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Differential Effect of Caffeine Consumption on Diverse Brain Areas of Pregnant Rats

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Background: It has previously been shown that during gestation, the mother's brain has an increase in glial fibrillary acidic protein (GFAP)-immunoreactivity (-ir) and a decrease in the mRNA level of A1 adenosine receptor. Little is known about the A2A adenosine receptor in the maternal brain, and whether caffeine consumption throughout gestational period modifies GFAP and adenosine receptor density in specific brain areas. This study was undertaken to investigate the protein density of GFAP and adenosine receptors (A1 and A2A subtypes) in different regions of pregnant rat brain and the possible effect of caffeine on these proteins.
Methods: For this purpose, we examined the GFAP-, A1- and A2A-ir in the cingulate cortex (Cg2), dentate gyrus (DG), medial preoptic area (mPOA), secondary somatosensory cortex (S2), and striatum (Str) of pregnant Wistar rats (drug-free tap water or water with 1g/L diluted caffeine).
Results: We show a consistent and highly significant reduction of GFAP-ir in caffeine-treated pregnant rats in most of the areas analyzed. Our data demonstrate that caffeine consumption induces a significant increase of A2A-ir in Str. Concerning A1 receptor, the observed changes are dependent on the region analyzed; this receptor density is increased in Cg2, DG, and mPOA and decreased in the somatosensory cortex and Str. The results were confirmed by Western blotting.
Conclusions: Our results suggest that chronic caffeine exposure could modify the physiolological situation of gestation by a reorganization of the neural circuits and the adenosine neuromodulator system.

Introduction

Caffeine is the most widely consumed psychoactive substance in the world, with nearly 70% of pregnant women consuming products containing caffeine (including beverages such as coffee, tea and soft drinks, chocolate, and some medications; the average intake is 125 mg/day).[1,2] The multiple effects of caffeine are mainly due to antagonism of endogenous adenosine that activates four specific receptors (A_1, A_{2A}, A_{2B}, and A_3). A_1 and A_{2A} adenosine receptors are the most predominantly expressed in the brain, and they are the preferential targets for caffeine. A_1 receptors are widely expressed in the brain, whereas A_{2A} receptors are mainly concentrated in the striatum (Str).[3–5] Although several clinical investigations have shown that high caffeine intake during pregnancy represents a danger to offspring and may be associated with a higher risk of growth retardation and sudden death,[6,7] little evidence of effects of this substance on the adenosine neuromodulator system of the maternal brain has been produced to date.

Pregnancy is a physiological state characterized by behavioral changes, hormonal level variations, and modifications in the number and sensitivity of receptors.[8–10] One of the most notable changes in the maternal brain, responsible for behavioral and physiological alterations during pregnancy, is the modification of neurotransmitter and receptor expression and the increase in astrocyte numbers.[11] It has been shown that adrenergic receptors are increased in the rat hippocampus,[12] and benzodiazepine and progesterone receptors are increased in the hippocampus and the medial preoptic nucleus, respectively, during pregnancy.[13,14] Changes in monoamine levels in the prefrontal area, hippocampus, and medial preoptic area (mPOA) have been reported in pregnant rats.[15] We previously described how metabotropic glutamate receptors (mGluR) are increased and adenosine A_1 receptors

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decreased in the whole rat brain at the end of pregnancy, associated with increased endogenous adenosine levels.[16,17] Nevertheless, there is no detailed study of protein immunoreactivity differentiating anatomical regions.

Astrocytes play a critical role in neuromodulation and neuroprotection in the central nervous system,[18] and quantitative changes in the expression of glial fibrillary acidic protein (GFAP), an exclusive protein of astrocytes,[19] have been observed in late pregnancy.[20] Furthermore, the passage from quiescent to reactive astrocytes, producing upregulation of GFAP, has been associated with neurodegenerative conditions and is used as a marker of neuronal damage.[21,22] However, the astrocytic response to caffeine during pregnancy remains unknown.

Therefore, the aim of the present study was to analyze the effect of caffeine during pregnancy on the distribution and density of A_1 and A_{2A} receptors and GFAP protein in various brain areas. For this purpose, a previously established animal model of caffeine consumption in rat was used, and the protein expression in five regions was examined: cingulate cortex (Cg2), dentate gyrus (DG), mPOA, secondary somatosensory cortex (S2), and Str.

Materials and Methods

Animals

Eighteen adult Wistar rats (3-months old) were used (12 for immunohistochemistry [IHC] assay and 6 for Western blotting assay). All animals were housed individually in a temperature-controlled room (23°C). The animals were subjected to a 12-hour light/dark cycle (08:00–20:00 lights on) and given free access to food and tap water. Following a previous protocol of caffeine administration by our group, nine pregnant rats were treated with caffeine (1 g/L) in the drinking water from gestational day 2 and during the entire gestational period. Nine control pregnant rats received drug-free tap water. As we previously reported,[23] daily water intake and rat weight during the gestational period were of the same order in the two animal groups (pregnant rats not treated and treated with caffeine).

