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Adult rats sired by obese fathers present learning deficits associated with epigenetic and neurochemical alterations linked to impaired brain glutamatergic signaling

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Abstract

Aim: Offspring of obese mothers are at high risk of developing metabolic syndrome and cognitive disabilities. Impaired metabolism has also been reported in the offspring of obese fathers. However, whether brain function can also be affected by paternal obesity has barely been examined. This study aimed to characterize the learning deficits resulting from paternal obesity versus those induced by maternal obesity and to identify the underlying mechanisms.

Methods: Founder control and obese female and male Wistar rats were mated to constitute three first-generation (F1) experimental groups: control mother/control father, obese mother/control father, and obese father/control mother. All F1 animals were weaned onto standard chow and underwent a learning test at 4 months of age, after which several markers of glutamate-mediated synaptic plasticity together with the expression of miRNAs targeting glutamate receptors and the concentration of kynurenic and quinolinic acids were quantified in the hippocampus and frontal cortex.

Results: Maternal obesity induced a severe learning deficit by impairing memory encoding and memory consolidation. The offspring of obese fathers also showed reduced memory encoding but not impaired long-term memory formation. Memory deficits in offspring of obese fathers and obese mothers were associated with a down-regulation of genes encoding NMDA glutamate receptors subunits and several learning-related genes along with impaired expression of miR-296 and miR-146b and increased concentration of kynurenic acid.

Conclusion: Paternal and maternal obesity impair offspring's learning abilities by affecting different processes of memory formation. These cognitive deficits are associated with epigenetic and neurochemical alterations leading to impaired glutamate-mediated synaptic plasticity.

KEYWORDS

developmental programming, glutamate signaling, kynurenine pathway, learning, paternal obesity

1 | INTRODUCTION

The fetal and neonatal periods of development are highly sensitive to environmental factors that can shape and durably modify brain structure and function. Observational and experimental studies indicate that many mental disorders, including autism, schizophrenia, and intellectual and learning disabilities, are related to adverse nutritional or psychological conditions experienced by the mother during pregnancy.^{1–3} These observations fall within the hypothesis of the Developmental Origins of Health and Disease (DOHaD) also known as developmental programming. According to this hypothesis, exposure to malnutrition, infection, stress, and other adverse events during in utero development and/or neonatal life, leads to a high risk of developing metabolic and mental disorders via epigenetic changes that translate effects of early, and transient, environmental stimuli leading to long-term changes in brain function and energy homeostasis.^{4,5}

In light of the current obesity epidemic, particular attention has been devoted during the last years to the analysis of the consequences of maternal obesity on the health of the offspring. Thus, a large number of clinical and experimental studies have reported that maternal obesity sensitizes the offspring to the development of obesity, insulin resistance, stroke, and other metabolic diseases.^{6–8} Likewise, maternal obesity impairs the cognitive function of the offspring. Namely, children born to obese mothers present lower scores on verbal cognitive tests,^{9,10} lower reading and mathematical skills,¹¹ and poor executive functioning that affects their attention, inhibitory control, and working memory.^{12,13} Similarly, animal studies have shown that the offspring born to obese dams display reduced working, spatial, and associative memory.^{14–17} Several mechanisms have been proposed to underpin the cognitive deficits in the offspring of obese mothers including impaired expression of BDNF and reduced neurogenesis and dendritic arborisation in the hippocampus.^{18–21}

There is also a large body of evidence demonstrating that paternal obesity predisposes the offspring to metabolic diseases.^{22–25} However, whether brain function can be affected by the obese state of the father, has been barely examined.

The present study aimed to elucidate whether paternal obesity induces alterations of the memory process in the offspring and whether these alterations are common to those induced by maternal obesity. In particular, we

sought to determine whether the learning deficits in the offspring of obese fathers can be underpinned by impaired glutamatergic signaling. We focused on glutamate neurotransmission because of its key role in the regulation of learning and memory processes and the results of the literature showing that maternal obesity impairs the expression of NMDA glutamate receptors in the brain of the offspring.^{20,26,27}

2 | RESULTS

2.1 | Metabolic alterations induced by the fc-HFHS diet in founder (FO) rats

Since our principal objective was to determine whether maternal and paternal obesity can compromise the cognitive function of the offspring, we first ensured that consumption of the fc-HFHS diet does induce an obese phenotype. Examination of F0 rats before mating, showed that male animals exposed to the fcHFHS diet displayed an increased body weight (597 ± 22 g vs. 520 ± 19 g, $p < 0.01$) and serum glucose (204.30 ± 8.63 ng/dL vs. 182.50 ± 4.87 ng/dL, $p < 0.01$) in comparison to male rats fed standard chow. F0 obese males also showed reduced glucose tolerance as indicated by the larger area under the GTT curve (obese = 7717 ± 394 vs. controls = 4085 ± 85 , $p < 0.0001$). Likewise, F0 female rats in the fcHFHS diet group were heavier (369 ± 25 vs. 293 ± 9 , $p < 0.05$) and presented an increased concentration of glucose in serum (100.20 ± 2.67 ng/dL vs. 87.60 ± 3.42 ng/dL, $p < 0.05$) in comparison with their control counterparts.

2.2 | Memory deficits induced by maternal and paternal obesity in offspring

All experimental groups spent the same time exploring object 1 versus object 2 during the sample phase of the NOR test (Figure 1A). However, clear memory deficits were observed in animals with an obese parent. Thus, the offspring from obese mothers were unable to distinguish the novel object from the familiar one during the two testing sessions performed 2 h [$F(2, 29) = 5.275$, $p = 0.0111$; Figure 1B] and 24 h [$F(2, 29) = 3.649$, $p = 0.0386$; Figure 1C] after the sample phase. In contrast, the offspring of obese fathers showed reduced memory capacity

