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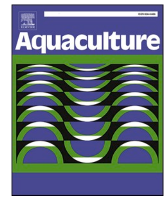

Fatty acids of different nature differentially modulate feed intake in rainbow trout

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A B S T R A C T

Feed intake is subjected to a complex regulation involving a plethora of signals, among which nutrients stand as one of the most important. In mammals, the gastrointestinal tract is able to sense nutrients in the lumen, and respond with the release of signaling molecules that ultimately modulate brain circuits governing appetite, resulting in decreased/increased feeding. Whether equivalent mechanisms operate in fish remains unknown. In a recent study, we described that the gastrointestinal tract of rainbow trout contains several sensors for free fatty acids (FAs), and that the luminal presence of FAs of different length and degree of unsaturation modulates the levels of key gastrointestinal hormones involved in feed intake regulation. In this study, our aim was to characterize the impact of such a luminal presence of FAs on brain appetite-regulatory centers, as well as its effects on rainbow trout feed intake. Major results from this study demonstrated that: (i) FAs of different length and degree of unsaturation [medium-chain (MCFAs, octanoate), long-chain (LCFAs, oleate), long-chain polyunsaturated (PUFA, α-linolenate), and short-chain (SCFA, butyrate) FAs] differentially modulate feed intake levels when administered intragastrically, (ii) intragastrically-administered FAs modulate the phosphorylation status of appetite-related transcription factors, as well as mRNA levels of key appetite-regulating neuropeptides, in the hypothalamus and/or telencephalon, (iii) luminal presence of FAs results in changes in the central abundance of mRNAs encoding gastrointestinal hormone receptors, and (vi) luminal FA-derived central changes in neuropeptide mRNAs are not observed (or are lessened) in vagotomized fish. Together, these results provide comprehensive evidence in favor of a gut-brain axis in fish. In addition, we observed different responses in terms of feed intake regulation depending on the type of fatty acid administered into the lumen, which is very relevant for aquaculture considering differences in fatty acid composition in aquafeeds.

1. Introduction

Optimising feed consumption is essential for good health, proper development and welfare of animals, particularly when raised in captivity. An optimal feedfeed intake reflects the achievement of nutritional balance or homeostasis, whose maintenance is governed by a dynamic and complex process involving several tissues and a plethora of signals of diverse nature (Guyenet and Schwartz, 2012). In mammals, there is unequivocal evidence that the gastrointestinal tract (GIT) is importantly involved in the homeostatic control of feedfeed intake and energy balance through the so called gut-brain axis (Cummings and Overduin, 2007). Within this axis, intestinal cells, mainly enteroendocrine cells (EECs), have the ability to sense nutrients (carbohydrates, proteins/amino acids and lipids/fatty acids) in the lumen, and respond releasing multiple regulatory peptides, such as ghrelin (GHRL), peptide tyrosine-tyrosine (PYY), cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). These peptides can act paracrinally, but more importantly they act as signals for gut-brain communication, either by vagal transmission or by classical endocrine communication through the systemic circulation (Janssen and Depoortere, 2013; Raka et al., 2019; Rasoamanana et al., 2012). Gut-derived information reaches the central nervous system (CNS) and is integrated, ultimately leading to changes in the production of key hypothalamic factors that control appetite, thus increasing or decreasing feed intake (Chambers et al., 2013).

