference arose between the two groups for HFS feed. However, during the second test with standard feed, WD animals tended to eat more than SD animals in terms of: energy ingested (W=112, *P*=0.067) (Figure 2C), numbers of button presses (W=112, *P*=0.068) (Figure 2D), break point (85.6 ± 4.1 *vs.*74.4 ± 3.3, W=110, *P*=0.086), and number of obtained rewards (86.2 ± 4.0 *vs.* 74.8 ± 3.4, W=111, *P*=0.077). No difference arose between sexes.

Brain glucose metabolism. The first-level analysis without *a priori* revealed a significantly lower basal brain activity in WD compared to SD group in the NAc and APFC of the left hemisphere (Table 2, Figure 4A). The second-level analysis with *a priori* on regions of interest highlighted the same deactivations in WD compared to SD group in the NAc and the APFC, and showed a deactivation in the right putamen (Table 3). Yet, no

497 difference appeared between the two groups in memory-related structures such as the
498 hippocampus or cingulate cortex, neither in other ROIs. Interestingly, separate sex analysis
499 showed that within the WD group, females contributed more than males to the deactivation in
500 the NAc, whereas males contributed more than females to the prefrontal cortex deactivation
501 (Table 4).

502 *DAT brain Imaging.* DAT-scan imaging revealed a trend for higher binding potential of
503 DA in the SD group compared to the WD group in the putamen ($F_{1,13}$=3.977, P=0.07), but no
504 significant difference in the caudate ($F_{1,13}$=2.593, P=0.1) (Figure 4B). SD showed a higher
505 binding potential in hippocampus ($F_{1,13}$=4.989, P=0.0437) and parahippocampal cortex
506 ($F_{1,13}$=7.468, P=0.0171).

507 *Obesogenic challenge: Food intake, weight and adiposity.* Food intake (kg/day) was
508 significantly higher in SD compared to WD pigs ($F_{4,65}$=5.433, P=0.037, Figure 5A),
509 particularly after 5 weeks of obesogenic diet. Relative food intake over the 5 weeks of the
510 obesogenic diet decreased in both groups ($F_{1,65}$=4.56, P<0.01), with a significant difference
511 between groups at W5 ($F_{1,13}$=8.318, P=0.0125, Figure 5B). Obesogenic challenge induced
512 significant weight gain in both SD and WD animals ($F_{1,28}$=240.027, P<0.001) and there was a
513 significant maternal diet × time interaction ($F_{1,28}$=8.836, $P < 0.001$, Figure 5C) highlighting
514 that SD animals tended to gain more weight that WD animals (P= 0.09 at week 5 of
515 obesogenic diet). Subcutaneous backfat thickness (mm) increased in both SD and WD groups
516 after 5 weeks of obesogenic diet ($F_{1,26}$=47.158, $P < 0.001$), without any difference between
517 dietary groups (Figure 5D).

518 *Lipid profile and glucose tolerance.* When lean, WD animals displayed a significant
519 higher glucose peak than SD during the IVGTT ($F_{1,14}$=10.39, P=0.0061), Figure6A), but no
520 difference arose between both groups according to SI ($F_{1,14}$=0.701, P=0.42, Table 5). Five
521 weeks of an obesogenic (high-fat high-sucrose) diet significantly impaired glucose tolerance

522 in both overweight SD and WD groups, significantly increasing glucose $AUCG_{0-30}$
523 ($F_{1,24}$=5.2873, *P*=0.03135) (Figure 6A), and significantly decreasing KG ($F_{1,24}$=6.0125,
524 *P*=0.023) compared to lean animals. This was concomitant with a significant increase in AIR
525 ($F_{1,24}$=8.5432, *P*=0.00788), AUCI ($F_{1,24}$=7.3942, *P*=0.01253) and AUCI0-30 ($F_{1,24}$=9.844,
526 *P*=0.004786, Figure 6B), highlighting a significant decrease in glucose effectiveness (SG,
527 $F_{1,24}$=7.3097, *P*=0.01506,) and in insulin sensitivity (S2: $F_{1,24}$=12.5817, *P*=0.0014) in
528 overweight SD and WD pigs compared to lean SD and WD pigs. Interestingly, $AUCG_{0-30}$ and
529 glucose peak increased more in overweight SD than in overweight WD as illustrated by a
530 significant maternal diet × status interaction ($F_{1,222}$=4.5938, *P*=0.043 and $F_{1,22}$=4.3280,
531 *P*=0.049 respectively) (Figure 6A). At the overweight status, WD pigs displayed a higher KG
532 ($F_{1,8}$=2.3591, *P*=0.046) demonstrating that overweight WD animals were less glucose
533 intolerant than overweight SD animals.
534 About lipid profile, lean WD showed higher Chol/TG ratio than lean SD animals ($F_{1,14}$=5.93,
535 *P*=0.029, Figure 6C). The obesogenic challenge also significantly degraded lipid profiles,
536 significantly increasing basal FFA levels in both overweight SD and WD animals
537 ($F_{1,24}$=6.2938, *P*=0.02) compared to lean animals, as well as a trend to increased
538 FFA/cholesterol ratio ($F_{1,24}$=3.4334, *P*=0.077, Figure 6D). Regarding inflammation, WD
539 overweight animals had a higher haptoglobin concentration than SD overweight animals
540 ($F_{1,8}$=5.96, *P*=0.041). All physiological data are summarized in Table 5.
541 *Microbiota activity:* Fecal SCFA concentrations were not globally different in lean WD and
542 SD pigs (Figure 6E). Total SCFA concentration increased transitorily during the second week
543 of obesogenic diet in SD compared to WD pigs ($F_{1,13}$=8.723, *P* = 0.01, Figure 4B). This
544 increase was due to a significant increase in acetate (19.8±3.9 *vs.* 43.4±9.4 nmol/kg in WD
545 and SD respectively, $F_{1,13}$=7.512, *P* = 0.018), propionate (6.3±1.4 *vs.* 30.6±11.04 nmol/kg in

WD and SD respectively, $F_{1,13}$=8.798, P = 0.012) and isobutyrate (0.9±0.2 *vs.* 2.4±0.7 nmol/kg in WD and SD respectively, $F_{1,13}$=7.471, P = 0.018).

4. Discussion

At the adult age, there was no difference between WD and SD animals in terms of memory scores during the holeboard cognitive task, and it is noticeable that the animals obtained very poor scores of reference memory. This poor performance could be indicative of an inappropriate device and/or task. In the young age the animals always entered by the same door into the holeboard arena, whereas at the adult age four doors were used and systematically alternated: this change of process might have been more difficult to integrate for animals. Other studies demonstrated a significant improvement in working and reference memories on the same type of test, along sessions, but also involved only a single entry (44) , or more trials, between 30 to 60 according to studies (46,57,58) while we used 20 acquisition trials only.

