

Article

Acetic Acid Activates Intracellular Calcium Responses in Astrocytes from the Rat Olfactory Bulb

Francisco Jonathan Pérez-Delgado ¹, Olimpia Ortega-Fimbres ¹, Miguel Angel Valencia-Núñez ¹,
Diana Monge-Sánchez ¹, Miriam Denisse García-Villa ¹, Angeles Edith Espino-Saldaña ², Daniel Reyes-Haro ²,
J. Abraham Domínguez-Avila ³, Gustavo A. González-Aguilar ⁴, Marco Antonio López-Torres ⁵,
Enrique De La Re-Vega ⁵ and Marcelino Montiel-Herrera ^{1,*}

- ¹ Department of Medicine and Health Sciences, University of Sonora, Building 7D, Boulevard Luis Donald Colosio and Reforma, Hermosillo 83000, Sonora, Mexico; jonathanpzd441@gmail.com (F.J.P.-D.); olimpiamof.3110@gmail.com (O.O.-F.); mavalnu21@gmail.com (M.A.V.-N.); dianamonge8@gmail.com (D.M.-S.); miriam.garcia@unison.mx (M.D.G.-V.)
- ² Institute of Neurobiology, National Autonomous University of Mexico, Juriquilla 76230, Querétaro, Mexico; aedith.espino@gmail.com (A.E.E.-S.); dharo@unam.mx (D.R.-H.)
- ³ SECIHTI-Research Center for Food and Development A. C., Carretera Gustavo Enrique Astiazaran Rosas No. 46, La Victoria, Building E, Hermosillo 83304, Sonora, Mexico; abrahamdominguez9@gmail.com
- ⁴ Research Center for Food and Development A. C., Carretera Gustavo Enrique Astiazaran Rosas No. 46, La Victoria, Building E, Hermosillo 83304, Sonora, Mexico; gustavo@ciad.mx
- ⁵ Department of Scientific and Technological Research, University of Sonora, Building 7G, Hermosillo 83000, Sonora, Mexico; marco.lopez@unison.mx (M.A.L.-T.); enrique.delare@unison.mx (E.D.L.R.-V.)
- * Correspondence: marcelino.montiel@unison.mx

Abstract

Background: Short-chain fatty acids (SCFAs) are metabolites produced by the gut microbiota after fiber fermentation. Some SCFAs, such as acetate, propionate, and butyrate, have been recognized as essential for human health, especially for the brain; however, the cellular mechanisms activated by these molecules in food-intake-related organs, such as the olfactory bulb, remain unclear. **Objective:** This study evaluates the effects of acetic acid (AA) and sodium butyrate on the physiology of Ca^{2+} metabolism in olfactory bulb cells (OBCs). **Methods:** Primary OBC cultures of the postnatal rat (P7-21) were made, and Ca^{2+} imaging experiments were performed to record the intracellular Ca^{2+} responses (iCaR) elicited by the application of AA and sodium butyrate (100 nM–1 mM). Immunocytochemical analyses were performed to identify GFAP⁺ cells and GPR41 and GPR43 receptors in OBCs. Endpoint RT-PCR analyses were made to identify GPR41 and GPR43 transcripts in OBCs. **Results:** Fewer than 10% of the OBCs tested responded to the application of AA and sodium butyrate with iCaR. Pharmacological studies (20 μM 2-aminoethyl diphenylborinate (2-APB); 10 nM GLPG0974, 120 nM AR420626) showed that iCaR were independent of inositol triphosphate (IP_3)-signaling pathways and that OBCs expressed both GPR41 and GPR43 receptors. Endpoint RT-PCR studies performed in both olfactory bulbs and primary OBC cultures confirmed the expression of the GPR41 receptor. **Conclusions:** This study shows that AA and butyrate induce intracellular Ca^{2+} responses activated by GPR41 and GPR43 receptors in a discrete cellular population of the rat olfactory bulb.

Keywords: olfactory bulb cells; short chain fatty acids; intracellular Ca^{2+} responses; GPR41; GPR43



Academic Editor: Parisa Gazerani

Received: 28 May 2026

Revised: 1 July 2026

Accepted: 4 July 2026

Published: 7 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

Fatty acids constitute a wide variety of molecules that can regulate metabolism and intracellular signaling in several cellular populations [1,2]. They can be generally classified as long-, medium-, and short-chain fatty acids (SCFAs) [3]. Acetate, propionate, and butyrate are the main SCFAs produced by the fermentation of dietary fiber by the gut microbiota [4,5] and absorbed by enterocytes to use as a primary energy source [6]. Nonetheless, a portion of the absorbed SCFAs reaches the bloodstream and arrives at other tissues of the body, where they play an important role in mammal physiology [7]. For instance, they participate in central nervous system development during embryonic gestation [8,9] and in pathological conditions related to stroke and its recovery processes [10], making SCFAs important mediators of gut-brain communication throughout development.

The biological effects of SCFAs rely on the expression of free fatty acid receptors and the intracellular signaling cascades initiated by their activation. In the nervous system, it has been shown that sympathetic neurons can detect SCFAs by expressing G-protein-coupled receptors 41 and 43 (GPR41 and GPR43) [11,12]. The activation of GPR43 (also known as Free Fatty Acid Receptor 2; FFAR2) leads to the production of IP₃ and increases in cytosolic Ca²⁺, while GPR41 (also known as FFAR3) can reduce the production of intracellular cAMP [13–15]. Altogether, these signaling cascades regulate hormone secretion and metabolism at the intestinal level, modulate communication networks, mainly in orexin/hypocretin neurons, and consequently regulate energy homeostasis and feeding [16].