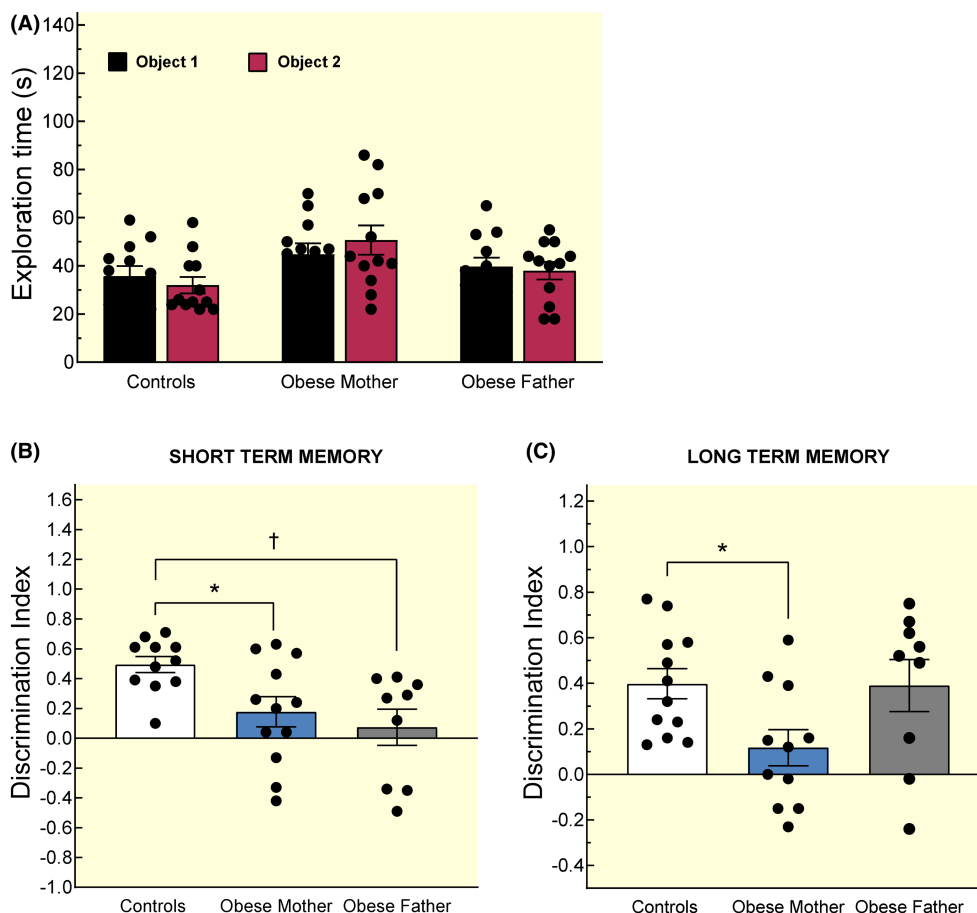


FIGURE 1 Impact of maternal and paternal obesity on the learning skills of the offspring. The learning abilities of adult control rats, born to obese mothers, or from the mating of an obese male with a female of normal body weight, were assessed using the Novel Object Recognition test. (A) Object exploration time during the training phase. Discrimination index during the test sessions conducted 2 and 24 h after the training session to measure, respectively, short-term (B) and long-term (C) memories. Note that rats born to obese mothers do not distinguish the novel object from the familiar one in any of the test sessions despite the fact that they spent the same amount of time as control animals exploring two identical objects in the sample phase during which the encoding of the information takes place. Learning tests were performed at the beginning of the dark phase of the animal's dark–light cycle. Data represent the mean \pm SEM of the data from a total number of 9–12 animals per experimental group. * $p < 0.05$, $^{\dagger}p < 0.01$ as determined by one-way ANOVA.

only during the test session carried out 2 h after the training session (Figure 1B). These results indicate that both maternal and paternal obesity impair the encoding stage of memory processing and that maternal obesity affects, in addition, the consolidation stage leading to impaired long-term memory formation.

2.3 | Maternal and paternal obesity alters the expression of genes involved in glutamatergic signaling and synaptic plasticity in the brain of the offspring

To establish the mechanisms by which the cognitive abilities of the offspring could be affected by parental obesity, the mRNA levels encoding for genes involved in glutamate signaling and synaptic plasticity were evaluated

in the hippocampus and frontal cortex of adult F1 rats by real-time PCR. The analysis of the data by one-way ANOVA showed that the expression of NMDA, but not of AMPA receptors, is downregulated in the hippocampus of offspring from obese mothers or obese fathers (Table 1, Figure 2A). Furthermore, F1 rats of obese fathers, just like their congeners born to obese mothers, show reduced expression of several genes involved in the regulation of synaptic plasticity and learning and memory processes (Table 1, Figure 2B).

Maternal obesity and paternal obesity also induced a reduced expression of NMDA receptors and of mRNAs coding for genes regulating synaptic plasticity in the frontal cortex of their offspring (Table 1, Figure 3A,B). In contrast to what was observed in the hippocampus, parental obesity reduced the expression of AMPA-type subunit 1 receptors (Table 1, Figure 3A).

TABLE 1 Statistical differences in the expression levels of genes coding for AMPA and NMDA receptor subunits and markers of glutamate-mediated synaptic plasticity in the brain of adult rats born to obese mothers or sired by obese fathers.

Gene	Hippocampus				Frontal cortex			
	DFn, DFd	F	p	Significance	DFn, DFd	F	p	Significance
Gria1	2, 12	2.088	0.1667	NS	2, 12	6.126	0.0147	*
Gria2	2, 12	2.809	0.0998	NS	2, 12	0.052	0.9490	NS
Grin1	2, 12	7.472	0.0078	†	2, 12	10.550	0.0023	†
Grin2A	2, 12	4.915	0.0276	*	2, 12	8.164	0.0058	†
Grin2B	2, 12	8.059	0.0060	†	2, 12	1.913	0.1901	NS
Arc	2, 12	26.59	<0.0001	¥	2, 12	8.481	0.0051	†
Dlg4	2, 12	14.45	0.0006	‡	2, 12	5.501	0.0202	*
Egr1	2, 12	6.655	0.0114	*	2, 12	2.984	0.8870	NS
miR-146b	2, 15	3.960	0.0416	*	2, 13	5.264	0.02120	*
miR-296b	2, 11	16.38	0.0005	‡	2, 13	23.450	<0.0001	¥

Note: The data correspond to the results of the one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Dfn and DFd are the degrees of freedom for the numerator and denominator, respectively.