The maze test was designed to assess cognitive functions without any motivation bias related to the use of food rewards. In this context, SD animals were more efficient than WD in terms of working memory and exploration during the acquisition phase. This test also has its limitations, mainly related to the anxiogenic testing environment. Indeed, the failures observed were mainly related to an exploration deficit of some WD animals, which travelled back and forth in the first section of the labyrinth near the gateway. WD animals thus either showed lower cognitive abilities or a higher susceptibility to stress, associated with higher variability in response to stress in WD group. No clear link between sugar consumption and behavior has been demonstrated yet (59,60), but there is evidence of fat exposure impact on mood and anxiety-like behaviors (61) in humans and non-human primates. Solutions to make the environment less anxiogenic while maintaining its aversive potential might be considered

571 to increase the motivation of animals to escape from the maze, such as deteriorating olfactory
572 and/or thermal conditions. Since the maze test involved only five males in each group, it will
573 be necessary to repeat the experiment on a larger cohort including both males and females to
574 confirm these results.

575 It is possible that differences in the level of stress between the maze test and the
576 holeboard task triggered different behavioral and cognitive outcomes. Accordingly, Joëls *et*
577 *al.* (62) suggested that the nature of stress and its context of perception could modulate
578 memory abilities of animals. A moderate stressor might help optimizing memory, whereas
579 intense stress might reduce retention capacities. The impact of stress on cognitive
580 performance might explain why differences between WD and SD animals in terms of
581 cognitive responses were observed in the maze task and not in the holeboard task, which was
582 much less stressful and even positively associated with the food rewards. Alternatively,
583 differences of performance between the two tasks might be explained by differences in the
584 nature of the rewards, the holeboard task being an appetitively-motivated task, whereas the
585 maze test is not.

586 The behavioral differences could be linked to altered brain glucose metabolism, that
587 was lower in WD compared to SD animals in the APFC and NAc, with a similar tendency in
588 the putamen, but no difference in the hippocampus. Furthermore, a decreased neurogenesis
589 was observed in the hippocampus in the young WD animals compared to the SD animals
590 (14). Immunohistochemical studies are necessary to confirm whether this decreased
591 neurogenesis persisted at the adult age in WD animals. Nevertheless, the absence of
592 difference in hippocampal metabolism in adult animals suggests that the observed behavioral
593 results, especially in the maze test, might not be explained by a direct alteration of
594 hippocampus-related cognitive abilities in the WD piglets, but more probably by the alteration
595 of ancillary functions indirectly shaping the learning and memory processes, such as goal-

596 oriented motivation, hedonism (according to the holeboard test), and emotional state
597 (according to the maze test). The striatum (63), is involved in the regulation of motivated
598 behaviors (64,65), and especially in the processing of information relative to the emotional
599 dimension associated with reward signals (66). The NAc is strongly connected with the
600 amygdala, the prefrontal cortex and hippocampus, and receives inhibitory inputs from the
601 APFC (67). Consequently, deactivation of the prefrontal cortex and the NAc, like those
602 observed in the WD animals, could be associated with altered reward sensitivity and cognitive
603 control of eating behavior. In humans, it is also known that a reduction of glucose metabolism
604 in the prefrontal cortex is associated with depressive disorder (68–70). Thus, a hypo-activated
605 prefrontal cortex might predispose the subjects to react more intensely to stress, which might
606 explain the putative emotional status of WD compared to SD animals in the maze test. The
607 effect of perinatal exposure to a WD diet on the susceptibility of offspring to anxiety,
608 depression and attention disorders has been supported (71). Interestingly, the same
609 deactivations were observed in obese minipigs exposed to a Western diet at the adult age (24)
610 and in obese humans (72,73), usually in combination with an alteration of the dopaminergic
611 system.

612 The higher amount of radioactivity signal detected by DAT-Scan in SD putamen can be
613 explained in two ways: 1) DAT was denser in these regions of interest in SD, as described in
614 a study involving obese rats exposed to high-fat diet and showing a lower reuptake of DA
615 compared to control group in striatum (74), or 2) DAT affinity was reduced in WD groups.
616 Such phenomenon has been observed in rats exposed to high-fat-diet-induced obesity, which
617 showed a lower striatal density of D2 receptor, and a lower DAT expression and function
618 (75). Ioflupane exhibits significantly more non-specific DAT/SERT binding in the pig
619 compared to others mammals (76). As a consequence, the hypotheses formulated above have
620 to be verified by immunohistological analyses carried out on brain sections of WD and SD

621 individuals, and by molecular biology analyses to quantify DAT expression. In both cases, it
622 implicates a lower synaptic DA reuptake in the WD group, which is consistent with literature
623 data, in obese-resistant mice presenting a lower density of DAT (20,77), or in rats where
624 high-fat diet fed individuals presented a loss of reuptake of DAT with increased extracellular
625 DA (75,78). Accordingly in humans, body mass index is negatively correlated with DAT
626 density in the striatum (79), and SERT deficiency impairs insulin action on peripheral tissues,
627 associated with a lower leptin secretion (80). A SERT deficiency could explain the higher
628 insulin concentration in WD animal at lean and overweight status. Our results suggest that
629 exposure to a high-fat high-sucrose diet during the perinatal period, even without further
630 overweight or obesity development, could alter the dopaminergic system even in lean adults,
631 with expected consequences on the reward system and food preferences. The WD showed
632 lower binding potential in hippocampus and parahippocampal cortex (PHC) than SD group.
633 Hippocampus and PHC are involved in learning, memory, and more specifically in learning
634 special configuration (81) and visuospatial memory (PHC) (82). Functional impairment in
635 these two regions should implicate poor performance in holeboard discrimination task in WD
636 group, but no difference was observed. A supplementary test without food reward is required
637 to confirm that spatial cognition was impaired by WD diet.
638 However, food preferences between WD and SD groups were similar: animals from both
639 groups preferred the standard known feed, and WD animals did not consume more high-fat
640 high-sucrose feed than SD animals. As a consequence, a high-fat high-sucrose maternal diet
641 during pregnancy and lactation did not modify, in our experimental paradigm, the preferences
642 for fat and sweetness in the offspring, neither at three months of age (14) nor in the adult age.
643 The intake of a new feed can trigger neophobia (83), a phenomenon frequently observed in
644 pigs, and probably expressed by the animals for the high-fat high-sucrose feed, and reinforced
645 by a change of environment (*e.g.* isolation in an individual cage). It is important to note that