Olfactive brain structures are important players in the food intake and reward systems due to the diversity of interneurons and brain cells that form communication networks with juxtacellular bodies and with other brain structures, such as the hypothalamus, amygdala, hippocampus, and cortex through long projection axons [17]. However, there is scarce information on the physiology of acetic acid (AA) receptors in the brain. According to Lein et al. (2007) [18], olfactory bulb cells (OBCs) may express SCFA receptors, yet their contribution to the physiology of the olfactory bulb is unknown.

This study used Ca²⁺ imaging recordings to show that the rat's OBCs produced intracellular Ca²⁺ responses to extracellular application of SCFAs. Pharmacological, immunocytochemical, and endpoint RT-PCR analyses were also used to show that the intracellular Ca²⁺ responses induced by AA and butyrate were produced through the activation of GPR41 receptors expressed mainly by olfactory bulb astrocytes. The reported data are intended to provide additional knowledge about gut-brain axis communication and the role of SCFAs as mediators of this process.

2. Materials and Methods

2.1. Animals

This study was reviewed and approved by the Research Ethics Committee of the University of Sonora (CEI-UNISON 012/2020, 1 July 2020) and followed the Mexican guidelines for the care and handling of laboratory animals (NOM-062-ZOO-1999). 7- to 21-day-old Wistar rats (males and females, $n = 10$) were obtained from the animal facility of the Department of Medicine and Health Sciences of the University of Sonora (Mexico); they were housed at 25 °C, with standard 12 h day/night cycles, and had ad libitum access to standard rodent diet and drinking water. Animal cages were cleaned up twice per week, and no animals other than rats were present in the room.

2.2. Olfactory Bulb Primary Cell Cultures

Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (120 mg/kg) and then euthanized. Both olfactory bulbs were dissected and placed in a

Petri dish containing ice-cold high-Mg²⁺ artificial cerebrospinal fluid (Mg²⁺-aCSF) with the following composition (in mM): 125 NaCl, 25 NaHCO₃, 3 KCl, 1.25 NaHPO₄·2H₂O, 10 glucose, 6 MgCl₂·6H₂O, 2 CaCl₂·6H₂O, at pH 7.4. Coronal olfactory bulb slices were then manually made to produce small fragments of OB tissue. These were transferred to an Eppendorf tube containing 300 µL of neurobasal medium supplemented with penicillin (100 U/mL), 2 mM l-glutamine, and 10% fetal bovine serum in order to make primary cell cultures, as described by Pérez-Delgado et al. (2024) [19], in a previously sterilized laminar flow hood (LABCONCO, Kansas City, MO, USA). Small tissue fragments were mechanically dissociated to obtain a suspended OBC solution; 100 µL of this solution was placed on a glass coverslip coated with 0.01% poly-l-ornithine contained in a sterile 35-mm Petri dish. After 2 min, 2 mL of the supplemented neurobasal medium was carefully added. Primary OBCs were maintained at 37 °C in a mixed air atmosphere with 5% CO₂ (New Brunswick incubator Galaxy 48S, Eppendorf Company, Hamburg, Germany). Culture medium was replaced every third day; OBCs were used for up to 10 days of culture.

2.3. Ca²⁺ Imaging

To record the effects produced by acetate and butyrate on the intracellular Ca²⁺ signaling of OBCs, 7–10-day-cultured OBCs contained in a glass coverslip were incubated for 10 min with Fluo-4/AM (10 µM; Invitrogen, Thermo Fisher Scientific, Hillsboro, OR, USA) diluted in aCSF; this solution was made daily from a frozen stock. Then, OBCs were placed in a custom-made recording chamber on the stage of a Leica DM500 microscope (Leica Microsystems, Heerbrugg, Switzerland) and continuously perfused with aCSF (rate 3 mL/min) composed of (in mM): 135 NaCl, 3 KCl, 10 glucose, 1 MgCl₂·6H₂O, 1.8 CaCl₂·6H₂O, and 5 HEPES. AA (A6283, Sigma-Aldrich, St. Louis, MO, USA) and sodium butyrate (303410, Sigma-Aldrich, St. Louis, MO, USA) were dissolved in aCSF daily prior to the experiments. To study if intracellular Ca²⁺ responses (iCaR) were dependent on extracellular Ca²⁺, we used zero-Ca²⁺ aCSF containing EGTA (1 mM; E4378, Sigma-Aldrich, St. Louis, MO, USA) and substituted CaCl₂ with 3 mM MgCl₂. 2-aminoethyl diphenylborinate (2-APB; 20 µM; D9754, Sigma-Aldrich, St. Louis, MO, USA) was used to analyze if AA-induced iCaR was produced in response to activation of IP₃ receptors. GLPG0974 (GLPG; 10 nM; SML2443, Sigma-Aldrich, St. Louis, MO, USA) and AR420626 (AR; 120 nM; SML1339, Sigma-Aldrich, St. Louis, MO, USA) were used to study the participation of GPR43 and GPR41 receptors, respectively.

OBCs were visualized through a 20×/0.8 NA immersion objective (Leica Microsystems, Wetzlar, Germany). iCaR produced by OBCs were recorded by scanning frames (512 × 512-pixel resolution) at a rate of 1 frame/s for 10 min using an Electro-Retiga CCD camera (Teledyne Photometrics, Thousand Oaks, CA, USA). Fluo-4/AM was excited (488 nm) with an X-cite XYLIS™ system (Excelitas Technologies, Mississauga, ON, Canada), and the emission (510 nm) was collected through a dichroic mirror. LED intensity was kept at 5% to avoid cell phototoxicity damage. Acquisition of the fluorescence data was performed using the PVCAM software version 3.8.3 (Teledyne Photometrics, Thousand Oaks, CA, USA). OBCs-iCaR were analyzed by drawing regions of interest (ROI) to obtain raw fluorescence data using ImageJ FIJI software Java 1.8.0 (NIH, USA). iCaR were illustrated with Origin Pro 9.65 (OriginLab Corporation, Northampton, MA, USA) and are depicted as changes in fluorescence intensity percentage (%ΔF, arbitrary units, AU). All data were normalized to the basal line to eliminate fluorescence noise variability between cells, according to Equation (1). AA and butyrate iCaR were considered when

$\Delta F/F_0 > \text{two standard deviations criteria}$. iCaR are presented as mean $\pm$ standard error (SE) of $n \geq 50$ cell records unless indicated otherwise.

$$\Delta F/F_0 = \frac{F - F_0}{F_0} \quad (1)$$

where F_0 , basal fluorescence; F , fluorescence response.