In fish, the knowledge on gut nutrient sensing mechanisms and their implication in feed intake regulation is still in its infancy. Only very few studies have offered evidence on functional aspects related to carbohydrate (Polakof et al., 2010; Polakof and Soengas, 2013; Ye et al.,

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2019) and amino acid (Calo et al., 2021) sensing in the GIT of fish species. However, to our knowledge, no information on gut fatty acid sensing mechanisms and their impact in fish appetite is available in the literature. Apart from all general functions of lipids in vertebrates (Santos and Preta, 2018), this type of nutrient is particularly important for fish as free fatty acids (FFAs) derived from triglycerides (as those in diet) are the major aerobic fuel source for energy metabolism of fish muscle (Tacon, 1987) and it is the main source of energy in aquaculture nutrition (Bell and Koppe, 2010). In addition, it must be highlighted that fish exhibit important differences with mammals in terms of lipid metabolism (e.g., fish are able to endogenously produce long-chain polyunsaturated fatty acids (PUFAs), essential for multiple physiological processes, while capacity for PUFA synthesis is very limited in most mammals (Kuah et al., 2015)), thus being of enormous interest to study if such differences could result in evolutionary variations in terms of sensing mechanisms.

With this background, to unravel the response of fish to ingested fatty acids would be of enormous interest to the aquaculture sector and could help develop novel feeds able to trigger a robust positive appetite response, thus maximizing feeding efficiency. In a recent study from our research group, we demonstrated that intragastrically-administered fatty acids of different length and degree of unsaturation modulate the expression of several fatty acid receptors and transporters, as well as the levels of gastrointestinal hormones (Ghrl, Cck, Pyy and Glp-1) mRNAs/protein, in the GIT of rainbow trout (*Oncorhynchus mykiss*) (Calo et al., *under review*). These observations suggest that the rainbow trout GIT, as is mammals, is able to sense the luminal presence of fatty acids and likely respond with the release of signaling molecules. However, whether such signaling molecules may impact brain appetite-regulatory centers and ultimately modulate feed intake remains unknown. Therefore, the objectives of this study were to assess the effects of intragastrically-administered fatty acids on rainbow trout feed intake, and to characterize putative changes in central parameters involved in appetite regulation in response to such a luminal presence of fatty acids. In addition, we aimed to study the afferent pathways used by intestinal signals to impact brain centres, including both vagal transmission and systemic circulation, thus aiming to characterize a functional gut-brain axis in fish.

2. Material and methods

2.1. Animals

Rainbow trout (*Oncorhynchus mykiss*) with a body weight (bw) of 100 ± 20 g were purchased from a local fish farm (A Estrada, Spain) and housed in 100 L tanks with aerated and dechlorinated tap water (15 ± 1 °C) in an open circuit. A number of 40 fish were housed in each tank during acclimation; then, for each experiment, the necessary number of fish (see each experimental designs for exact numbers) were redistributed in separated tanks. Photoperiod was set at 12 h light:12 h darkness (12 L,12D) (lights on at 08:00 h). Fish were offered feed from a commercial dry pellet diet (proximate analysis: 2.5% carbohydrates, 44% crude protein, 21% crude fat, and 17% ash; 20.2 MJ kg^{-1} of feed; Biomar, Dueñas, Spain) daily at 11:00 until visual apparent satiety, for at least two weeks before the experiments. All experiments performed followed the ARRIVE Guidelines, adhered to the guidelines of the Spanish Government (RD 53/2013) and the European Union Council (2010/63/UE) for the use of animals in research, and were approved by the Ethics Committee of the Universidade de Vigo.