646 average 36±7.1% of animals still avoided HFF food during the last test (from 29% to 57% ate
647 less than 10g HFF food), which explains the high variability observed in HFF food
648 consumption in both SD and WD groups. As a consequence, the fact that SD animal increased
649 their HFF food differently to WD animals during tests (Figure A) could indicate a lesser level
650 of neophobia in SD compared to WD, which is consistent with the assumption that WD
651 perinatal diet could be linked with higher stress/anxiety level; but a simple food preference
652 difference cannot be ruled out. Nevertheless, 10% of all the individuals never pressed the
653 button to get feed, and 20% of all the individuals did not do so at the end of the habituation
654 phase, revealing poor learning of the task. Lean WD animals tended to consume more feed
655 than SD individuals during the test involving standard feed.
656 Consistently, plasma analyses showed that WD animals were therefore less tolerant
657 to glucose, with a higher glucose peak, a trend for an increase in AUCI0-30, and an increase
658 in the chol / TG ratio indicating an increase in the LDL fraction at lean status. Paradoxically,
659 SD animals ate more during the obesogenic diet challenge and gained more weight than WD
660 animals. If both SD and WD animals displayed glucose intolerance and decreased insulin
661 sensitivity after 6 weeks of obesogenic diet, WD animals appeared to cope better with the
662 obesogenic diet than SD animals as they displayed higher plasmatic glucose disappearance
663 rate after an IV glucose challenge than SD, and their glucose AUC increased less than SD
664 animals (significant interaction of maternal diet*obesogenic diet). These results are consistent
665 with the adaptive hypothesis stipulating that one is better adapted to the environment it has
666 experienced in early life (84). Studies have shown that maternal undernutrition was associated
667 with the development of metabolic syndrome in the progeny confronted with an over-
668 energetic nutritional environment (2,85), which matches the idea of a compensation
669 phenomenon of the organism when facing a possible scarcity. In the opposite situation, *i.e.* in
670 the case of maternal over-nutrition associated with obesity, the development of metabolic

671 syndrome and obesity was also observed in the offspring (86). However, the absence of
672 obesity in the mothers and offspring in the first part of our study might explain the
673 discrepancy between our results and those previously published. It is also possible that a
674 caloric intake surfeit during the perinatal period, in the absence of pathological condition such
675 as obesity, might have conferred an adaptive advantage to the progeny in the context of a
676 transition towards a high-energy diet. Similar results were found in offspring of rats exposed
677 to high-fat diet without obesity development: high-fat diet pups showed a lower weight gain
678 than control rats, despite altered hypothalamic leptin signaling (87). Maternal high-fat diet can
679 also protect progeny against obesity (88), or endothelial dysfunction (89). In the same way, it
680 was demonstrated that maternal obesity is necessary to program the effects of a high-fat diet
681 on the offspring's health (90), even in offspring exposed to a diet-induced obesity at the adult
682 age. It is important to note that animals did not become really obese but overweight during the
683 obesogenic challenge, showing an average 37.2 % weight gain, corresponding in the human
684 to an overweight status according to the BMI classification. These studies suggest a higher
685 energy expenditure programmed by high-fat diet in non-obese mothers. This high energy
686 expenditure should be linked to the lower microbiota activity in WD 2 weeks after the start of
687 obesogenic diet, involving a lower absorption of SCFA, contrary to results observed in mice
688 ob-ob compared to lean (91). Of course, our obesogenic challenge was applied for only six
689 weeks, and our results do not augur what would happen in these animals after a longer
690 obesogenic-diet exposure. Further studies with a longer-term challenge are necessary to assess
691 whether this apparent adaptive advantage would persist over time.

692 **Conclusion**

693 In normal-weight adults, a maternal Western diet did not appear to alter cognitive
694 processes *per se,* but probably had an effect on other functions, *i.e.* affective (stress) and
695 motivational, that could modulate learning performances of minipigs. WD females were more

motivated for the food rewards than SD females in the holeboard task, and this was confirmed in progressive ratio tests. SD males seemed to be more efficient than WD males in reward search in a non-aversive, stress-free holeboard task, whereas WD animals expressed a high stress level in the anxiogenic alley-maze test, with a lower success compared to SD animals. Brain imaging provided interesting data contributing to the interpretation of the behavioral results. Lower basal activity in WD compared to SD animals was observed in the nucleus accumbens, anterior prefrontal cortex and putamen, as well as a tendency for lower DAT density in WD compared to SD animals in the putamen. These findings suggest altered reward and cognitive control processes in WD animals, with different effects between females and males. In overweight adults, surprisingly, SD animals showed higher feed intake, weight gain and glucose intolerance than WD animals, suggesting that perinatal exposure to a maternal Western diet in the absence of maternal obesity might help the offspring to better cope with a diet enriched in calories, fat and sugar at the adult age, in terms of weight and metabolism management. SCFA analysis suggested a role of microbiota in the differential adaptation of overweight SD and WD minipigs to a Western diet.

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725

726 **Author contributions**

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728 and Caroline Clouard designed research; Yentl Gautier, Isabelle Luneau, Nicolas Coquery,
729 and Sylvie Guérin performed research; Yentl Gautier, Isabelle Luneau, Nicolas Coquery, Paul
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731 Henri Malbert developed new brain imaging procedure and analytic tools; Yentl Gautier and
732 David Val-Laillet wrote the paper; all co-authors red and revised the paper.

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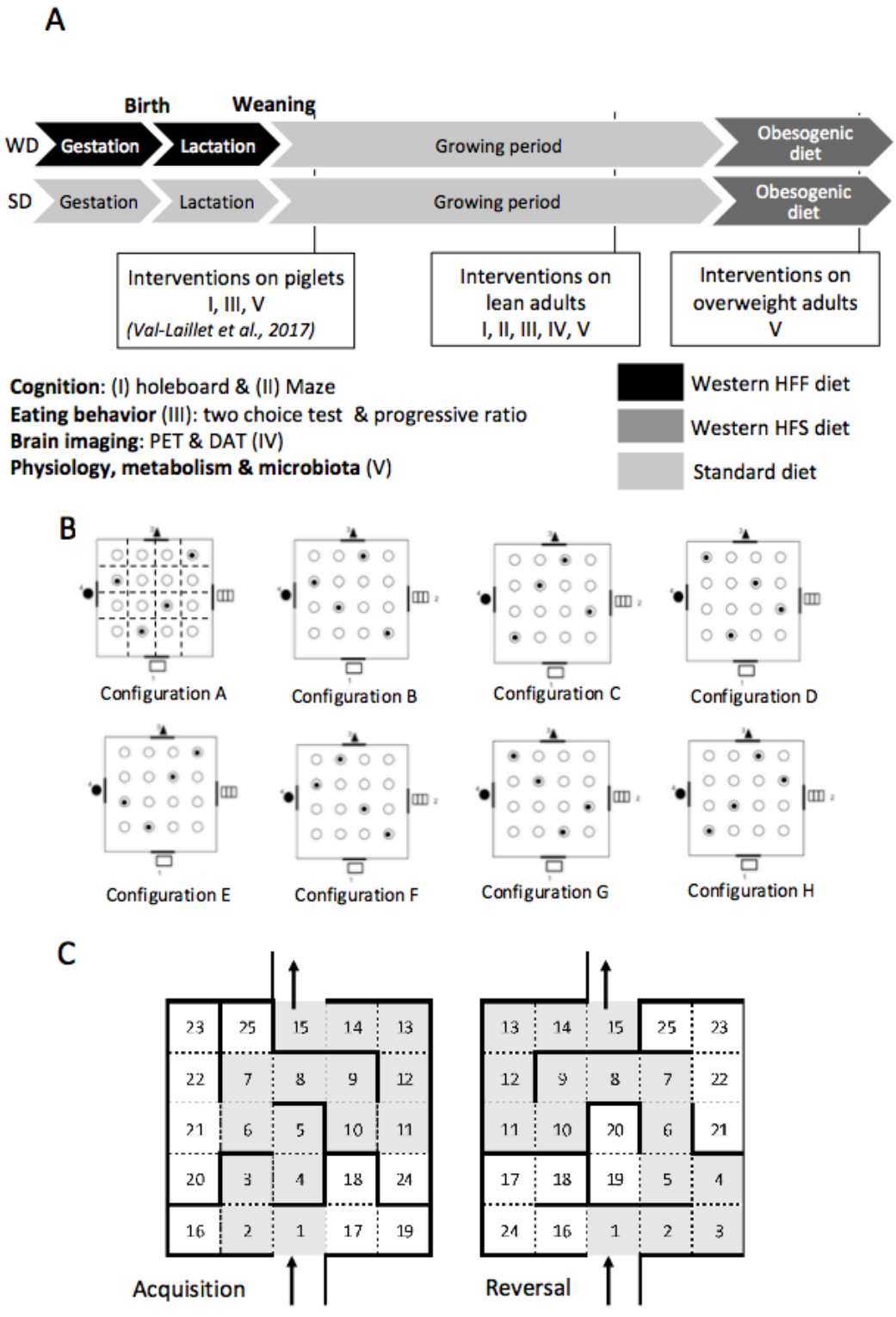