2.4. Immunofluorescence Assay

The immunofluorescence assays were performed as described by Montiel-Herrera et al. (2006) [20], with minor modifications. Briefly, after AA and butyrate iCaR were recorded, cultured OBCs were placed in cold methanol (-20°C) for 2 h. Antibody information is presented in Table 1. All antibodies were prepared by diluting 10% fetal bovine serum in aCSF solution. Primary antibodies (anti-GPR41 and anti-GPR43) were incubated individually for 6 h, washed out three times with aCSF solution, followed by the secondary antibody incubation for 1 h, washed as before, and examined with the same equipment mentioned previously (Section 2.3) to determine the percentage of immunoreactive cells. All procedures were conducted at room temperature in duplicate.

Table 1. Antibody resource identification.

Antibody	Immunogen	Manufacturer Information	Concentration
Anti-glial fibrillary acidic protein (anti-GFAP) produced in rabbits	Glial Fibrillary Acidic Protein	Sigma-Aldrich Cat# G9269 RRID: AB_477035 Produced in rabbit Polyclonal	1:200
Anti-free fatty acid receptor 3	GPR41	ELK Biotechnology (Sugar Land, TX, USA) Cat# ES5618 Produced in rabbit Polyclonal	1:200
Anti-free fatty acid receptor 2	GPR43	ELK Biotechnology Cat# ES5620 Produced in rabbit Polyclonal	1:200
Fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG	Rabbit IgG (whole molecule)	Sigma-Aldrich Cat# F0382 RRID: AB_259384 Produced in a goat Polyclonal	1:300

2.5. Endpoint RT-PCR

Total RNA was extracted from freshly isolated olfactory bulbs and OBCs primary cultures, using a Direct-zol RNA MiniPrep kit (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. The results were also compared with the extraction of total RNA from other brain structures and organ tissues (cerebellum, hippocampus, corpus callosum, amygdala, brainstem, hypothalamus, meninges, olfactory epithelium, and small intestine). The total RNA obtained was dissolved in nuclease-free water and quantified in a Nanodrop apparatus (Thermo Fisher, Wilmington, DE, USA). RNA was reverse transcribed into cDNA using the GoScript reverse transcription system (Promega, Madison, WI, USA), according to the manufacturer's instructions, and quantified in a Nanodrop. Primer sequences are shown in Table 2. Endpoint PCR experiments were carried out in a C1000 thermal cycler (Bio-Rad, Foster City, CA, USA) to determine the

expression of GPR41 and GPR43 receptors, using β -actin as a reference gene. PCR products were visualized in 2% agarose gel electrophoresis. RT-PCRs were conducted in triplicate.

Table 2. Oligonucleotides sequences used to identify GPR41 and GPR43 in the rat brain.

Gene	Sequences 5' to 3' (Forward, Reverse)	Amplicon (bp)	GenBank ID
GPR41	ACTACCCCTCAACGTGATGG CAGGTCCGAAATGGTCAGGTT	103	NM_001108912.1
GPR43	ATCAGCATAGAACGCTACCTGG TGCTCGGTTGAGTTCAGGTAC	158	NM_001005877.1
β -actin	TCGTGCGTGACATTAAAGAG TGCCACAGGATTCCATAC	198	NM_031144.3

2.6. Data Analysis

All data from Ca^{2+} experiments are shown as mean $\pm$ standard error of $n \geq 50$ recorded cells, unless indicated otherwise. Immunofluorescence and endpoint RT-PCR results represent at least two replicates. Figures were made in OriginPro 9.65 (OriginLab Corporation, Northampton, MA, USA). A Shapiro–Wilk test was performed to identify the normality of the data, $p = 0.001$. Non-parametric statistical analyses were performed using the Mann–Whitney U-test to determine differences in AA- and butyrate-responding OBCs, $p < 0.05$.

3. Results

3.1. Acetic Acid and Butyrate Induce Intracellular Ca^{2+} Responses in OBCs

The extracellular application of AA and butyrate resulted in a discrete number of OBCs generating iCaR with similar characteristics; Figure 1 shows a representative iCaR. The iCaR were studied by perfusing (0.1–1000 μM) OBCs with AA or butyrate to identify their effects on intracellular Ca^{2+} activity. First, the AA-iCaR were recorded in OBCs, which showed that extracellular perfusion of 0.1 μM of AA generated responses in 9% of cells ($n = 3/35$), 0.5 μM in 11% of cells ($n = 4/35$), 1 μM in 4% of cells ($n = 7/170$) (Figure 1A), 100 μM in 5% of cells ($n = 2/41$), 500 μM in 8% of cells ($n = 6/74$), and 1000 μM in 12% of cells ($n = 5/41$) (Figure 1B). The co-application of 500 μM AA and 20 μM 2-APB induced iCaR in 3 out of 6 OBCs (Figure 1C,D). Moreover, the GPR43 antagonist GLPG0974 (10 nM) did not completely block the iCaR (Figure 1E) in any of the AA-responding cells ($n = 8/71$ cells). Nonetheless, experiments recorded in the presence of 2-APB or GLPG0974 produced a variation in AA-iCaR latencies by showing a decay (τ) from 36 ± 8 s to 17 ± 4 s ($p = 0.005$). Interestingly, this phenomenon was recorded in both conditions, showing differences only in the physical form of responses, where co-application of AA with 2-APB or GLPG0974 induced a shorter calcium response. GPR41 agonist AR420626 (120 nM) evoked iCaR (Figure 1F) in 6% of the OBCs ($n = 3/48$ cells). In all cases, the number of cells that responded to AA was not different from each other ($p = 0.873$).