Abbreviations: Arc, activity-regulated cytoskeleton-associated protein; Dlg4, discs large MAGUK scaffold protein 4; Egr1, early growth response 1; Gria1, glutamate ionotropic receptor AMPA type subunit 1; Gria2, glutamate ionotropic receptor AMPA type subunit 2; Grin1, glutamate ionotropic receptor NMDA type subunit 1; Grin2A, glutamate ionotropic receptor NMDA type subunit 2A; Grin2B, glutamate ionotropic receptor NMDA type subunit 2B. * $p < 0.05$;

† $p < 0.01$; ‡ $p < 0.001$; ¥ $p < 0.0001$; NS = non significant.

2.4 | Maternal and paternal obesity alters the expression in the brain of the offspring of microRNAs that target NMDA receptors or that negatively regulate learning

Several studies have shown that the metabolic alterations induced by maternal obesity in offspring are associated with epigenetic modifications, including impaired miRNAs expression.^{27–29} To determine whether the previously described decrease in mRNAs encoding NMDA receptors in the offspring of obese parents could be mediated by epigenetic modifications, the expression of miR-296 and miR-146b was assessed by RT-PCR. The glutamatergic receptors Grin2A and Grin2B are targets of miR-296³⁰ and inactivation of miR-146b gene expression in mice results in increased learning capabilities.³¹

As shown by one-way ANOVA analysis followed by Dunnett's multiple comparison test (Table 1), and illustrated in Figure 4A, offspring of obese mothers or obese fathers, showed increased expression of miR-296 in the hippocampus. Pairwise comparison of the miR-146b expression data by unpaired t test, revealed that maternal and paternal obesity also increase the levels of miR-146b in the hippocampus of the offspring ($p=0.04$ for maternal obesity; $p=0.02$ for paternal obesity). However, only the difference between control and obese father offspring reached statistical significance when the data were analyzed by one-way ANOVA (Table 1, Figure 4B). In contrast to the increase in miR-296 expression observed in

the hippocampus in response to parental obesity, the offspring of obese mothers or fathers showed reduced levels of miR-296 in the frontal cortex (Table 1, Figure 4C). However, as in the hippocampus, animals sired by obese fathers showed increased expression of miR-146b in the frontal cortex in comparison to their control counterparts (Table 1, Figure 4D).

2.5 | Effect of maternal and paternal obesity on the metabolism of tryptophan in the brain of the offspring

Tryptophan is an essential amino acid that serves as a precursor for several bioactive compounds via its catabolism through the serotonin (5-HT) and kynurenine (KYN) pathways. 5-HT is known for its enhancing or inhibitory effects on memory depending on the type of 5-HT receptor activated and the experimental paradigm used to assess learning,^{32,33} while kynurenic acid (KA) and quinolinic acid (QA), two by-products of KYN metabolism, inhibit the learning and memory processes mediated by glutamate and acetylcholine. Indeed, KA blocks the actions modulated by $\alpha 7$ nicotinic acetylcholine ($\alpha 7$ nAChR) and NMDA receptors, whereas excessive activation of NMDA receptors by QA leads to neurotoxicity. In a previous study, we found that obesity-induced memory deficits are linked to increased concentrations of KA and QA in the brain.³⁴ Therefore, we sought to determine whether the learning deficits in the offspring of obese parents could be