Figure 1. A) General design of the study including three steps: (1) in the young age (just after weaning) previously published in Val-Laillet *et al.* (2017), (2) at the adult age in non-obese animals, and (3) after an obesogenic dietary challenge. The present paper corresponds to the last two steps. On Step 2 in adult lean minipigs, behavioral tests (I, II & III), brain imaging (IV) and biological sampling (V) were performed. In overweight animals on Step 3, only

biological sampling was performed (V). **B)** Holeboard design: 2D plans of the holeboard apparatus indicating the different reward configurations **C)** Design of the alley-maze apparatus with the respective configuration for the acquisition phase (left) and reversal phase (right). The optimum path is indicated in grey, detours or wrong paths being indicated in white. Virtual delimitations are represented with dashed lines; real walls are represented with bold continuous lines. The starting zone was the number 1, the last zone the number 15. Number of virtual zones crossed was used to calculate a performance score (15 being the perfect score and minimum number of zones to cross before leaving the apparatus).

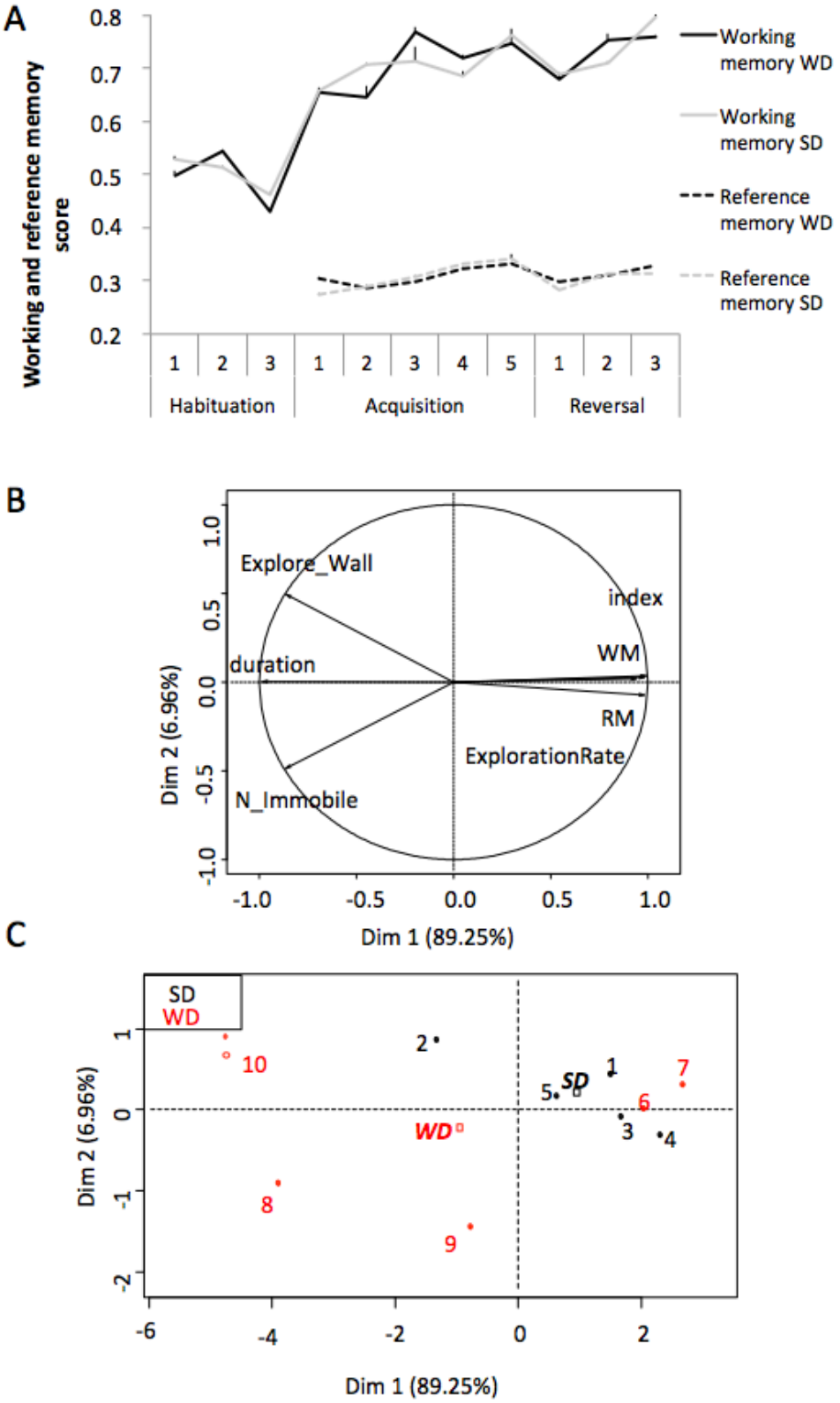

Figure 2. A) Reference (down, dashed lines) and working (top, continuous lines) memory scores obtained during the holeboard discrimination task, in WD (black) and SD (grey) groups. **B)** Variables graphic from PCA analysis for maze test. With WM: working memory, RM: reference memory, Exploration rate corresponding to park exploration by animals,

N_Immobile as occurrences of immobility, duration as test duration, Explore_Wall representing the time of wall exploration by animals, and Index as index of performance of the maze. **C)** Individuals graphic from PCA analysis for maze test. With 5 WD individuals (red) and 5 SD individuals (black)