To perform the IHC assay, the last day of gestation, embryonic day 21, six animals per experimental group were intracardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB; 0.08 M K_2HPO_4, 0.02 M NaH_2PO_4; pH 7.4), and the brains were removed. All the brains were immersed in a graded sucrose solution after fixation and stored in a cryprotectant solution at −20°C. Serial sections (50-μm thick) of the cortical tissue were obtained using a vibratome, and the sections were processed for immunohistochemical staining. Sections (at least six per animal and region) were selected and matched for anatomical location. Alphabetically, the areas chosen for study were the cingulate cortex, the polymorph layer of the dentate gyrus, medial preoptic area, secondary somatosensory cortex, and striatum (Cg2, DG, mPOA, S2, and Str according to Paxinos and Watson[24]).

For Western blot assay, six pregnant rats (three of them receiving drug-free tap water and the other three with caffeine) were sacrificed, and the brains were removed, frozen in N_2 liquid, and stored at −80°C.

All experiments were performed in accordance with the guidelines established by the European Union regarding the use and care of laboratory animals, the Spanish law on animal experiments, and the Animal Research Ethics Committee of the Castilla-La Mancha University.

Immunohistochemistry

Free-floating selected sections were pretreated for 30 minutes with a solution of 0.5% hydrogen peroxide and 50% ethanol in PB to remove the possible endogenous peroxidase activity. Subsequently, sections were rinsed in PB and preincubated for 1 hour at room temperature in a stock solution containing 3% normal serum (normal horse serum for slices used with those primary antibodies made in mouse and normal goat serum for rabbit anti-A_1; Vector Laboratories) in PB with 0.25% Triton X-100. Thereafter, the sections were incubated for 48 hours at 4°C in the same stock solution containing mouse antineuron-specific nuclear protein (neuronal nuclei [NeuN]), mouse anti-GFAP, rabbit anti-A_1, and mouse anti-A_{2A} (1:2000; Chemicon, 1:400; Sigma-Aldrich, 1:250; Calbiochem, and 1:1000; Upstate, respectively; dilutions used and immunostaining procedure were the same as described in detail elsewhere[25,26] or recommended for IHC application included on the datasheet of the company). The sections were then washed in PB, incubated in secondary biotinylated antibodies (1:200; Vector), and processed using the horse anti-mouse or goat anti-rabbit avidin-biotin-peroxidase method of the Vectastain ABC immunoperoxidase kit (Vector). Staining was visualized histochemically with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and 0.01% hydrogen peroxide, and the sections were rinsed in PB, mounted onto glass slides, dehydrated, cleared with xylene, and cover-slipped. Adjacent sections stained with thionine (staining of Nissl bodies) or immunostained with the neuronal marker NeuN were used to reveal the borders between different areas and quantified Nissl- or NeuN-stained cells. To demonstrate specificity, separate sections were treated following the IHC protocol, but omitting primary antibody incubation (see Supplementary Fig. S1 for further details; Supplementary Data are available online at www.liebertpub.com/caf).

Microscopy and quantitative analysis

For quantitative analysis of IHC, sections from each animal were analyzed in two parameters: the intensity of staining and the area occupied by the staining. The experimenter was unaware of the experimental group. Photomicrographs (6–10 fields per animal and region of analysis) for figures and quantification were captured using a digital camera (Olympus DP70) set up on an Olympus light microscope (Olympus BX51). First, the light and contrast settings were adjusted to normalize the distribution of gray levels in the digitized image using Adobe Photoshop software (version 11.0; Adobe Systems). Brightness levels of each image histogram were adjusted pushing the black-and-white-point sliders of the level tool to the edges of the histogram. Once these settings were adjusted, they remained constant for all measurements of a given section. Intensity measurements were taken using National Institutes of Health (NIH) ImageJ software version 1.44p.[27] As previously described by other groups,[28,29] we also analyzed the surface area occupied by the staining in the same regions and fields used in the analysis of intensity. Briefly, we estimated the surface area occupied by stained microstructures per μm^2 of the tissue. For this purpose, images were analyzed using same software as in the intensity parameter. The surface area occupied by staining was expressed as a percentage of the reference area (image area).

The total number of cells (including pyramidal neurons, nonpyramidal neurons, and glia) was averaged in Nissl-stained sections, and the total number of neurons (including pyramidal and nonpyramidal neurons) was estimated by positive NeuN immunostaining. Concisely, adjacent sections were used for these two stainings. Photomicrographs (six fields per animal and region of analysis) were captured using a digital camera (Olympus DP70) set up on an Olympus light microscope (Olympus BX51). An unbiased counting frame with an area of 2044.7 μm^2 was used. The counting frame was systematically and randomly moved through the region of interest until a total of six fields were acquired. Cells were manually counted using NIH ImageJ software version 1.44p (Plugin: cell counter).