Following the same set of experiments, the perfusion of butyrate to OBCs produced iCaR. The application of 0.1 and 0.5 μM butyrate induced iCaR in 2/48 cells, 1 μM in 8/55 cells (Figure 2A), 100 μM in 2/45 cells, 500 μM in 5/47 cells, and 1 mM in 6/52 cells (Figure 2B). The co-application of 500 μM butyrate and 20 μM 2-APB induced iCaR in 2 out of 7 OBCs ($n = 56$) (Figure 2C,D). Moreover, the GPR43 antagonist GLPG0974 (10 nM) blocked the iCaR (Figure 2E) produced by butyrate-responding cells ($n = 27$ cells). GPR41 agonist AR420626 (120 nM) evoked iCaR in 7% of the OBCs ($n = 2/27$ cells; Figure 2F). The amplitudes of iCaR induced by butyrate on OBCs were not different from each other ($p = 0.681$), and the number of iCaR recorded with AA and butyrate ($p = 0.597$). These

results suggested that OBCs responded to SCFAs, with variations across all tested drugs, potentially by activating GPR41 and GPR43 receptors, whose responses were associated with cytosolic mechanisms linked to Ca^{2+} release from intracellular reservoirs.

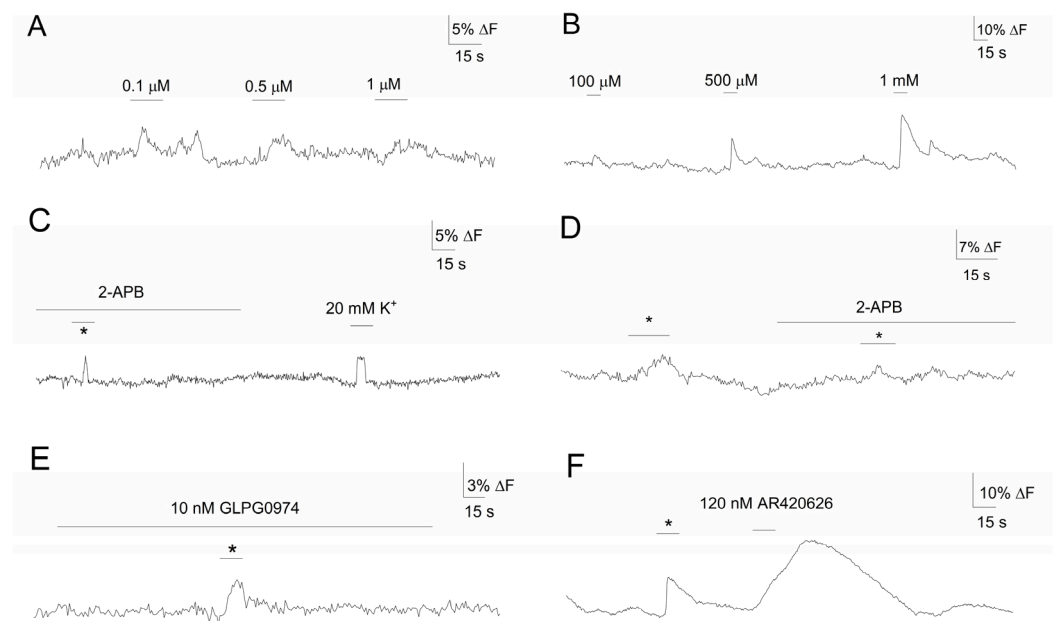


Figure 1. Representative iCaR activated by AA in OBCs (A,B). AA-evoked iCaR in the presence of 2-APB (C,D), and 20 mM K^+ was used as a control. Pharmacological experiments were performed to identify the participation of GPR43 (GLPG0974, (E)) and GPR41 (AR420626, (F)). Lines and * indicate the application and co-application of the drugs and AA, respectively.

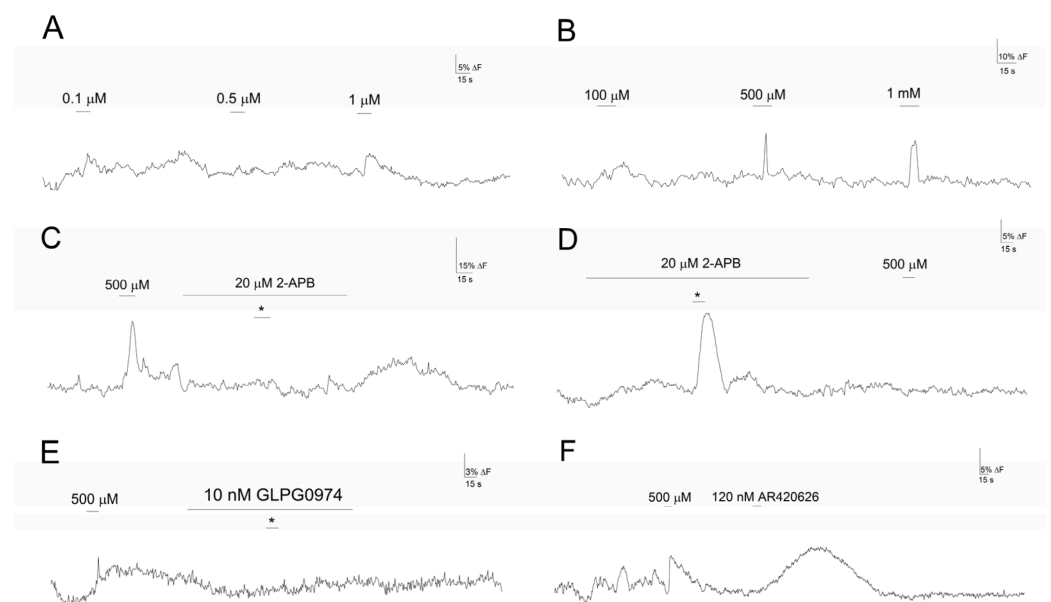


Figure 2. Representative iCaR activated by butyrate in OBCs (A,B). Butyrate-evoked iCaR in the presence of 2-APB (C,D). Pharmacological experiments were performed to identify the participation of GPR43 (GLPG0974, (E)) and GPR41 (AR420626, (F)). Lines and * indicate the application and co-application of the drugs and butyrate, respectively.