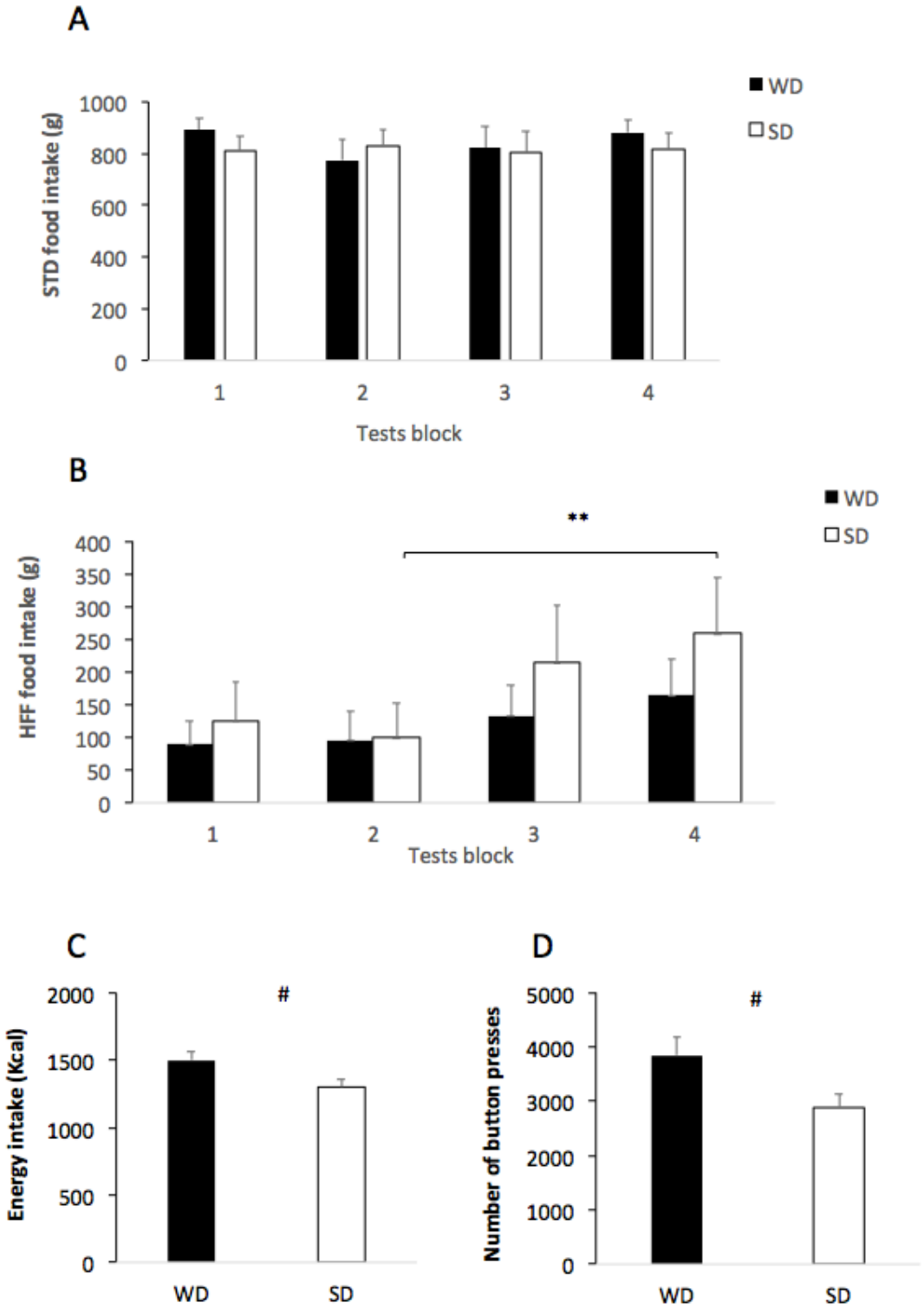

Figure 3. A) Quantity of STD food intake by both SD and WD animals during the 4 sessions of the two-choice test. **B)** Quantity of HFF food intake by both SD and WD animals during the 4 sessions of the two-choice test. **C)** Total energy intake by both SD and WD animals during the two-choice tests ($P < 0.1$). **D)** Number of button presses in progressive ratio test ($P < 0.1$).