Plasma membrane preparation

The frontal cortex, Str, and hippocampus were dissected according to Chiu's procedure.[30] Plasma membranes from these regions were purified through sucrose gradients as described previously.[31] Briefly, previously dissected tissue was homogenized in 20 volumes of isolation buffer (10 mM Tris–HCl, pH 7.4, 0.1 g/L bacitracin, and 100 μM phenyl methyl sulfonyl fluoride) containing 8.5% sucrose using a Potter-Elvehjem homogenizer. The crude homogenate was centrifuged for 2 minutes at 1500 *g* in a Beckman J2-21 centrifuge. The supernatant was spun for 10 minutes at 20,000 *g*, and the pellet (P2 fraction) was resuspended in an isolation buffer. The suspension obtained was gently homogenized in a Dounce homogenizer (10× A, 10× B) and stored at 0°C for 2 hours to permit lyzing. The homogenate was layered over a discontinuous sucrose gradient (40%/28%) in the isolation buffer and spun for 45 minutes at 65,000 *g* in a Beckman L7-55 centrifuge. The interface was collected as the plasma membrane fraction and spun for 60 minutes at 100,000 *g*. The pellet was finally resuspended in the isolation buffer.

Western blotting assays

For Western blot assays, 5 μg of purified plasma membranes was mixed with the Laemmli buffer containing 0.125 M Tris (pH 6.8), 20% glycerol, 10% β-mercaptoethanol, 4% sodium dodecyl sulfate (SDS), and 0.002% bromophenol blue; the mixture was then heated at 95°C for 5 minutes (each gel always included samples from different groups for direct comparison). The protein was electrophoresed on a 10% SDS-PAGE gel using a miniprotean system (Bio-Rad) with prestained molecular weight markers (Bio-Rad). The protein was transferred to nitrocellulose membranes in the iBlot™ Dry Blotting System (Invitrogen). Membranes were blocked with phosphate-buffered saline (PBS; 14 mM NaCl, 2 mM KCl, 12 mM Na_2HPO_4, 1.7 mM KH_2PO_4; pH 7.4) containing 0.1% Tween 20 (PSB-T) and 5% skimmed milk for 1 hour at room temperature and then incubated with the same primary antibodies as for IHC at 4°C overnight (1:1000 dilution for anti-A_1, anti-A_{2A}, and GFAP; 1:5000 dilution for anti-β-actin; Abcam). After three washing periods for 10 minutes with PBS-T, the membranes were incubated with the horseradish peroxidase-conjugated anti-rabbit or anti-mouse (Bio-Rad) at a dilution of 1:5000 in PBS-T containing 5% skimmed milk for 30 minutes. The antigen was visualized using the Lumi-Light Western Blotting Substrate (Roche). Luminescent signal was recorded and quantified with the Syngene G:Box (Syngene) monitored with Gene Snap (Syngene) Software (version 7.08). Specific bands were quantified with densitometry using Gene Tools Software (version 4.01; Syngene). Membranes were stripped for a second probe by incubating them three times with a medium stripping buffer (0.15% glycine, 0.1% SDS, 1% Tween 20, pH 2.2) for 5 minutes at room temperature. After removing the stripping buffer and washing them twice with PBS, membranes were ready for the blocking stage.

Protein determination

Protein concentration was measured with Lowry's method,[32] using bovine serum albumin as standard.

Statistical analysis

Data are mean ± standard error of the mean. SPSS version 15.0 for Windows (SPSS, Inc.) was used for two-way analysis of variance with Tukey's *post hoc* test. Differences between mean values were considered statistically significant at $p < 0.05$.

Results

IHC assay

Adenosine A_1 and A_{2A} receptors. When we analyzed A_1 receptor density, the caffeine-treated experimental group (pregnant caffeine [PC]) exhibited a significant increase in optical density for Cg2, DG, and mPOA regions (61%, 16%, and 15%, respectively), whereas for Str and S2 areas, there was a significant decrease in the labeling intensity (5% and 18%, respectively) when compared with control rats taking drug-free tap water (pregnant water [PW]). We tried to elucidate whether these observed changes in the density of labeling were parallel to the variation in the amount of stained microstructures. In this case, every comparison between different areas of the PW and PC groups showed a similar profile (Fig. 1A, C; see Supplementary Table S1 and Supplementary Fig. S2 for representative photomicrographs).