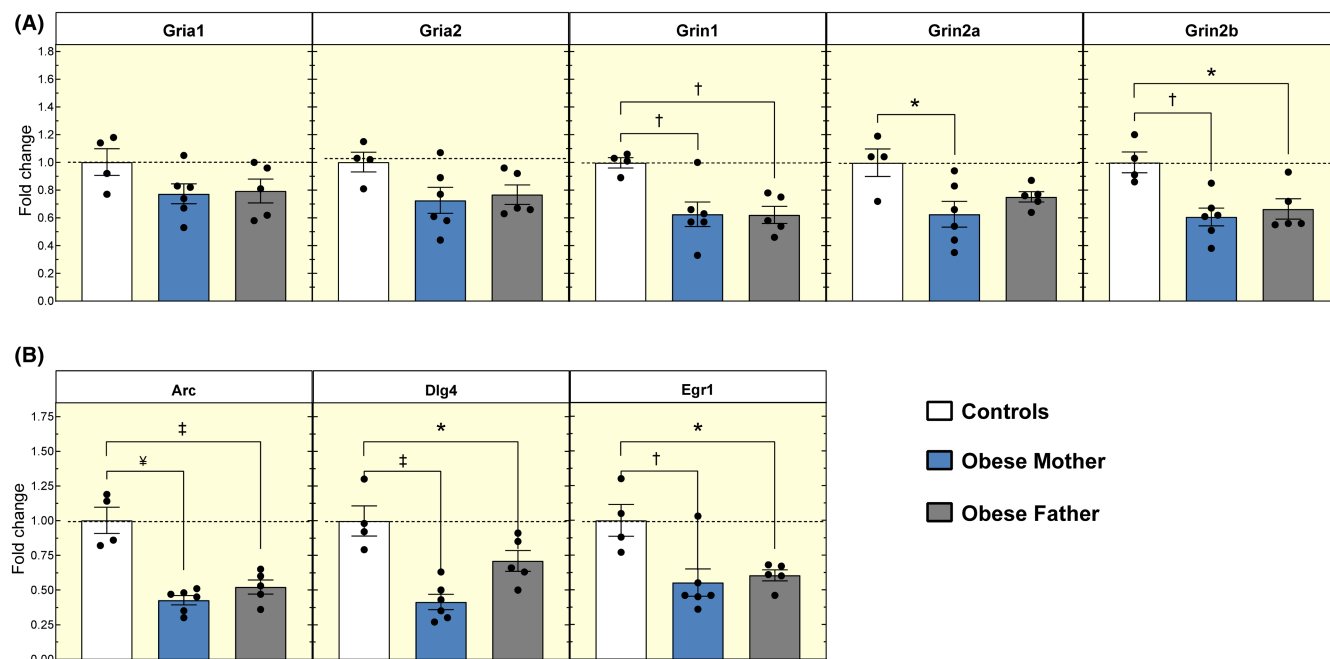


FIGURE 2 Expression levels of mRNAs that encode for AMPA and NMDA receptor subunits (A) and markers of glutamate-mediated synaptic plasticity (B) in the hippocampus of offspring born to obese mothers or sired by obese fathers. Gene expression levels were determined by real-time quantitative PCR. Variations in gene expression were calculated using the comparative Ct method and the expression of control animals as a calibrator. Data represent the mean \pm SEM of the data from a total of 4–6 animals per experimental group. * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$, § $p < 0.0001$ as determined by one-way ANOVA followed by Dunnett's multiple comparisons test. Gria1, glutamate ionotropic receptor AMPA type subunit 1; Gria2, glutamate ionotropic receptor AMPA type subunit 2; Grin1, glutamate ionotropic receptor NMDA type subunit 1; Grin2A, glutamate ionotropic receptor NMDA type subunit 2A; Grin2B, glutamate ionotropic receptor NMDA type subunit 2B; Arc, activity-regulated cytoskeleton-associated protein; Egr1, early growth response 1; Dlg4, discs large MAGUK scaffold protein 4.

underpinned by impaired production of these two neuroactive compounds.

Maternal obesity resulted in higher KA levels in the hippocampus [$F(2, 15) = 4.209$, $p = 0.0354$; Figure 5A], and the frontal cortex [$F(2, 14) = 4.965$, $p = 0.023$; Figure 5B], of the offspring, as well as in increased hippocampal concentration of QA [$F(2, 27) = 3.464$, $p = 0.0458$; Figure 5C]. The offspring of obese fathers exhibited also an upregulation of KA levels in the hippocampus and frontal cortex (Figure 5A,B). However, paternal obesity induced no changes in the concentration of QA neither in the frontal cortex or in the hippocampus (Figure 5C,D).

3 | DISCUSSION

This study shows that both maternal and paternal obesity impair the learning abilities of the offspring by disrupting different steps of the memory process. More precisely, offspring born to obese mothers were unable to distinguish a novel object from a familiar one during the two learning sessions designed to measure short and long-term memory. These findings indicate that maternal obesity impairs the encoding and retrieval processes of memory. In

contrast, animals born from the mating of an obese male rat with a nonobese female did not distinguish the novel object from the familiar object during the first test session but they clearly showed a preference to explore the novel object during the test session performed 24 h after training, indicating that their capacity to encode new information is impaired but that their ability to form long-term memories remains intact.

It is generally accepted that memory encoding and consolidation are based on synaptic plasticity, of which long-term potentiation is the cellular counterpart.³⁵ The key regulator of this process is glutamate through its interaction with α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. These receptors are tetramers of intertwined subunits that form a non-selective cation channel.³⁶ The AMPA receptors are composed Gria1, 2, 3, and 4 subunits, while NMDA receptors are tetramers of two obligatory Grin1 subunits and two Grin2 and/or Grin3 subunits. At the resting membrane potential, the NMDA ion channel is blocked by physiological levels of extracellular Mg^{2+} .³⁷ The process of long-term potentiation, and therefore of memory

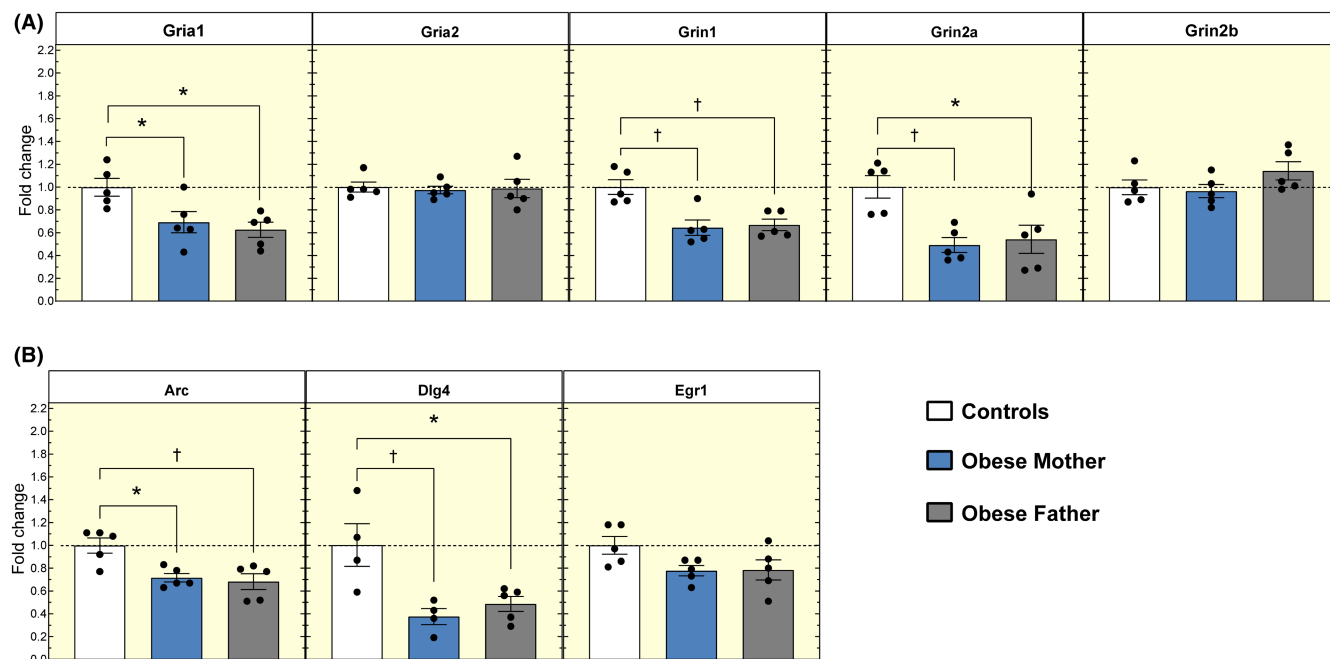


FIGURE 3 Expression levels of mRNAs that encode for AMPA and NMDA receptor subunits (A) and markers of glutamate-mediated synaptic plasticity (B) in the frontal cortex of offspring born to obese mothers or sired by obese fathers. Gene expression levels were determined by real-time quantitative PCR. Variations in gene expression were calculated using the comparative Ct method and the expression of control animals as a calibrator. Data represent the mean \pm SEM of the data from a total of 4–5 animals per experimental group. * $p < 0.05$, † $p < 0.01$ as determined by one-way ANOVA followed by Dunnett's multiple comparisons test. Gria1, glutamate ionotropic receptor AMPA type subunit 1; Gria2, glutamate ionotropic receptor AMPA type subunit 2; Grin1, glutamate ionotropic receptor NMDA type subunit 1; Grin2A, glutamate ionotropic receptor NMDA type subunit 2A; Grin2B, glutamate ionotropic receptor NMDA type subunit 2B; Arc, activity-regulated cytoskeleton-associated protein; Egr1, early growth response 1; Dlg4, discs large MAGUK scaffold protein 4.