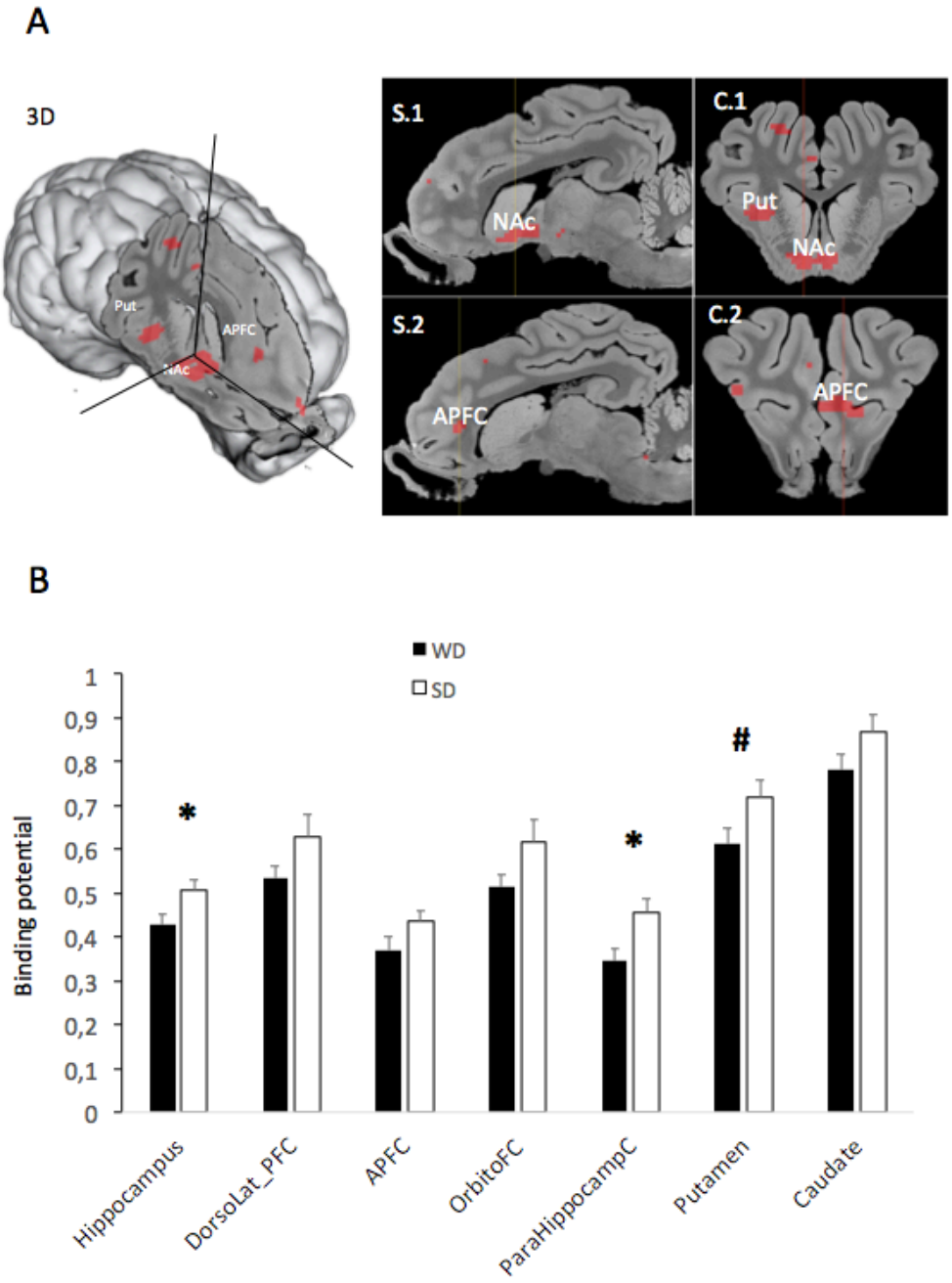

Figure 4. A) Differences in metabolic activity from TEP imaging, in WD compared to SD groups: SD > WD. Left panel: Three-dimensional representation (3D) of brain areas with significant differences in metabolism between the WD and SD groups revealed by the first-level of analysis (P = 0.005, pFDR = 0.01, T = 2.87), Put, Putamen; NAc, Nucleus accumbens; APFC, anterior prefrontal cortex. Right panel: Two-dimensional representations in sagittal section (S1,S2) and coronal section (C1, C2) of the right putamen and bilateral nucleus accumbens (S1, C1), and of the left anterior prefrontal cortex (S2, C2) differential

activations. **B)** Binding potential (mean ± SEM) of DAT in VOIs in WD (black) and SD (white) animals. *, $P < 0.05$. #, $P < 0.1$.

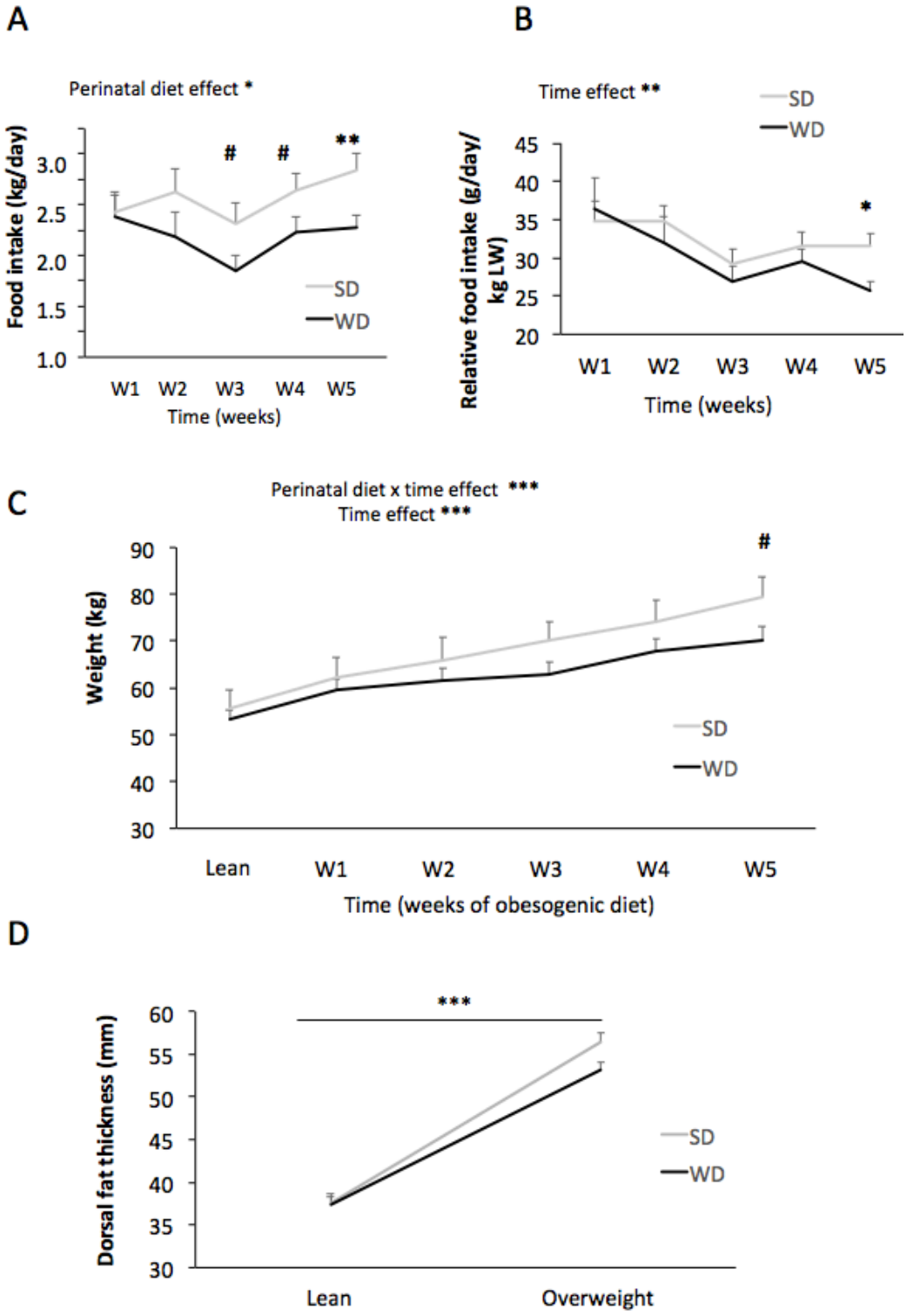

Figure 5. A) Raw food intake (kg/day) during the obesogenic dietary challenge. **B**) Relative daily food intake (g/day/kg live weight) per week of obesogenic dietary challenge. *, $P < 0.05$. **, $P < 0.01$. #, $P < 0.1$. **C)** Weight gain (kg) during the obesogenic dietary challenge (from week 1 to week 6). Free Fatty Acids concentration (FFA, mmol/L) in lean (full) and

overweight animals (hatched) animals. **D)** Dorsal backfat thickness evolution between lean and overweight status in SD (grey) and WD (black) animals.