A detailed examination of the A_{2A}R-immunoreactivity (A_{2A}R-ir) in the Str revealed that there was a notable increase in the immunostaining density as an effect of caffeine treatment in pregnant rats (Fig. 1E). With respect to the surface area occupied by this staining, there was a significant increase in the size of labeled regions modified by the caffeine treatment (see Fig. 1G and Supplementary Fig. S2 for representative photomicrographs). We were unable to detect A_{2A}-ir signal in the other regions analyzed in this study.

GFAP immunostaining. When we analyzed the effect of caffeine during gestation in the labeling intensity of GFAP protein, there was observed an increase in the S2 cortical area (16%), and a significant decrease in the Cg2 and DG regions (11% and 15%, respectively). However, no significant changes were detected in the Str and mPOA regions between treated and untreated pregnant rats (Fig. 1B).

When we analyzed the surface area occupied by GFAP-ir, we observed a significant increase in the occupied surface of GFAP-ir in mPOA, S2, and Str. Cg2 and DG did not present this parameter modified in PC animals (see Fig. 1D and Supplementary Fig. S3 for representative photomicrographs).

Western blotting assay

We wanted to confirm the IHC results with Western blotting assays of the plasma membrane. We found that the

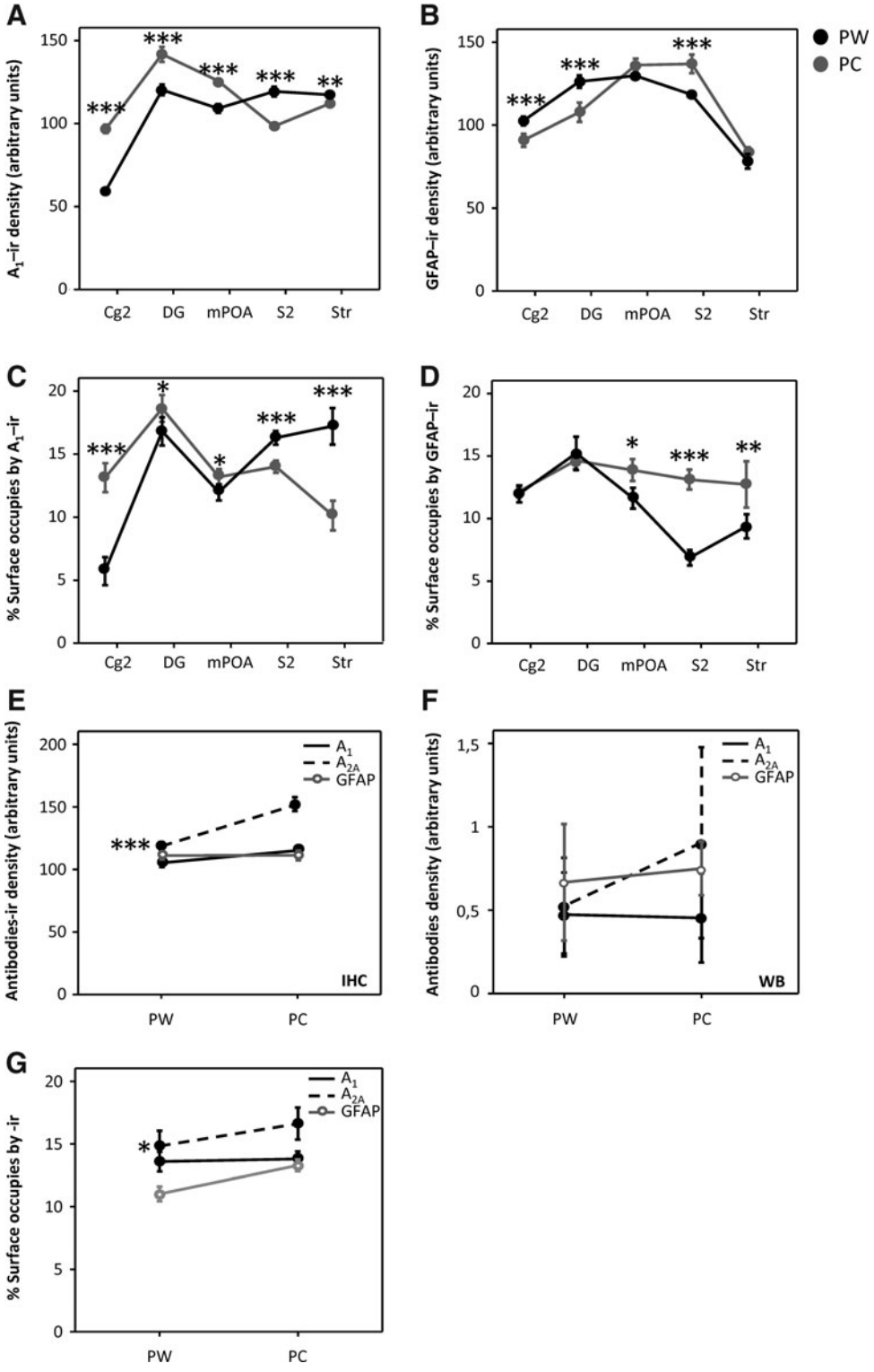

FIG. 1. Graphs representing mean ± SEM of A_1-**(A, C)**, A_{2A}-**(E–G)**, and GFAP-staining **(B, D)** by IHC. Optical density in arbitrary measurement **(A, B, E)** and percentage of the occupied surface area by staining **(C, D, G)** in every analyzed region and the combined totals **(E–G). (A)** An increase in the A_1 receptor density in Cg2, DG, and mPOA and a