formation at the cellular level, starts with the activation of AMPA receptors by glutamate leading to a Na^+ influx, and a small K^+ efflux. This provokes depolarization of the postsynaptic neurone leading to the removal of the Mg^{2+} block of NMDA receptors and, consequently, to the influx of Ca^{2+} through the NMDA receptor channel.³⁸ The increased intracellular concentration of Ca^{2+} results in the activation of several Ca^{2+} -binding proteins together with enhanced gene expression, as those of the Early Growth Response 1 (Egr1), and the Activity Regulated Cytoskeleton Associated Protein (Arc), genes. These intracellular events ultimately lead to increased synaptic efficacy through the accumulation of additional AMPA receptors to synapses and dendritic growth.^{39,40} The spatial proximity, and therefore the functional link, between glutamatergic receptors and their signaling molecules within the synapse is ensured by the Discs Large MAGUK Scaffold Protein 4 (Dlg4). Hence, any alteration in the number of AMPA and NMDA receptors and/or the expression of the genes involved in their signaling pathways can alter the memory process and lead to cognitive deficits.

The impaired learning resulting from maternal and paternal obesity was accompanied by an altered expression of NMDA receptors and other markers of synaptic

plasticity in the brain. Specifically, offspring born to obese dams show a downregulation both in the hippocampus and frontal cortex of the mRNAs coding for the Grin1 and Grin2A subunits of NMDA receptors together with a decreased expression of Arc, Dlg4, and Egr1. A very similar pattern of changes in gene expression was observed in the hippocampus and frontal cortex of F1 rats of obese fathers. Although other studies have shown that maternal obesity impairs glutaminergic signaling in the brain of the offspring, this is the first study to report that paternal obesity also impairs glutamate receptor-mediated synaptic plasticity associated with learning and memory.

The alteration of the epigenetic regulation of gene expression is the most common mechanism advanced to explain the long-term effects induced by the temporary exposure to adverse environmental stimuli during early development and, indeed, variations in DNA methylation levels or histone binding at the promoter site of several genes have been described in offspring born to obese mothers or sired by obese fathers.^{41–46} Changes in the amounts of miRNAs in various tissues of animals born to obese dams have also been found.^{28,47–49} miRNAs are small noncoding RNAs that negatively modulate gene expression including that of genes belonging to the epigenetic machinery. Here we found that the memory deficits

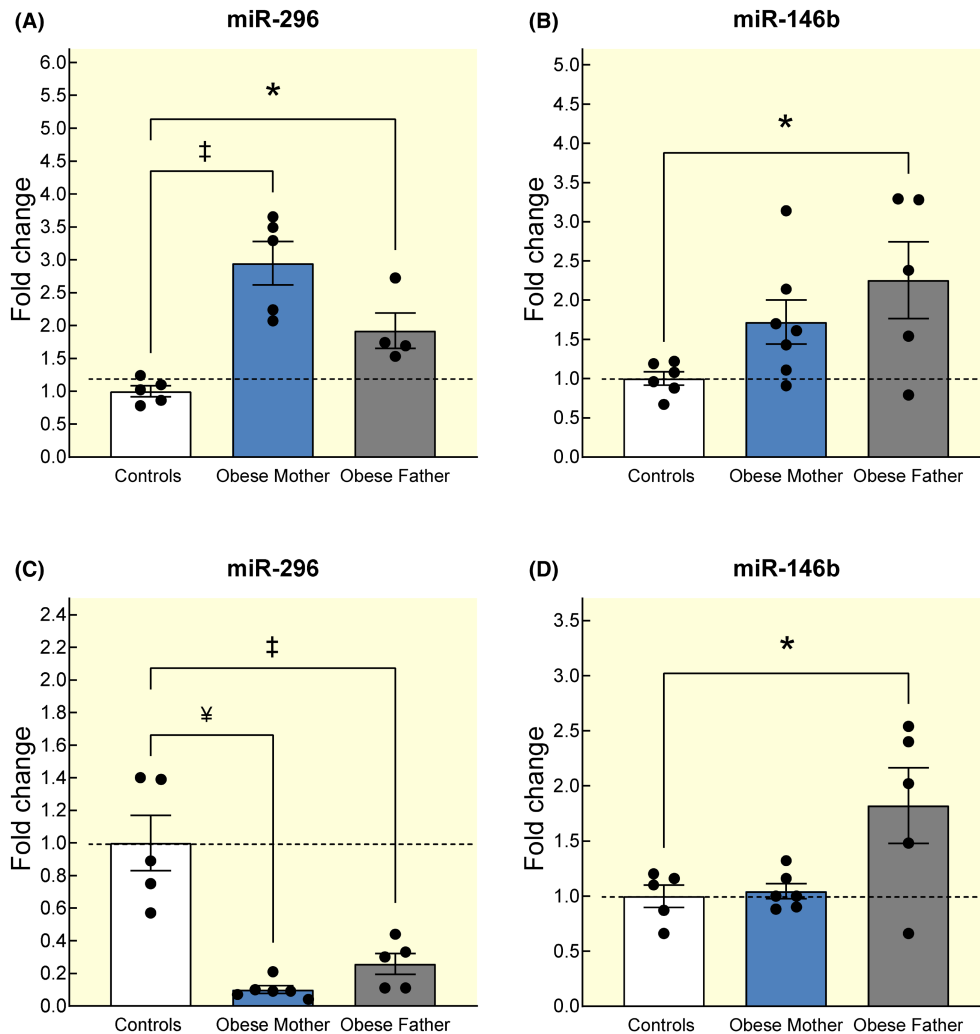


FIGURE 4 Differences in miR-296 and miR-146b expression levels in the hippocampus (A, B), and frontal cortex (C, D) of control rats or of animals born to obese mothers or sired by obese fathers. Data are expressed as fold change in relation to the control group as calculated using the comparative Ct method and the amplifications of Let-7d and Let-7g performed in the same real-time PCR run as a reference. * $p < 0.05$, † $p < 0.001$, ‡ $p < 0.0001$ as determined by one-way ANOVA.