3.2. Immunocytochemical Analysis of Olfactory Bulb Cells

In order to know which type of brain cell was responding to the application of SCFAs and which GPR41 and GPR43 receptors were being expressed by it, they were identified immunocytochemically. We found that a discrete number of OBCs that activated iCaR

induced by AA (2/11 cells; Figure 3A,B) and butyrate (3/18 cells; Figure 3C,D) were GFAP⁺ cells, and that both receptors (GPR41 and GPR43) were expressed by OBCs (Figure 3E,F). It is important to mention that other cell populations of OBCs responded to AA and butyrate that were not GFAP⁺ cells.

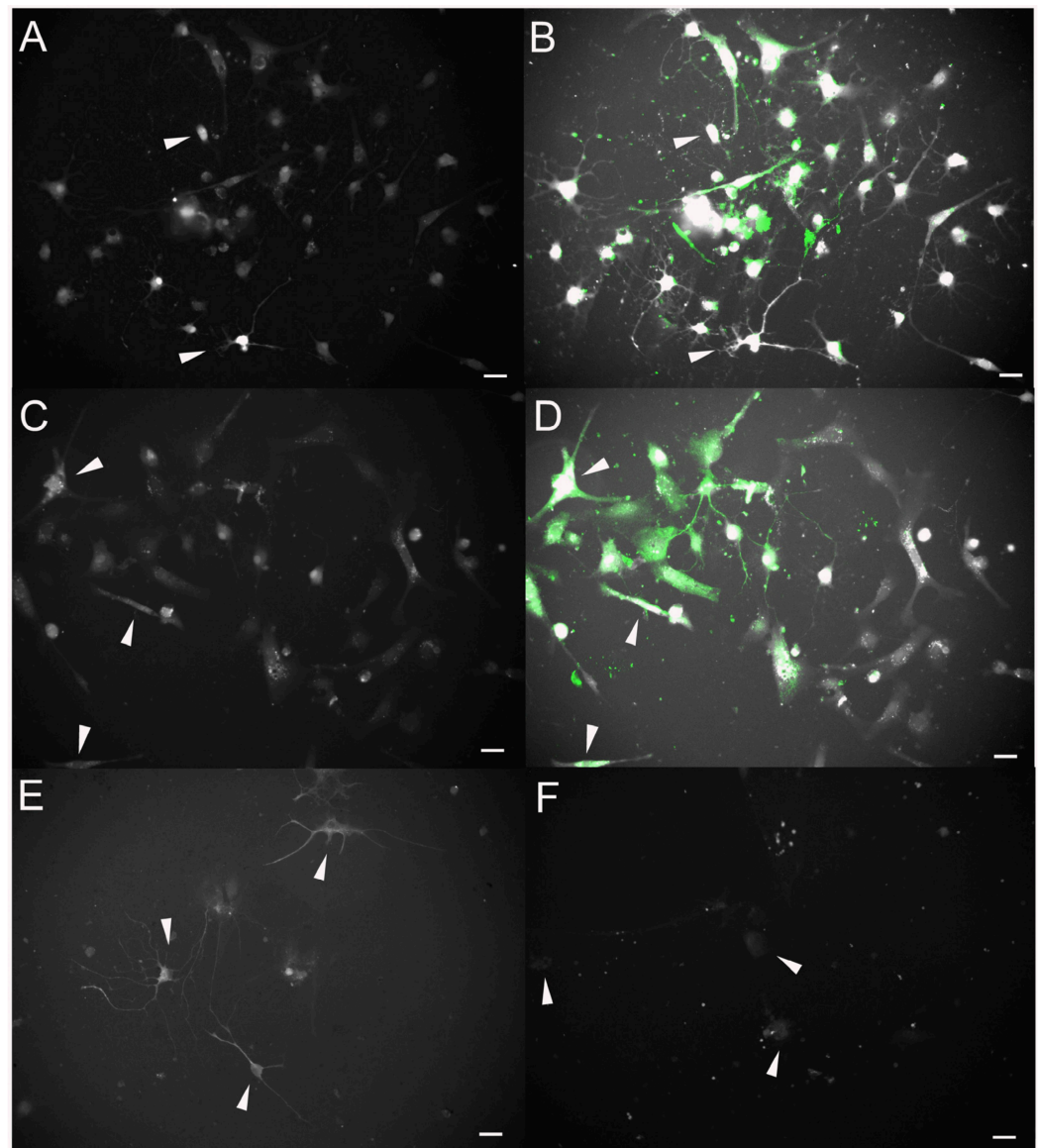


Figure 3. Immunocytochemical analysis was performed on OBCs (loaded with Fluo-4/AM) that responded to the application of AA and butyrate. (A) OBCs activated an iCaR by the perfusion of 500 μM of AA (arrowheads). (B) OBCs immunolabeled with GFAP antibody (green) showed that two astrocytes (same arrowheads as in A) activated iCaR. (C) OBCs perfused with 500 μM of butyrate generated iCaR (arrowheads). (D) OBCs immunolabeled with GFAP antibody (green) showed that three astrocytes (same arrowheads as in C) activated iCaR. (E) OBCs positive to GPR41 (FFAR3) receptor (arrowheads). (F) OBCs positive to GPR43 (FFAR2) receptor (arrowheads). Scale bar = 5 μm.