decrease in S2 and Str, as effects of caffeine treatment during gestation. These changes are parallel to changes in the surface occupied by immunoreactivity **(C)**. In Str, the A_{2A} receptor density **(E)** and its percentage of occupied surface area **(G)** are increased with caffeine treatment. GFAP-ir is decreased in Cg2 and DG and increased in S2 **(B)**, in PC animals, with an increase in the percentage of the occupied surface area by immunoreactivity in mPOA, S2, and Str **(D)**. Combining the results in totals by technical approaches, IHC and Western blot (WB), we observed the same total effect of caffeine; $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ significantly different from PW rats. GFAP, glial fibrillary acidic protein; Cg2, cingulate cortex; S2, secondary somatosensory cortex; DG, dentate gyrus; mPOA, medial preoptic area; PC, pregnant caffeine; PW, pregnant water; ir, immunoreactivity; SEM, standard error of the mean; WB, Western blot; IHC, immunohistochemistry.

density of the three analyzed proteins was different in the analyzed groups depending on the area studied (Fig. 2). The linearity of the Western blotting method was checked using Na^+/K^+-ATPase as a specific plasma membrane marker (see Supplementary Fig. S4 for further details).

When we measured adenosine A_1 receptors in the plasma membranes obtained from the frontal cortex, a significant increase (124%) in the A_1 receptor density was observed in rats that received caffeine during gestation as compared to control rats. On the other hand, caffeine consumption induced a significant decrease (63%) of these receptors in the hippocampus. Nevertheless, a nonsignificant decrease of these receptors was reported when the A_1 receptor density was measured in Str.