exhibited by the offspring of obese dams or obese fathers are associated with increased expression of miR-296 and miR-146b in the hippocampus. miR-296 downregulates Grin2A and Grin2B NMDA receptors,³⁰ and mice in which the miR-146b encoding gene has been deleted display better memory skills than wild-type mice³¹ indicating that miR-146b negatively modulates the learning process. Therefore, the deleterious effects on learning in the offspring of maternal and paternal obesity could be underpinned by the negative epigenetic effects of miR-296 and miR-146b on the expression of NMDA receptors, on the one hand, and on cognition on the other hand. It should be noted, however, that in the frontal cortex, the levels of miR-296 are reduced, as are those of glutamate receptors. Consequently, a direct cause-and-effect relationship cannot be established between the changes in miR-296 expression and the downregulation of NMDA receptors in the frontal cortex.

In addition to miRNA-mediated epigenetic modifications, the cognitive shortfall produced by maternal and paternal obesity could be mediated by an impairment of brain tryptophan metabolism through the kynurenine pathway. Actually, the concentration of kynurenic acid was increased in F1 rats from both obese mothers and obese fathers, and quinolinic acid levels above those observed in control animals were also detected in the hippocampus of offspring born to obese dams.

Elevated brain levels of KA or QA have been associated with cognitive impairment in previous studies.^{50–52} Conversely, reducing the brain concentration of KA has been shown to improve memory performance.^{53,54} Several lines of evidence indicate that the negative effects of these metabolites on learning are related to their ability to modulate the glutamatergic system. Indeed, KA blocks the actions of glutamate through its binding at the glycine site of NMDA receptors whereas QA

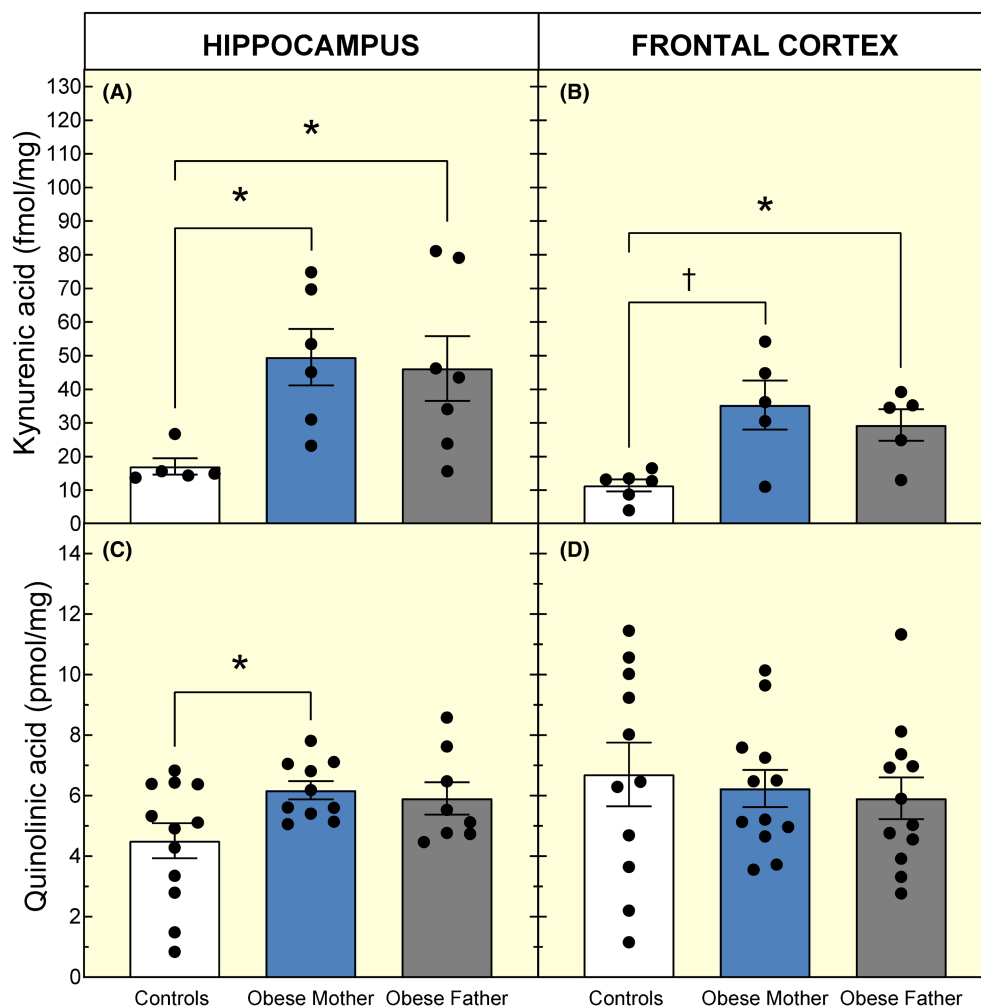


FIGURE 5 Impact of maternal and paternal obesity on tryptophan metabolism through the kynurenine pathway in the hippocampus and frontal cortex of the offspring. Bars represent the mean \pm SEM of the concentration of kynurenic acid (A, B) and quinolinic acid (C, D) in the hippocampus and frontal cortex of adult control rats or born to obese mothers or from the mating of an obese male with a female of normal body weight. The concentrations of KA and QA were determined by liquid chromatography–tandem mass spectrometry. * $p < 0.05$, † $p < 0.01$ as determined by one-way ANOVA.