2.2. Effects of intragastrically-administered fatty acids on feed intake

A first set of four experiments were carried out to evaluate the effects of fatty acids on feed intake levels. In each experiment, following a 2-week acclimation period, 75 rainbow trout were divided into five experimental groups (15 fish/group), placed in separate tanks, and levels of feed intake by each group of fish was registered for 7 days to evaluate basal levels. For this, fish were offered pre-weighed amounts of feed in 3-min intervals (accounting for ~3% bw in total) until visual satiation. Then, the uneaten feed (if any) was recovered, dried and weighed. Feed consumed by all fish in each tank was calculated as previously described as the difference from the feed offered (De Pedro et al., 1998). During the experiment, fish were slightly anaesthetized with 2-phenoxyethanol (0.02% *v*/v), weighed and intragastrically offered 1 mL per 100 g^{-1} bw of vehicle (distilled water containing 5% EtOH) alone (control) or containing 50 μmol per mL^{-1} of octanoate, oleate, α-linolenate (ALA) or sodium butyrate (all from Sigma-Aldrich, San Luis, USA). Octanoate, oleate and ALA were chosen as representative MCFA, LCFA and PUFA, respectively, based on previous studies from our research group demonstrating their effectiveness as modulators of food intake levels and/or related parameters in rainbow trout or Senegalese sole (Librán-Pérez et al., 2012; Velasco et al., 2017). No previous studies are available demonstrating a role for SCFAs in food intake control in fish; thus, among the main SCFAs, butyrate was selected as representative in this study. Administration was carried out with a 13 cm-long cannula attached to a blunt-tip syringe. We visually monitored for putative regurgitation, and observed none during administration of solutions. The fatty acid dose was calculated based on a typical amount of oleate (selected because it has important effects on feed intake in rainbow trout (Librán-Pérez et al., 2015b, 2014, 2012)) ingested daily by a trout fed a standard commercial diet (Librán-Pérez et al., 2015a). We used an equimolar dose for the remaining fatty acids. Following administration, fish were returned to their corresponding tanks and allowed to recover. They were then offered feed at 2, 6 and 24 h post-administration. Feed intake at each time point was quantified as described above. This experiment was carried out four times; in each experiment, each groups of fish was treated with a different fatty acid. For each experiment, basal levels of feed intake were registered again, and treatments were not administrated until basal levels were stable (typically, one week). Results in graphs correspond to the mean values of the four experiments.

2.3. Characterization of the response of central appetite-regulatory mechanisms to luminal fatty acids

The effect of fatty acids on central appetite-regulatory mechanisms was evaluated in a separate experiment using separate fish as those in Section 2.2. This experiment was performed during two days. For both days, fish that will be used for experiment (kept in acclimation tanks) were feed-deprived for 48 h to ensure intestinal emptying and the achievement of basal levels of hormones with a role in the metabolic control of apeptite. Each day of experiment, 30 fish were captured in batches of 6 (accounting for $n = 6$ per treatment), slightly anaesthetized with 2-phenoxyethanol (0.02% *v*/v), and intragastrically offered 1 mL per 100 g^{-1} bw of vehicle (distilled water containing 5% EtOH) alone (control) or containing 50 μmol per mL^{-1} of octanoate, oleate, ALA or sodium butyrate, as described in the previous section. After administration, fish from each experimental group were recovered in individual tanks. After 2 h, fish were sacrificed by decapitation, and the hypothalamus and telenchepalon were collected. Of the 12 fish used per treatment, samples from 6 fish were used for analysis of mRNA levels (see Section 2.5) and samples from the other 6 fish for quantification of protein levels (see Section 2.6).

2.4. Study of the putative involvement of the vagus nerve in the fatty acid-sensing signaling from the gut to the brain

A third experiment was carried out to analyse the putative

involvement of the vagus nerve in the fatty acid-sensing signaling from the gut to the brain. 60 fish (30 control/sham and 30 vagotomized) were used in this experiment. The day of surgery, 24 h-fasted animals were individually anaesthetized (in water with tricaine methanosulfate; 150 mg l^{-1}; Sigma-Aldrich; and sodium bicarbonate; 300 mg l^{-1}), and placed on a surgery table, with well aerated freshwater containing anesthetic continuously irrigating the gills. Then, fish were vagotomized by bilaterally cutting the vagi, as explained by Seth and Axelsson (Seth and Axelsson, 2010). Briefly, we performed a 0.5 cm dorsoventral incision in the tissue connecting the fourth gill arch and the cleithrum; this gives access to the vagus nerve, which would appear visible below the connective tissue. The vagus was subsequently sectioned. Sham surgery was performed following the same procedure except for vagus nerve transection. Sham and vagotomized fish were placed back in acclimation tanks and allowed to recover for 48 h.