3.3. Expression of GPR41 and GPR43 in OBCs

As shown before, the application of AA and butyrate to OBCs produced iCaR produced by GPR41 and GPR43. Endpoint RT-PCR experiments revealed that OBCs from OB and primary cell cultures expressed GPR41. We also explored other brain structures, such as the cortex, hippocampus, and brainstem, which also expressed GPR41 (Figure 4). However, the cerebellum, corpus callosum, amygdala, olfactory epithelia, hypothalamus, and meninges did not express GPR41, and GPR43 was not expressed in any brain structures (Figure S1).

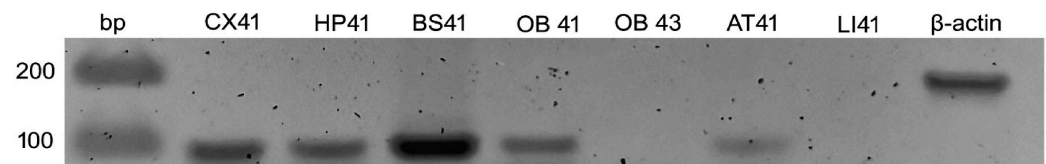


Figure 4. Gene expression of GPR41 and GPR43 in the rat brain. OB, olfactory bulb; CX, cortex; HP, hippocampus; BS, brain stem; AT, adipose tissue; LI, large intestine. β -actin was used as a positive control. Note: OB primary cell cultures also expressed GPR41; 41 and 43 stand for GPR41 and GPR43, respectively.

4. Discussion

Glial cells play a key role in brain physiology, primarily contributing to brain homeostasis. In the central nervous system, they maintain systematic and reciprocal communication with neurons and other brain cells to maintain cellular networks that control blood flow, cellular differentiation, and maturation, among other processes [21–23]. Astrocytes are known for their major roles in metabolic processes and modulating neuronal communication, even at the synaptic level. In this sense, it would be expected that brain cells could sense and use SCFAs like AA to communicate aspects of brain physiology. For instance, it is known that acetate plays a major role in brain cell physiology due to its utilization in metabolic pathways like the tricarboxylic acid cycle, as well as its role in GABA synthesis [24]. However, it would be less expected that some SCFAs like AA could be used as bioactive transmitters to promote cellular signaling between neurons and glial cells by activating SCFA receptors expressed on their membranes. Nonetheless, previous *in situ* hybridization studies have shown the expression of SCFA receptors in the mouse brain, such as the GPR41 receptor [18], while other authors have reported that systemically activating these GPR41 receptors leads to increased cytosolic Ca^{2+} concentrations, by intracellular mechanisms linked to intracellular Ca^{2+} release from different reservoirs [25]. We therefore tested if OBCs from the rat olfactory bulb express SCFA receptors and found that at least a discrete number of astrocytes express both GPR41 and GPR43 receptors. Interestingly, a minor population of responding cells does not mean that SCFA have less physiological significance; rather, we hypothesize that there may be cells that accomplish specific metabolic functions through SCFA signaling in the olfactory system that deserve further characterization. Although SCFAs could activate GPR41 in OBCs, this remains to be confirmed due to the lack of a commercially available antagonist for this receptor. In this sense, this study explored the intracellular calcium responses of OBCs to the extracellular application of AA. We strongly believe this work is novel because very little is known about SCFAs signaling in the central nervous system, particularly in the olfactory system. Nonetheless, further research is needed to properly characterize SCFAs receptors' signaling in the mammalian olfactory system. These results provide new insight into SCFA receptor signaling in glial OBCs, suggesting active participation of these receptors in the metabolic and intercellular functions of the rat olfactory bulb [26], where AA and butyrate may contribute to brain cell proliferation and maturation processes [27]. For example, it has been reported that propionate and butyrate (1–4 mM) induced iCaR in pituitary cells and sympathetic neurons of the celiac-mesenteric ganglia, with approximately 20% and 12% of cells, respectively, responding to these SCFAs and activating intracellular Ca^{2+} mobilization [15,28,29]. It is also known that activation of the GPR41 receptor modulates the intracellular concentration of cAMP, whereas the activation of the GPR43 receptor is associated with IP_3 production and intracellular Ca^{2+} release pathways [30,31]. In sympathetic neurons, the activation of these receptors can increase intracellular Ca^{2+} by diverse intracellular mechanisms that involve the production of IP_3 and Ca^{2+} release pathways, as well as PLC-MAPK signaling coupled to the voltage-gated Ca^{2+} -channel $\text{Cav}2.2$ and Ca^{2+} -induced Ca^{2+} -release path-

ways [3,28,32]. Additionally, Forte et al. (2024) [16] described that activation of GPR43 receptors changed the excitability of orexin/hypocretin hypothalamic neurons in mice by enhancing GABAergic transmission, while their pharmacological blockade did not produce significant changes in the excitability of neurons. This suggests that GPR43 plays a role in increasing neurons' cytosolic Ca^{2+} and, thus, in modulating their excitability.

In summary, this work shows that OBCs respond to AA and butyrate by inducing intracellular Ca^{2+} mobilization through the activation of SCFA receptors. Pharmacological, immunocytochemical, and RT-PCR analyses indicate the participation of GPR41 and GPR43 receptors in these processes. These findings deserve further pharmacological and behavioral investigation to better understand the role of SCFAs in the physiology of the rat olfactory system and in other brain structures, as revealed by our RT-PCR experiments.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/neuroglia7030022/s1>.