produces neurotoxic effects by overstimulating this same type of receptors.^{55–57} Moreover, the administration of an NMDA glutamate receptor antagonist prevents the learning deficits induced by the intracerebral infusion of QA.⁵² Similarly, the reduction of the brain concentration of KA via the pharmacological or genetic inactivation of the kynurenine aminotransferase II (KAT II) gene, leads to enhanced glutamate levels and to improved cognitive performance.^{53,58} The causal link between the cognitive deficits and the modifications of the glutamatergic system induced by KA is further reinforced by the results of the literature showing that the intra-cerebral administration of KYN or KA leads to a reduction in the extracellular concentration of glutamate in the hippocampus along with decreased learning performance and that these two actions are blocked by the pharmacological inhibition of KA synthesis.^{59,60} On the basis of these

observations, one can reasonably suggest that the cognitive deficits in the offspring of obese parents described in the present study, result in part from the disturbances of the glutamatergic system following the enhanced production of KA and QA. To fully substantiate this hypothesis, it would be necessary to assess to what extent the memory deficits exhibited by animals of obese parents are improved by the pharmacological blockade of KA synthesis and/or QA production.

In the brain, Tryptophan metabolism via the kynurenine pathway takes place in astrocytes and microglia. In a first step common to both cell types, Tryptophan is transformed to KYN by the enzyme indoleamine 2,3-dioxygenase (IDO). Subsequently, KYN is converted to KA in astrocytes and to 3-hydroxykynurenine (3-HK) in microglia through the action of, respectively, kynurenine aminotransferase (KAT), and

kynurenine-3-monooxygenase (KMO).^{61–63} 3-HK serves as an intermediate for the production of xanthurenic acid, quinolinic acid, nicotinic acid, and ultimately NAD⁺. In a previous study, maternal obesity was shown to upregulate the expression of KMO and, conversely, reduce the levels of the mRNAs coding for KAT in the hippocampus of the offspring and these gene expression changes were associated with anxious behavior.⁶⁴ Unfortunately, in the present study, it was not possible to assess the expression of the genes encoding for these enzymes. Therefore, it remains unclear whether the herein-observed elevated levels of QA and KA reflect an overexpression of KMO and KAT.

Maternal obesity during pregnancy increases the number of astrocytes in the hypothalamus of the offspring both during the fetal period and after birth.²¹ Likewise, enhanced expression of microglia activation markers in the hippocampus along with anxiety behavior and reduced spatial learning have been observed in the offspring born to obese dams.⁶⁵ The increased levels of KA and QA in the brain of the offspring documented in the present study could therefore result from a reactive gliosis process triggered by the exposure during fetal development and neonatal life to inflammatory agents derived from maternal obesity. In addition, considering that rats start consuming food at the age of 18 days,⁶⁶ and that, in our experiments, they were weaned at 21 days, the effects of consuming the FcHFHS diet short before weaning could accumulate with those of maternal obesity to trigger an overactivation of microglia that endured until adulthood.

In summary, both maternal and paternal obesity impair offspring learning ability but to different degrees and by interfering in different ways with the process of memory formation. Specifically, maternal obesity impairs the encoding step of memory as indicated by the inability of rats born to obese mothers to distinguish a novel object from a familiar one during the two learning sessions designed to measure short and long-term memory. In contrast, only the ability to encode new information is affected by paternal obesity.

Despite these differences, the cognitive deficits induced by maternal and paternal obesity are associated with impaired NMDA receptor-mediated synaptic plasticity resulting possibly from the decreased expression of Grin1, Grin2a, and Grin2b coding genes and the increased production in the brain of KA and QA, two by-products of the kynurenine pathway known to have deleterious effects on learning and memory through their interaction with NMDA receptors. The reduced expression of NMDA receptors could be consecutive to the enhanced expression of miR-296 that targets the Grin2a and Grin2b NMDA receptor subunits.

4 | MATERIALS AND METHODS

4.1 | Animal model of maternal and paternal obesity

All experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the principles of laboratory animal care (NIH publication no. 85–23, revised 1985) and were approved by the Ethical Committee for Animal Experimentation of the Pays de la Loire (authorization number 2017012013318453/APAFIS 8215).

A group of founders (F0) female and male animals was first constituted by feeding Wistar rats (Janvier Labs, Le Genest Saint Isle, France) from weaning (21 days old) either with a standard chow diet (control parents) or with a free choice-High Fat/High Sugar diet (fc-HFHS) to promote an obese phenotype (obese parents). The fc-HFHS diet was a modification of the diet used by la Fleur et al.⁶⁷ and was composed of four separated elements: (1) A 30% sugar/water solution prepared with sweetened condensed milk, (2) powdered standard chow mixed with pig fat in the proportion of 30 g of fat per 70 g of food, (3) standard chow, and (4) tap water. Animals were maintained under an inverted 12–/12-h dark–light cycle (lights off at 7:00 AM) and at 21 ± 1°C with food and water ad libitum throughout the experiment. When animals reached the age of 100 days, they were mated to constitute the following first (F1) generation experimental groups: (1) Control Parents, born from the mating of a control male with a control female, (2) Obese Mother, born from the mating of a control male with an obese female, and (3) Obese Father, born from the mating of an obese male with a control female. Female F0 rats continued consuming the same diet (standard chow or fc-HFHS diet) during pregnancy and lactation. In total, 12 control and 6 obese female rats that were mated with an identical number of control and obese male animals were used. All animals, including pregnant rats, were housed 4 per cage until they reached 300 g of body weight and 2 per cage thereafter. Three days before delivery, pregnant rats were housed individually and 1 day after birth, litters were homogenized to eight pups. Only two males from each litter of the F1 generation were kept after weaning and all animals from the three groups were fed standard chow until the end of the experiment.

4.2 | Glucose tolerance test

A glucose tolerance test (GTT) was performed in F0 animals before mating to search for metabolic disturbances.

The test was performed during the dark phase of the 12-/12-h dark-light cycle after 6 h of fasting. In brief. A drop of blood was taken by making a small incision in the tip of the tail immediately before the oral administration of glucose (2 g of glucose/kg of body weight) and 20, 40, 80, and 120 min after. Blood glucose was determined with a blood glucose monitor (Accu-Check® Active, Roche Diagnostics, Vercors, Meylan, France).