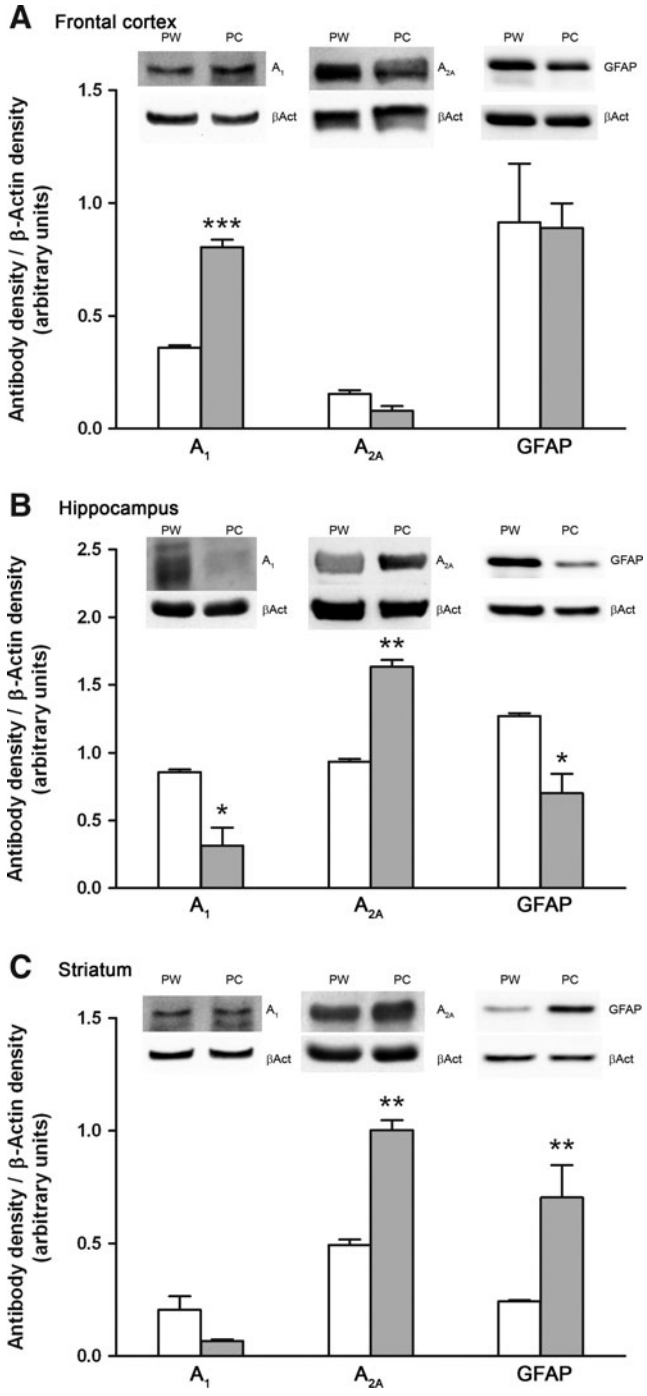

FIG. 2. WB of A_1, A_{2A}, and GFAP performed in plasma membranes isolated from the frontal cortex **(A)**, hippocampus **(B)** and Str **(C)** of nontreated pregnant rats, PW, or treated with caffeine, PC, *Upper panel* shows a representative WB assay, showing the bands corresponding to A_1 37 kDa, A_{2A} 45 kDa, and GFAP 50 kDa. *β*-actin 42 kDa, which was used as loading control, is shown below. *Lower panel* shows the histogram obtained from densitometric analysis of bands. In summary, caffeine treatment during gestation produces a significant increase in the A_1 receptor density in the frontal cortex and the A_{2A} receptor density in the hippocampus and Str, and a significant decrease in the A_1 receptor and GFAP densities in hippocampus. The results are mean ± SEM using arbitrary units, of three animals in each group. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ significantly different from PW rats. Str, striatum.

Changes opposite to those reported in the density of A_1 receptors were found in the density of the adenosine A_{2A} receptors. In frontal cortex plasma membranes, a decreasing, but not significant, tendency (49%) was observed when PC membranes were compared to PW membranes. On the other hand, when A_{2A} receptor densities were studied in the hippocampus and Str, caffeine consumption during pregnancy induced an increase in the receptor density as compared to PW rats (74% for hippocampus and 103% for Str), which was statistically significant in both cases (Fig. 2).

Finally, when GFAP was studied in these brain areas, we observed that caffeine consumption during pregnancy induced different changes in this protein depending on the area analyzed. In the frontal cortex, caffeine consumption did not induce any quantitative changes in the density of GFAP. However, in rats treated with caffeine during pregnancy, the density of GFAP was altered in an opposite way in the hippocampus and Str: increased (190%) in Str and decreased (45%) in hippocampus.

When the optical density data obtained by IHC and Western blot were put together in an overall quantity per experimental group, the comparison between methods demonstrated the same profile (Fig. 1E, F).

Number of cells/neurons

Counting of Nissl-stained cells demonstrated a significant loss of cellular bodies in Cg2, DG, and mPOA (44%, 50%, and 45%, respectively) in animals that consumed caffeine during gestation as compared to those that drank caffeine-free water. In brain sections stained with an anti-NeuN (neuronal marker) with IHC assay, we observed a slight, nonsignificant loss of neuronal bodies in Cg2 and Str (Fig. 3 and Supplementary Table S1).

Discussion

Treatment with caffeine during pregnancy produces dissimilar changes in adenosine receptor and GFAP levels depending on the brain region analyzed. Antagonism of adenosine receptors induces loss of cells in Cg2, DG, and mPOA, associated with a decrease in glial cells and astroglial immunoreactivity in Cg2 and DG. Together, these data provide evidence that chronic caffeine exposure produces an area-specific reorganization of the adenosine neuromodulator system in the maternal brain, which could be involved in the restructuring of cellular circuits.

Caffeine during pregnancy produces dissimilar changes in the adenosynergic system depending on the brain region analyzed

Concerning adenosine receptors during gestation, previous work in the whole brain by our group established a downregulation of A_1 receptors in the same animal model of caffeine treatment.[16] In addition, the present study reveals that this downregulation is area specific. A decrease in A_1 receptor is observed in the somatosensory cortex and Str, while in Cg2, DG, and mPOA, there is an increase in these receptors. Additionally, the protein density of A_{2A} receptor, mainly concentrated in the Str, is increased by caffeine treatment at the end of pregnancy in this region.

Results of IHC are also consistent with Western blotting analysis, in which the same profile of changes is confirmed, except for the optical density of A_1 receptor in the hippocampus.