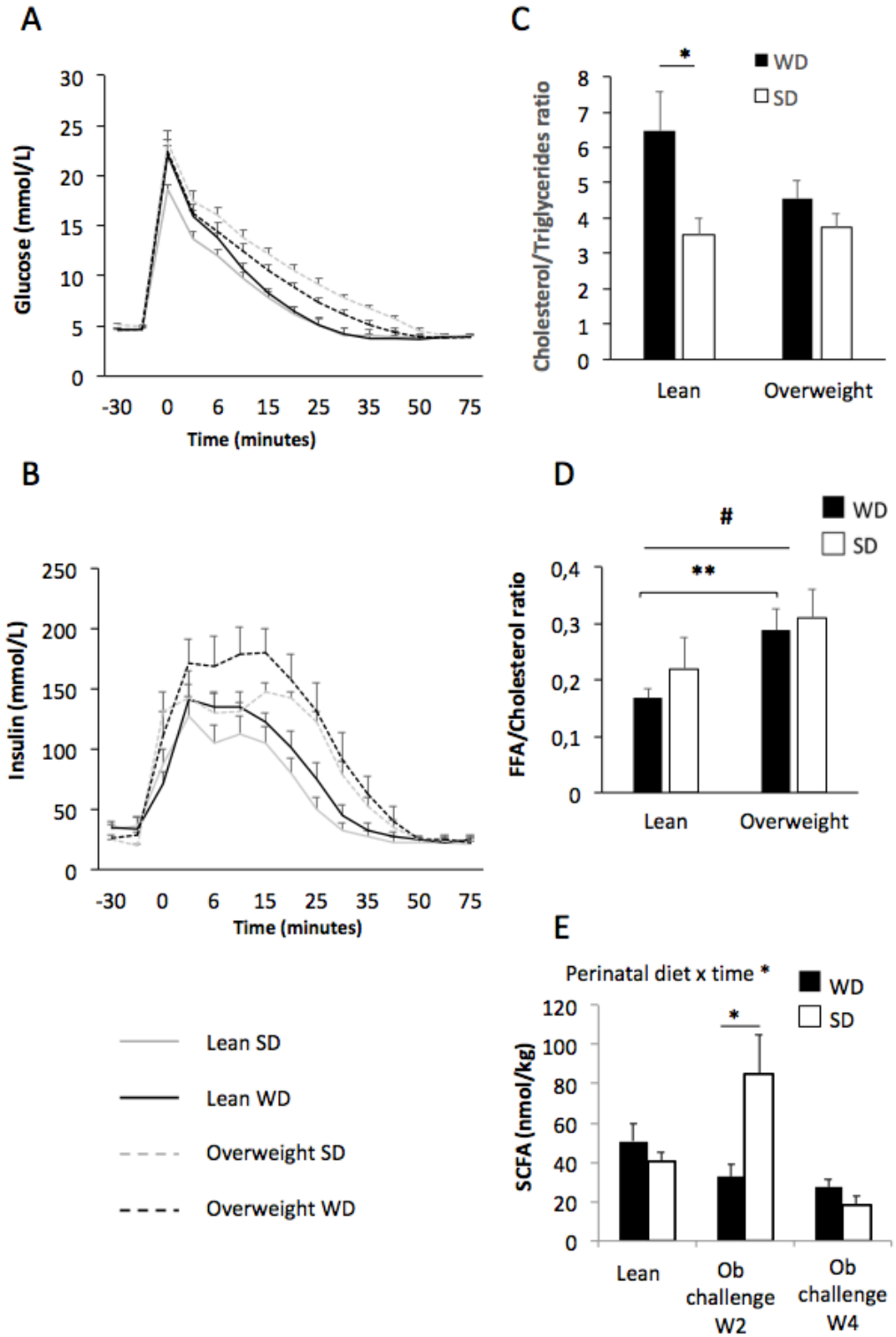

Figure 6. A) Glucose plasma concentrations during IVGTT (mmol/L), in SD (grey) and WD (black), at lean status (full lines) and 5 weeks after start of the obesogenic dietary challenge (doted lines), with t0 glucose injection. **B)** Insulin response during IVGTT (mmol/L). SD (grey) and WD (black), at lean status (full lines) and 5 weeks after start of the obesogenic dietary challenge (doted lines), with t0 glucose injection. **C)** Cholesterol/triglycerides ratio

(Chol/TG) in lean SD (white) and WD (black) minipigs. **D)** Free Fatty Acid / Cholesterol ratio (FFA/Chol) in lean (full) and overweight animals (hatched). **E)** Total SCFA concentration (nmol/kg) in lean status, then after 2 weeks and 4 weeks of obesogenic diet, in WD (black) and SD (white) groups. Two-way ANOVA, effect of time × diet: P=0.14, effect of diet: P=0.002, effect of time: P=0.002.

Table 1a. Composition and nutritional values of the feeds used for sows and offspring. The amount of minor components such as amino-acids and other additives is not reported in this table. Standard (SD) sows were fed the gestation and lactation STD feeds. WD sows were fed a high-fat-fructose (HFF) feed during gestation and lactation. All piglets were fed the STD pre-starter diet from weaning to PND (post-natal day) 15, and then the STD starter diet from PND 15 to the end of the first part of the experiment. Then, animals were given a STD diet for adult minipigs. During the obesogenic dietary challenge, they were exposed to a high fat-sucrose (HFS) feed during 6 weeks.

	Sows' diet			**Offspring's diet from weaning to obesogenic challenge**	**Offspring's diet during the obesogenic challenge**
Composition (%)	Standard (STD)	Lactation (LD)	High-fat fructose (HFF)	Standard (STD)	High-fat sucrose (HFS)
Wheat	10.00	25,6	6.00	10	6.25
Corn		12.0			
Barley	33.00	25.7	12.00	33	12
Wheat bran	25.00	10.0	14.00	25	14
Soybean meal	6.00	18.0	9.00	6	12
Sunflower meal	10.00	2.0	8.00	10	8
Soybean hulls	12.00		11.00	12	8
Molasses	1.00	3.0		1	
Corn starch			6.50		6.5
Sucrose			9.25		20
Fructose			9.25		
Lard oil			12.00		10
Bicalcium phosphate	0.60	1.02	0.60	0.60	0.60
Calcium carbonate	1.30	1.20	1.30	1.30	1.30
NaCl	0.60	0.45	0.60	0.60	0.60
Oligo-vitamins	0.50	0.50	0.50		
Mineral Vitaminic Complement	0.5			0.5	0.75
Total	100	100	100	100	100
Metabolisable energy (MJ/kg)	10.31	13.14	18.46	10.31	14.09
Net energy (MJ/kg)	7.27	9.41	14.52	7.27	10.80
		Nutritional value (%)			

Dry matter	87.7	NA	89.64	87.7	91.8
Cellulose	11.05	NA	8	11.05	7.49
Carbohydrates (starch)	28.23	NA	NA	28.23	38.34
Lipids	2.17	NA	13.45	2.17	11.38
Nitrogen matter	15.22	16.40	12.18	15.09	12.74
Mineral content	6.81	6.10	5.30	6.81	5.97

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1110 **Table 1b.** Macronutrient composition of maternal diets (NE: net energy).