4.3 | Novel Object Recognition test

The learning capabilities of F1 animals were assessed by the Novel Object Recognition test (NOR). The test was divided into three sessions: habituation, training, and testing. During the habituation session, rats were allowed to freely explore an empty open-field arena (50 × 50 × 40 cm) for 20 min for three consecutive days. On the fourth day, two identical objects were placed into the arena before introducing the rat, and each animal was allowed to explore the objects for 7 min. It is during this sample phase that memory encoding takes place. The consolidation and retrieval processes of memory were determined in two testing sessions performed 2 and 24 h after the initial training session. During the testing sessions, one of the familiar objects was exchanged for a new one. As during training, animals were allowed to explore the objects for 7 min. The time exploring each object was recorded and the capacity to remember the familiar object was determined from the discrimination index (DI). This was calculated by dividing the difference in exploration time of the novel object versus the familiar one over the total exploration time. A positive DI indicates the existence of recognition memory, whereas a DI equal to or below zero suggests no memory recognition. Memory tests were performed under red light, 2 h after the beginning of the dark phase of the animal's light-dark cycle (09:00 am) in a laboratory adjacent to their housing room. All sessions were recorded using the Viewpoint Videotrack system (Point Grey Research Inc., Richmond, BC, Canada) for offline analysis by two investigators blind to the experimental group of the animals. Object exploration was defined as sniffing or touching the object with the vibrissae or when the animal's head was oriented toward the object with the nose placed at a distance of less than 2 cm from the object.

4.4 | Biological samples collection and processing

The day following the last NOR testing session, F1 animals were sacrificed under deep isoflurane anesthesia

by cervical dislocation 2 h after the beginning of the dark phase of their dark-light cycle. The hippocampus and frontal cerebral cortex were collected and immediately frozen in liquid nitrogen and stored at -70°C until analysis.

4.5 | Real-time quantitative PCR

A conventional Trizol extraction method was used to obtain total RNA. Thereafter, 1 µg of purified RNA was treated with DNase and reverse transcribed using the MultiScribe™ Reverse Transcriptase kit from Thermo Fisher or polyadenylated and reverse-transcribed using the Applied Biosystems™ TaqMan™ Advanced miRNA cDNA Synthesis Kit. The resulting cDNA was used as a template for the amplification of genes and miRNAs involved in the regulation of glutamate signaling and synaptic plasticity using SYBR® Green qPCR. Relative expression levels were calculated using the comparative threshold cycle (Ct) method⁶⁸ and β-microglobulin and GAPDH as housekeeping genes and Let-7d and Let-7g as a reference for miRNA amplification. The sequences of the primers and miRNAs used for amplification are the following. β-microglobulin, forward: GAT GGC TCG CTC GGT GAC, reverse: CGT AGC AGT TGA GGA AGT TGG; GAPDH: forward: CAG TAT GAC TCT ACC CAC GGC A, reverse: ATC TCG CTC CTG GAA GAT GGT G; glutamate ionotropic receptor AMPA type subunit 1 (Gria1), forward: ACA ACT CAA GCG TCC AGA ATA GG, reverse: GCC CTC ATA GCG GTC ATT GC; glutamate ionotropic receptor AMPA type subunit 2 (Gria2), forward: GAG AAA GAA TAC CCT GGA GCA CAC, reverse: TCA TCA CTT GGA CAG CAT CAT ACG; glutamate ionotropic receptor NMDA type subunit 1 (Grin1), forward: GGT GGC AGA TGG CAA GTT TGG, reverse: CGC TCA TTG TTG ATG GTC AGT GG; glutamate ionotropic receptor NMDA type subunit 2A (Grin2A), forward: TTG GTG ATG GTG AGA TGG AGG AG, reverse: AGA TGA AGG TGA TGA GGC TGA GG; glutamate ionotropic receptor NMDA type subunit 2B (Grin2B), forward: 5' TTC CAT CGT CAC CAC CTA CTT CC, reverse: CTT AGA GTC GCC ATC GTC CAG AG; activity-regulated cytoskeleton-associated protein (Arc), forward: CCG AAG TGT CCA AGC AGG TG, reverse: CAT AGC CGT CCA AGT TGT TCT CC; early growth response 1 (Egr1), forward: GGG TAG TTT GGC TGG GAT AAC, reverse: TAC GAG CAC CTG ACC ACA GA; discs large MAGUK scaffold protein 4 (Dlg4), forward: GAC AGT GAG ACC GAC GAC ATT G, reverse: GAT GAT GAT GGG ACG AGC ATA GTG; rno-miR-296-5p: AGG GCC CCC CCT CAA TCC TGT; rno-miR-146b-5p: TGA GAA CTG AAT TCC ATA GGC

TGT; rno-let-7d-5p: AGA GGT AGT AGG TTG CAT AGT T; rno-let-7g-5p: TGA GGT AGT AGT TTG TAC AGT T.

4.6 | Quantification of kynurenic acid and quinolinic acid in the brain

Kynurenic acid (KA) and quinolinic acid (QA), two metabolites generated by the catabolism of tryptophan through the kynurenine pathway, were quantified in the hippocampus and frontal cortex by ultra-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) using the methodology described in detail by Honório de Melo Martimiano et al.⁶⁹

4.7 | Statistical analysis

Experimental results were analyzed using the software GraphPad Prism version 9. Data were first checked for normality by the Shapiro–Wilk normality test and the statistical differences were assessed by Student's *t*-test or one-way ANOVA followed by Dunnett's multiple comparisons test. Statistical significance was set at $p < 0.05$. Data are expressed as means \pm SEM of the data.

AUTHOR CONTRIBUTIONS

Francisco Bolaños-Jiménez: Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; supervision. **Carla Elena Mezo-González:** Investigation; writing – original draft; writing – review and editing. **Juan Antonio García Santillán:** Investigation; writing – review and editing. **Bertrad Kaeffer:** Investigation; writing – review and editing; methodology. **Mathilde Gourdel:** Investigation; writing – review and editing. **Mikaël Croyal:** Investigation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study, experimental materials, and analytic methods are available from the corresponding author upon reasonable request.

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