After 48 h, fish were captured in batches of 6 (accounting for $n = 6$ sham and n = 6 vagotomized per treatment), anaesthetized (2-phenoxyethanol; 0.02% *v*/v), and intragastrically offered 1 mL per 100 g^{-1} bw of vehicle (distilled water containing 5% EtOH) alone (control) or containing 50 μmol per mL^{-1} of octanoate, oleate, ALA or sodium butyrate, as described in sections above. After 4 h, fish were sacrificed by decapitation, and the hypothalamus and telencephalon were collected.

2.5. Quantification of mRNA abundance by reverse transcription – quantitative polymerase chain reaction (RT-qPCR)

Total RNA from tissues (n = 6 fish) was purified with Trizol reagent (Life Technologies, Grand Island, USA) and subsequently incubated with RQ1-DNAse (Promega, Madison, USA) following commercial instructions. Optical density (OD) absorption ratio (OD 260 nm/280 nm) was determined using a NanoDrop 2000c (Thermo, Vantaa, Finland) and used to determine RNA purity. Then, 2 μg of total RNA were transcribed into cDNA using Superscript II reverse transcriptase (Promega) according to the manufacturer's instructions. Lastly, mRNA abundance was determined by RT-qPCR using MAXIMA SYBR Green qPCR Mastermix (Life Technologies), with forward and reverse primers shown in Table 1, all purchased from IDT (Leuven, Belgium). PCRs were run in 96-well plates containing 1 μL cDNA (or water and RNA for controls), 500 nM of primer forward and 500 nM of primer reverse, in a 10 μL final volume. We used duplicate wells for each sample. The protocol used was the following: initial step of 95 °C - 10 min, and 40 cycles of 95 °C - 30 s + 60 °C (except for *npy*, whose annealing temperature is of 59 °C; see Table 1) - 30 s. Specificity of reactions was checked by running a melting curve (temperature gradient at 0.5 °C/5 s from 65 to 95 °C) following each run. All qPCRs were performed in an iCycler iQ (Bio-Rad, Hercules, USA). Efficiency of all reactions was 95–100% and R^2 was 0.97–1. Amplified cDNA was run on 1.5% agarose gels to confirm amplicon specificity. For the tissue distribution, we also run on agarose gels representative samples of each tissue to include a gel picture showing a qualitative estimation of expression levels. The relative abundance of target transcripts was calculated using the 2-ΔΔCt method (Livak and Schmittgen, 2001), using *actb* (gene encoding β-actin) and *ee1af1* (gene encoding elongation factor 1α) as reference genes. Both genes were stably expressed in this experiment.

2.6. Analysis of protein levels by Western blot

Tissue samples from 6 fish were used for Western blot analysis. Extraction and quantification of protein were carried out following previous protocols (Calo et al., 2021). Then, 20 μg protein were added to 4× Laemmli buffer (containing 0.2% 2-mercaptoethanol, Bio-Rad) and denatured at 95 °C during 10 min. Samples were then electrophoresed in Stain-Free −20% acrylamide gels (Bio-Rad) and, using the Trans-Blot Turbo transfer system (Bio-Rad), they were transferred to a 0.2 μm pore-size nitrocellulose membrane (Bio-Rad). Membranes were then blocked using Pierce Protein-Free T20 (PBS) Blocking Buffer (ThermoFisher) for 60 min, and subsequently incubated with specific primary antibody overnight. Primary antibodies used for protein detection were: anti-CREB (48H2) (1:500, Cat # 9197, Cell Signaling, Danvers, USA), anti-phospho-CREB (Ser133) (1:500, Cat # 9198, Cell Signaling), anti-phospho-FoxO1 (Thr24) (1:500, Cat # 9464, Cell Signaling), anti-FoxO1 (L27) (1:500, Cat # 9454, Cell Signaling), and anti-BSX (1:500, Cat # ab236983, Abcam, Cambridge, United Kingdom). These antibodies were previously validated in rainbow trout (Conde-Sieira et al., 2018). Dilution of antibody was 1:500 in all cases. The following day, membranes were washed and incubated with secondary antibody (goat anti-rabbit IgG (H + L) HRP conjugate; Cat # ab205718, Abcam) at a 1:5000 dilution. Protein were visualized in a ChemiDoc Touch imaging system (Bio-Rad), using Clarity Western ECL substrate (Bio-Rad). We quantified protein bands by densitometry, relative to total protein amount, using Image Lab software.