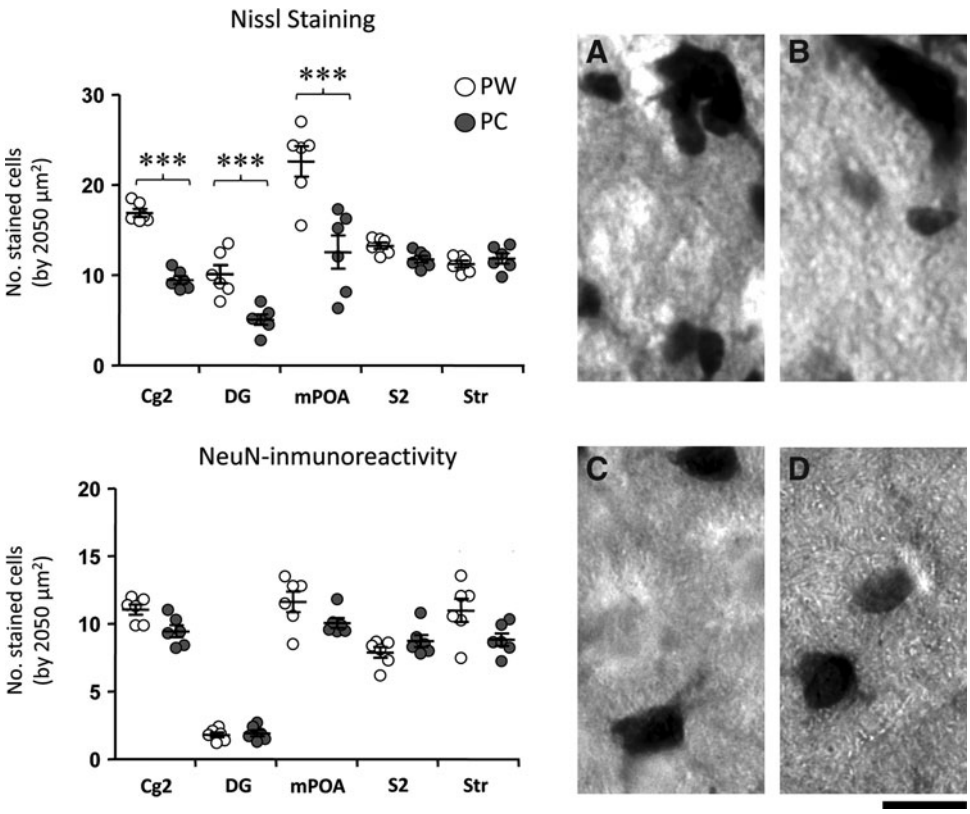

FIG. 3. Graphs representing mean ± SEM of the number of Nissl-stained or NeuN-immunopositive cells in the five analyzed regions. Representative photomicrographs of Nissl-stained **(A, B)** or NeuN-immunopositive **(C, D)** cells of the polymorph layer of the DG in control situation **(A, C)** and caffeine-treated **(B, D)** rats. In summary, there is a significant loss of cellular bodies in Cg2, DG, and mPOA. ***$p < 0.001$ significantly different from PW rats. NeuN, neuronal nuclei.

With respect to this apparent discrepancy in the results, several studies have demonstrated opposing changes in hippocampal Ammon's horn subfields versus DG in diverse situations; i.e., estrogen receptor expression is significantly increased in the CA1 and CA3 regions in lactating rats after treatment with kainic acid, but not in the DG.[33] Similarly, there is an increase in the number of granular cells in DG, but not in other hippocampal subfields after maternal separation.[34] Consequently, the significant increase observed in A_1-ir of DG after caffeine treatment would be masked in the overall decrease when it is analyzed as a part of the whole hippocampal formation using Western blot.

A number of behavioral, physiological, and neural changes occur in mammal females during pregnancy.[35] Maternal behavior, such as nest building, and the accompanying hormonal alterations affect various measures of plasticity in brain areas that are necessary for the full expression of maternal behaviors. The maternal experience is associated with, among other signs of neural plasticity, suppression of cell proliferation in DG,[36] plasticity in the spine density, and cell architecture in the hippocampus[37,38] and medial prefrontal cortex, or glial plasticity in Cg2 and mPOA.[20,39] For example, there is evidence from early studies that an intact Cg2 is critical for the rapid onset of maternal behavior in rats,[40,41] and this region is involved in recognition of the offspring and the onset of maternal behavior.[42] The increase demonstrated here in A_1-ir receptor in DG, Cg2, and mPOA reveals a new putative indicator of plasticity in regions directly involved in the maternal behavior.

To sample another brain area clearly involved in gestation, we analyzed S2. In this neocortical region, changes in the A_1 adenosine receptor density, as a result of caffeine treatment, are in contrast to those observed in the other cortical region analyzed, Cg2. This might explain a specific effect of caffeine in an adult brain and a dependence on regional function.

Changes in the optical density of immunostaining and the absence of modifications in the surface area occupied may imply that the same microstructures are expressing different quantities of protein, which suggests that the distribution of protein expression in brain areas remains constant, unlike the amount. Modification of the stained surface in the same way as the intensity parameter denotes a change in the optical density of staining that could be due to an increase/decrease in the amount of labeled microstructures. Further studies using electron microscopy are necessary to verify this hypothesis.