Author Contributions: Experimental procedures and funding acquisition, F.J.P.-D. and M.M.-H. Conceptualization, methodology, formal analysis, A.E.E.-S., D.R.-H., G.A.G.-A., J.A.D.-A., M.A.L.-T., E.D.L.R.-V., D.M.-S., M.A.V.-N., O.O.-F., F.J.P.-D., M.D.G.-V. and M.M.-H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI), CF-2023-I-905, CF-2023-I-672. The APC was funded by the University of Sonora.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the University of Sonora (CEI-UNISON 012/2020, 1 July 2020).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated during this study are available from the corresponding author upon reasonable request.

Acknowledgments: FJPD thanks Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for the scholarship received (CVU 1006147). The authors thank Karla Zavalza Ortega and Alejandra Lopez Vazquez for their technical assistance.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

SCFAs	Short Chain Fatty Acids
AA	Acetic acid
OB	Olfactory bulb
OBCs	Olfactory bulb cells
iCaR	intracellular Ca^{2+} responses
GPR41	G-protein coupled receptor 41
GPR43	G-protein coupled receptor 43

References

1. Segatto, M.; Pallottini, V. Facts about Fats: New Insights into the Role of Lipids in Metabolism, Disease and Therapy. *Int. J. Mol. Sci.* **2020**, *21*, 6651. [CrossRef] [PubMed]
2. Papackova, Z.; Cahova, M. Fatty Acid Signaling: The New Function of Intracellular Lipases. *Int. J. Mol. Sci.* **2015**, *16*, 3831–3855. [CrossRef] [PubMed]
3. Kimura, I.; Ichimura, A.; Ohue-Kitano, R.; Igarashi, M. Free Fatty Acid Receptors in Health and Disease. *Physiol. Rev.* **2020**, *100*, 171–210. [CrossRef] [PubMed]

4. Silva, Y.P.; Bernardi, A.; Frozza, R.L. The Role of Short-Chain Fatty Acids from Gut Microbiota in Gut-Brain Communication. *Front. Endocrinol.* **2020**, *11*, 25. [[CrossRef](#)] [[PubMed](#)]
5. Pascale, A.; Marchesi, N.; Marelli, C.; Coppola, A.; Luzi, L.; Govoni, S.; Giustina, A.; Gazzaruso, C. Microbiota and Metabolic Diseases. *Endocrine* **2018**, *61*, 357–371. [[CrossRef](#)] [[PubMed](#)]
6. Cong, J.; Zhou, P.; Zhang, R. Intestinal Microbiota-Derived Short Chain Fatty Acids in Host Health and Disease. *Nutrients* **2022**, *14*, 1977. [[CrossRef](#)] [[PubMed](#)]
7. Dalile, B.; Van Oudenhove, L.; Vervliet, B.; Verbeke, K. The Role of Short-Chain Fatty Acids in Microbiota–Gut–Brain Communication. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 461–478. [[CrossRef](#)] [[PubMed](#)]
8. Qin, X.; Zhang, M.; Chen, S.; Tang, Y.; Cui, J.; Ding, G. Short-Chain Fatty Acids in Fetal Development and Metabolism. *Trends Mol. Med.* **2025**, *31*, 625–639. [[CrossRef](#)] [[PubMed](#)]
9. Ye, Q.; Cai, S.; Wang, S.; Zeng, X.; Ye, C.; Chen, M.; Zeng, X.; Qiao, S. Maternal Short and Medium Chain Fatty Acids Supply during Early Pregnancy Improves Embryo Survival through Enhancing Progesterone Synthesis in Rats. *J. Nutr. Biochem.* **2019**, *69*, 98–107. [[CrossRef](#)] [[PubMed](#)]
10. Sadler, R.; Cramer, J.V.; Heindl, S.; Kostidis, S.; Betz, D.; Zuurbier, K.R.; Northoff, B.H.; Heijink, M.; Goldberg, M.P.; Plautz, E.J.; et al. Short-Chain Fatty Acids Improve Poststroke Recovery via Immunological Mechanisms. *J. Neurosci. Off. J. Soc. Neurosci.* **2020**, *40*, 1162–1173. [[CrossRef](#)] [[PubMed](#)]
11. Nøhr, M.K.; Pedersen, M.H.; Gille, A.; Egerod, K.L.; Engelstoft, M.S.; Husted, A.S.; Sichlau, R.M.; Grunddal, K.V.; Poulsen, S.S.; Han, S.; et al. GPR41/FFAR3 and GPR43/FFAR2 as Cosensors for Short-Chain Fatty Acids in Enteroendocrine Cells vs FFAR3 in Enteric Neurons and FFAR2 in Enteric Leukocytes. *Endocrinology* **2013**, *154*, 3552–3564. [[CrossRef](#)] [[PubMed](#)]
12. Nøhr, M.K.; Egerod, K.L.; Christiansen, S.H.; Gille, A.; Offermanns, S.; Schwartz, T.W.; Møller, M. Expression of the Short Chain Fatty Acid Receptor GPR41/FFAR3 in Autonomic and Somatic Sensory Ganglia. *Neuroscience* **2015**, *290*, 126–137. [[CrossRef](#)] [[PubMed](#)]
13. Kimura, I.; Inoue, D.; Maeda, T.; Hara, T.; Ichimura, A.; Miyauchi, S.; Kobayashi, M.; Hirasawa, A.; Tsujimoto, G. Short-Chain Fatty Acids and Ketones Directly Regulate Sympathetic Nervous System via G Protein-Coupled Receptor 41 (GPR41). *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 8030–8035. [[CrossRef](#)] [[PubMed](#)]
14. Koh, A.; De Vadder, F.; Kovatcheva-Datchary, P.; Bäckhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **2016**, *165*, 1332–1345. [[CrossRef](#)] [[PubMed](#)]
15. Perry, R.J.; Peng, L.; Barry, N.A.; Cline, G.W.; Zhang, D.; Cardone, R.L.; Petersen, K.F.; Kibbey, R.G.; Goodman, A.L.; Shulman, G.I. Acetate Mediates a Microbiome–Brain– β -Cell Axis to Promote Metabolic Syndrome. *Nature* **2016**, *534*, 213–217. [[CrossRef](#)] [[PubMed](#)]
16. Forte, N.; Marfella, B.; Nicois, A.; Palomba, L.; Paris, D.; Motta, A.; Pina Mollica, M.; Di Marzo, V.; Cristino, L. The Short-Chain Fatty Acid Acetate Modulates Orexin/Hypocretin Neurons: A Novel Mechanism in Gut-Brain Axis Regulation of Energy Homeostasis and Feeding. *Biochem. Pharmacol.* **2024**, *226*, 116383. [[CrossRef](#)] [[PubMed](#)]
17. Nagayama, S.; Homma, R.; Imamura, F. Neuronal Organization of Olfactory Bulb Circuits. *Front. Neural Circuits* **2014**, *8*, 98. [[CrossRef](#)] [[PubMed](#)]
18. Lein, E.S.; Hawrylycz, M.J.; Ao, N.; Ayres, M.; Bensinger, A.; Bernard, A.; Boe, A.F.; Boguski, M.S.; Brockway, K.S.; Byrnes, E.J.; et al. Genome-Wide Atlas of Gene Expression in the Adult Mouse Brain. *Nature* **2007**, *445*, 168–176. [[CrossRef](#)] [[PubMed](#)]
19. Pérez-Delgado, F.J.; García-Villa, M.D.; Fernández-Quiroz, D.; Villegas-Ochoa, M.; Domínguez-Avila, J.A.; Gonzalez-Aguilar, G.A.; Ayala-Zavala, J.F.; Martínez-Martínez, A.; Montiel-Herrera, M. Clicking Gallic Acid into Chitosan Prolongs Its Antioxidant Activity and Produces Intracellular Ca^{2+} Responses in Rat Brain Cells. *Int. J. Biol. Macromol.* **2024**, *277*, 134343. [[CrossRef](#)] [[PubMed](#)]
20. Montiel-Herrera, M.; Miledi, R.; García-Colunga, J. Membrane Currents Elicited by Angiotensin II in Astrocytes from the Rat Corpus Callosum. *Glia* **2006**, *53*, 366–371. [[CrossRef](#)] [[PubMed](#)]
21. Allen, N.J.; Lyons, D.A. Glia as Architects of Central Nervous System Formation and Function. *Science* **2018**, *362*, 181–185. [[CrossRef](#)] [[PubMed](#)]
22. Verkhratsky, A.; Nedergaard, M. Physiology of Astroglia. *Physiol. Rev.* **2018**, *98*, 239–389. [[CrossRef](#)] [[PubMed](#)]
23. Verkhratsky, A.; Parpura, V.; Vardjan, N.; Zorec, R. Physiology of Astroglia. *Adv. Exp. Med. Biol.* **2019**, *1175*, 45–91. [[CrossRef](#)] [[PubMed](#)]
24. Rowlands, B.D.; Klugmann, M.; Rae, C.D. Acetate Metabolism Does Not Reflect Astrocytic Activity, Contributes Directly to GABA Synthesis, and Is Increased by Silent Information Regulator 1 Activation. *J. Neurochem.* **2017**, *140*, 903–918. [[CrossRef](#)] [[PubMed](#)]
25. Miyamoto, J.; Hasegawa, S.; Kasubuchi, M.; Ichimura, A.; Nakajima, A.; Kimura, I. Nutritional Signaling via Free Fatty Acid Receptors. *Int. J. Mol. Sci.* **2016**, *17*, 450. [[CrossRef](#)] [[PubMed](#)]
26. Kimura, I.; Inoue, D.; Hirano, K.; Tsujimoto, G. The SCFA Receptor GPR43 and Energy Metabolism. *Front. Endocrinol.* **2014**, *5*, 85. [[CrossRef](#)] [[PubMed](#)]