2.7. Statistical analysis

First, normality and homogeneity of variance were checked for all data using Shapiro-Wilk's and Levene's tests, respectively. If any of these failed, data were log-transformed and re-checked. Then, for the in vivo intragastric approach, statistical differences between each treatment and control group were determined by *t*-test. For the vagotomy experiment, we used two-way ANOVA and Holm-Sidak multiple comparison test, considering vagotomy (performed, not performed/sham) and fatty acid treatment (none, octanoate/oleate/ALA/butyrate) as main factors, to assess statistical differences. Significance was considered when $p < 0.05$. SigmaPlot (Systat Software Inc., San Jose, CA, USA) was used to carry out all analyses.

Table 1
Primers used for determining mRNA abundance.

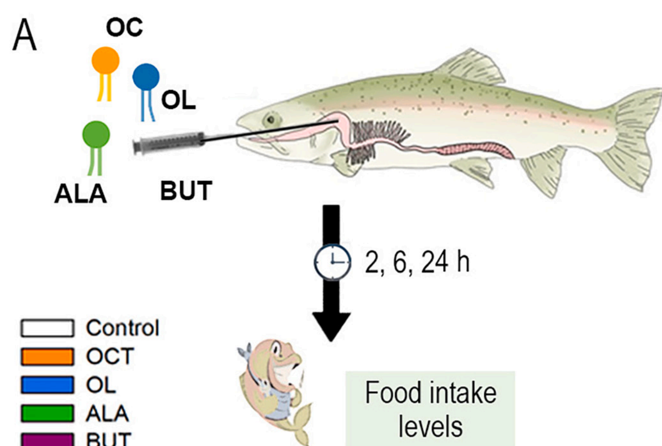
Gene	GenBank accession number	Sequence (5′ to 3′)	Reverse primer (5′ to 3′)	Amplicon size (bp)	Annealing temperature (°C)
actb	NM 001124235.1	GATGGGCCAGAAAGACAGCTA	TCGTCCCAGTTGGTGACGAT	105	59
agrp	NM_001146677	ACCAGCAGTCCTGTCTGGGTAA	AGTAGCAGATGGAGCCGAACA	87	60
cartpt	XM_021596562.2	ACCATGGAGAGCTCCAG	GCGCACTGCTCTCCAA	275	60
cckbr	XM_021579294.2	CCGTGAGTATCTCCACCTTCA	CGTGACTTTAGTGGGTTGCAG	79	60
eef1a1	AF498320	TCCTCTTGGTCGTTTCGCTG	ACCCGAGGGACATCCTGTG	159	59
ghsr1a	NM_001124594.1	TTCGTGCGCTCATCCTCTTT	ACTGGGTGGGTTTACACTCG	171	60
glp1r	XM_021582023.1	GCCGTGATGTCAGAGGAACA	GGATGGCAACCAGTAGACCC	100	60
npy	NM_001124266	CTCGTCTGGACCTTTATATGC	GTTCATCATATCTGGACTGTG	247	59
npy2r	DQ231509.1	GGAGTACGGGACGTTTGACC	AGATGCGGATGTAGGCGAAG	161	60
pomca1	XM_036963012.1	CTCGCTGTCAAGACCTCAACTCT	GAGTTGGGTTGGAGATGGACCTC	118	60

actb, β-actin; *agrp*, agouti-related protein; *cartpt*, cocaine- and amphetamine-regulated transcript prepropeptide (gene encoding Cart); *cckbr*, cholecystokinin B receptor; *eef1a1*, elongation factor 1α; *ghsr1a*, growth hormone secretagogue receptor type 1a (Ghrl receptor); *glp1r*, Glp-1 receptor; *npy*, neuropeptide Y; *npy2r*, NPY receptor type 2 (Pyy receptor); *pomca1*, proopiomelanocortin a1.