Changes in glial processes are opposite to those in A_1 receptor protein expression

It is well known that changes in ovarian steroids (estrogen and progesterone) and lactogenic (prolactin) hormone levels occur during gestation.[43,44] These sex hormone modifications have been related to quantitative changes in the expression of GFAP in three of the five analyzed regions (Cg2, DG, and mPOA[42,45]). For example, Salmaso *et al.* linked the fluctuations in GFAP protein in Cg2 of pregnant rats with recognition of the offspring and the onset of maternal behavior.[39]

The change of the GFAP protein pattern is linked to modifications in the number of synaptic inputs to neurons. This growth of astrocytic processes, identified by a GFAP-ir increase, produces a covering of neuronal membranes,[46,47] regulating the functional activity of neighboring neurons and affecting synaptic transmission.[48–53] Therefore, the decrease observed here in GFAP-ir in two regions from the maternal brain could be

related to a decrease in the astrocytic processes that might affect the formation and maintenance of synaptic contacts.

Curiously, regions where a decrease in GFAP-ir are demonstrated in this article presents a parallel increase of A_1 receptor-ir. These data suggest a kind of inverse relationship between the two proteins. The polymerization and depolymerization of GFAP are modulated by cytosolic Ca^{2+}, and the agonist stimulation of group II metabotropic glutamate receptors (mGluR II) produces a phosphorylation of GFAP that is inhibited by caffeine (nonspecific agonist of ryanodine receptors and nonselective antagonist of adenosine receptors).[54] Previous work by our group demonstrated that chronic caffeine exposure during the gestational period can affect transduction pathways other than adenosine receptors, such as the transduction pathway of mGluR/PLC.[55] Thus, the changes in GFAP-ir after caffeine treatment during gestation might be explained as a direct effect on RyRs, for high caffeine doses, and/or a relationship with mGluR II via crosstalk with the adenosynergic system.

Moreover, various studies have demonstrated a relationship between drug exposure and cytogenesis in several regions of adult rat brain.[56,57] In particular, exposure to moderate-to-high doses of caffeine has been shown to alter adult neurogenesis in DG, by depressing proliferation and reducing the number of activated microglia.[58,59] Here, we have demonstrated, with the comparison of Nissl and NeuN quantification, a decrease in the number of glial cells in animals with caffeine consumption, in just three areas, in two of which, Cg2 and DG, we observed an increase in A_1 receptor-ir and a decrease in GFAP-ir. These data suggest that results in GFAP-ir are due to a decrease in the number of cells.

Caffeine effect during gestational period: neurotoxicity or neuroprotection? That is the question

It has been suggested that chronic (but not acute) caffeine treatment attenuates brain injury, by adenosine receptor-mediated suppression of glutamate release, mediated by the A_{2A} receptor in Parkinson's and Alzheimer's diseases,[60–63] by A_1 receptors in ischemic and immunological brain injury models,[64,65] and through a downregulation of glial activation.[66] Antagonists of adenosine receptors, particularly A_{2A} receptor antagonists, have the ability to restore memory dysfunction associated with aging and neurodegenerative diseases.[67–69] However, caffeine, when given acutely, potentiates the increase of GFAP in the Str and enhances the acute toxicity and lethalness of 3,4-methylenedioxymethamphetamine (MDMA).[70,71] Moreover, it has been demonstrated that other psychostimulant drugs affect the expression of GFAP and induce strong changes in the astrocyte proliferation rate (cocaine and MDMA exposure),[71–73] generating many reactive species enabling neurodegeneration.[73,74]

Besides the double effect of caffeine on glial cells, up- or downregulation, it is postulated that an upregulation of adenosine receptors takes place after chronic caffeine exposure.[64,75–78] Nevertheless, the effect of caffeine on these receptors depends on the dose, administration method, and frequency of treatment. With our results of chronic caffeine treatment during gestation, we add a new role for this psychoactive substance in the adenosine receptors in a nonpathological situation. We may conclude that the regular microanatomical changes produced by pregnancy in the maternal brain (which partially explain maternal behavior and the neuroprotection of the mother's brain in a stressful situation) are modified by caffeine consumption. Taken together, our findings provide data reflecting the vulnerability of an adult brain. There are studies describing effects on fetuses' or neonates' brain whose mothers have consumed caffeine during gestation or lactation. However, the effects on mothers' brain seem to be forgotten. Metabolism of caffeine is slow during pregnancy, mainly during the third trimester of gestation, increasing caffeine concentration in mother's blood. This maintained increase can affect some transduction pathways and areas in the brain that are necessary to sustain the status of gestation, and in turn can affect not only fetuses and neonates but also mother's health. Perhaps, the same caution that applies to cigarette smoking during pregnancy could be taken in consideration to caffeine. Nevertheless, epidemiological studies are needed to evaluate the effects of caffeine consumption on the brain of pregnant women.

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Author Disclosure Statement

No competing financial interest exists for any of the authors.

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