	WD	**SD**
%Carb	61.7	75.3
%Prot	15.1	22.5
%Fat	23.2	2.2
NE (MJ/kg)	10.8	7.27

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Table 2. Brain regions with differential activation between WD and SD groups; using level-1 SPM analysis, without *a priori*, Whole Brain method, FDR (False Discovery Rate) correction (R, right; L, left). Stereotaxic coordinates (x,y,z) of the voxel with the highest *t-value* are indicated for each brain region. They were referenced to the *commissura anterior-commissura posterior* plane with the origin set at *commissura posterior* according to the stereotactic reference selected by (92), (50), for the pig brain stereotactic atlas.

	Hemisphere	Number of voxels	Coordinates			t-value	Voxel P (uncorrected)	P FWE (corrected)	P FDR (corrected)
ROIs H=WD<SD			x	y	z				
Nucleus accumbens	L	151	-1	20	-4	5.26	0.0001	NA	**0.047**
Anterior prefrontal cortex	L	42	-4	32	1	5.16	0.0001	NA	**0.047**

Height threshold: T = 2.88, p = 0.005. Extent threshold: k = 20 voxels, pFDR = 0.01.

Table 3. Brain region with differential activation between WD and SD groups; using level-2 SPM analysis with *a priori*, SVC method, FWE (Family Wise Error) correction (R, right; L, left). Stereotaxic coordinates (x,y,z) of the voxel with the highest *t-value* are indicated for each brain region. They were referenced to the *commissura anterior-commissura posterior* plane with the origin set at *commissura posterior* according to the stereotactic reference selected by (50),(92) for the pig brain stereotactic atlas. Only significant results are mentioned.

	Hemisphere	Number of voxels	Coordinates			t-value	Voxel P (uncorrected)	P FWE (corrected)	P FDR (corrected)
)ls H=WD<SD			x	y	z				
Nucleus accumbens	L	**33**	-1	20	-4	5.26	0.0001	**0.001**	**0.013**
	R	**28**	3	22	-5	3.93	0.0001	**0.013**	0.163
Anterior Prefrontal Cortex	L	**26**	-4	32	1	5.16	0.0001	**0.018**	0.116
	R	-	-	-	-	-	-	*NS*	*NS*
Caudate nucleus	L	8	-2	21	-4	4.08	0.0001	0.065	0.141
	R	7	4	19	-3	3.83	0.0001	0.099	0.432
Putamen	L	-	-	-	-	-	-	*NS*	*NS*
	R	**49**	11	19	5	4.77	0.0001	**0.018**	0.08

eight threshold: T = 2.88. p = 0.005 ; Extent threshold: k = 20 voxels

Table 4. Brain region with differential activation between WD and SD groups in separate sex analysis with F for females and M for males; using level 1 SPM analysis, without *a priori*, using Whole Brain method, FWE approach (R, right; L, left). Stereotaxic coordinates (x,y,z) of the voxel with the highest *t-value* are indicated for each brain region. They were referenced to the *commissura anterior-commissura posterior* plane with the origin set at *commissura posterior* according to the stereotactic reference selected by (50)(92) for the pig brain stereotactic atlas. Only significant results are mentioned.

		Sex	Hemisphere	Number of voxels	Coordinates			t-value	Voxel P (uncorrected)	P FWE (corrected)
ROIs H=WD<SD					x	y	z			
	Nucleus accumbens	F	L	1	-1	22	6	3.86	0.001	**0.006**
			R	5	2	22	-6	3.79	0.001	**0.006**
		M	L	-	-	-	-	-	*NS*	*NS*
			R	-	-	-	-	-	*NS*	*NS*
	Anterior prefrontal cortex	F	L	-	-	-	-	-	*NS*	*NS*
			R	-	-	-	-	-	*NS*	*NS*
		M	L	**37**	-7	41	-2	6.78	0.0001	**0.017**
			R	-	-	-	-	-	*NS*	*NS*

Height threshold: T = 2.88, p = 0.005. Extent threshold: k = 20 voxels

Table 5. Metabolic parameters of minipigs from IVGTT. Values are mean ± SEM. IVGTT, intravenous glucose tolerance test. $P < 0.05$, significant. $0.05 < P < 1$, tendency. NS, not significant.

	Lean		Overweight		P - value (type III ANOVA)		
	SD	**WD**	**SD**	**WD**	**Perinatal diet**	**Status**	**Interaction**
Effective	8	8	4	6			
Lipidic profile							
FFA (mmol/L)	0.41 ± 0.08	0.35 ± 0.04	0.67 ± 0.1	0.61 ± 0.05	*ns*	**<0.001**	*ns*
TG (mmol/L)	0.69 ± 0.16	0.39 ± 0.06	0.59 ± 0.03	0.52 ± 0.08	***<0.05***	*ns*	*ns*
Chol (mmol/L)	2.02 ± 0.18	2.08 ± 0.08	2.20 ± 0.22	2.20 ± 0.18	*ns*	*ns*	*ns*
Glucose tolerance							
glucose peak (mmol/L)	14.05 ± 0.4	17.42 ± 1.0	18.31 ± 1.1	17.72 ± 1.2	**0.05**	**<0.05**	**<0.05**
AUCG	136.92 ± 21.5	155.42 ± 10.0	262.01 ± 16.9	210.01 ± 18.8	*ns*	**<0.001**	0.07
AUCG0-30	130.2 ± 15.3	154.44 ± 9.4	242.34 ± 15.7	203.23 ± 16.4	*ns*	**<0.001**	**<0.05**
KG (%/min)	4.85 ± 0.6	5.21 ± 0.4	2.9 ± 0.2	3.57 ± 0.2	*ns*	**<0.01**	*ns*
SG (10-2.min-1)	4.41 ± 1.2	4.67 ± 0.4	1.38 ± 0.4	1.78 ± 0.4	*ns*	**<0.01**	*ns*
Insuline response							
AIR	71.3 ± 10.5	80.92 ± 4.7	113.6 ± 12.7	123.34 ± 14.8	*ns*	**<0.001**	*ns*
AUCI	1801.2 ± 282.2	2253.62 ± 327.5	3771.44 ± 138.7	4287.07 ± 699.3	*ns*	**<0.001**	*ns*
AUCI0-30	1671.97 ± 280.3	2187.31 ± 287.1	3987.35 ± 170.1	4697.27 ± 596.5	**0.099**	**<0.001**	*ns*
SI (/min/(µIU/mL))	18.65 ± 8.18	9.18 ± 2.03	5.25 ± 0.95	4.70 ± 1.33	*ns*	**0.05**	*ns*
S2 (ml/min.(µIU/ml)/kg)	7.16 ± 1.5	5.07 ± 0.8	2.02 ± 0.2	2.45 ± 0.5	*ns*	**<0.001**	*ns*
HOMA	7.11 ± 0.96	7.50 ± 1.72	4.65 ± 0.24	5.71 ± 0.52	*ns*	*ns*	*ns*
QUICKY	0.46 ± 0.01	0.47 ± 0.02	0.50 ± 0.01	0.48 ± 0.01	*ns*	*ns*	*ns*
Inflammation							
Haptoglobin	3,36±0,56	3,9±0,52	2.03± 0.39	3.08± 0.24	*ns*	**0.06**	*ns*