27. Wu, J.; Zhou, Z.; Hu, Y.; Dong, S. Butyrate-Induced GPR41 Activation Inhibits Histone Acetylation and Cell Growth. *J. Genet. Genom. Yi Chuan Xue Bao* **2012**, *39*, 375–384. [[CrossRef](#)] [[PubMed](#)]
28. Colina, C.; Puhl, H.L.; Ikeda, S.R. Selective Tracking of FFAR3-Expressing Neurons Supports Receptor Coupling to N-Type Calcium Channels in Mouse Sympathetic Neurons. *Sci. Rep.* **2018**, *8*, 17379. [[CrossRef](#)] [[PubMed](#)]
29. Miletta, M.C.; Petkovic, V.; Eblé, A.; Ammann, R.A.; Flück, C.E.; Mullis, P.-E. Butyrate Increases Intracellular Calcium Levels and Enhances Growth Hormone Release from Rat Anterior Pituitary Cells via the G-Protein-Coupled Receptors GPR41 and 43. *PLoS ONE* **2014**, *9*, e107388. [[CrossRef](#)] [[PubMed](#)]
30. Hua, Y.; Liu, D.; Zhang, D.; Wang, X.; Wei, Q.; Qin, W. Extracellular AMP Suppresses Endotoxemia-Induced Inflammation by Alleviating Neutrophil Activation. *Front. Immunol.* **2020**, *11*, 1220. [[CrossRef](#)] [[PubMed](#)]
31. Milligan, G.; Shimpukade, B.; Ulven, T.; Hudson, B.D. Complex Pharmacology of Free Fatty Acid Receptors. *Chem. Rev.* **2017**, *117*, 67–110. [[CrossRef](#)] [[PubMed](#)]
32. Li, C.; Horn, J.P. Differential Inhibition of Ca²⁺ Channels by Alpha2-Adrenoceptors in Three Functional Subclasses of Rat Sympathetic Neurons. *J. Neurophysiol.* **2008**, *100*, 3055–3063. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.