3. Results

3.1. Intragastric administration of octanoate, ALA and butyrate differentially modulates feed intake levels in rainbow trout

48 h-fasted fish were intragastrically administered with octanoate, oleate, ALA or butyrate, and feed intake levels was determined at 2, 6 and 24 h post-administration (Fig. 1A). As shown in Fig. 1B, intragastric administration of octanoate significantly reduced feed intake at 2 h, but not 6 or 24 h, post-administration. An anorexigenic response for ALA was also observed at 2 h, but the opposite effect (i.e., an increase in feed intake) was detected at 24 h. Butyrate significantly increased feeding at 6 h and 24 h. No differences in levels of feed intake were detected in fish treated with oleate compared to control fish.



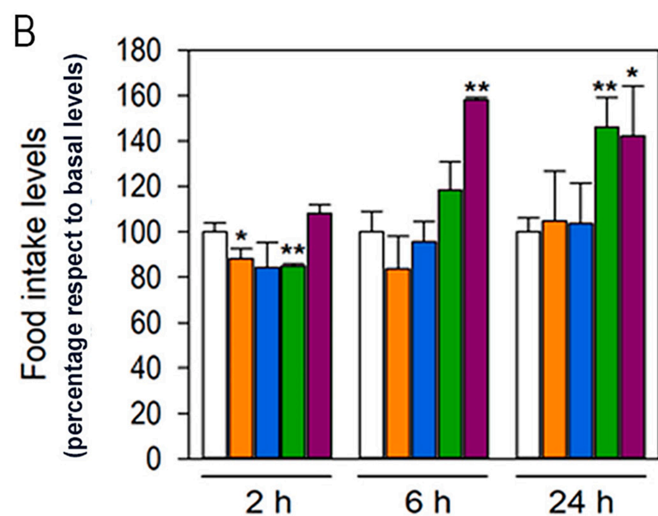


Fig. 1. Effects of the intragastric administration of octanoate, oleate, ALA and butyrate on rainbow trout feed intake. (A) Rainbow trout were intragastrically offered 1 mL per 100 g^{-1} bw of vehicle (distilled water containing 5% EtOH) alone (control) or containing 50 µmol per mL^{-1} of octanoate, oleate, ALA or sodium butyrate, and feed intake levels were evaluated at 2, 6 and 24 h post-administration. (B) Feed intake levels in trout 2, 6 and 24 h after intragastric administration of vehicle (control), octanoate, oleate, ALA and sodium butyrate. Feeding levels are represented as mean + SEM of the amount (in percentage) of feed ingested with respect to baseline levels (determined as the feed intake average the 3 days previous to experiment). Results shown are the mean + SEM of the results obtained in four different experiments. Asterisks indicate significant differences between control and treated groups (*t*-test; * $p < 0.05$, ** $p < 0.01$).

3.2. Intragastric fatty acid administration impacts on the hypothalamic and telencephalic abundance of mRNAs encoding appetite-regulating neuropeptides

To assess whether the luminal presence of fatty acids affects central circuits governing feed intake, expression of genes encoding key appetite-regulating neuropeptides was assessed in the hypothalamus and telencephalon of rainbow trout intragastrically-administered with fatty acids (Fig. 2A). As shown in Fig. 2B-E, the luminal presence of fatty acids led to changes in the mRNA levels of key appetite-regulating neuropeptides, namely the orexigens neuropeptide Y (NPY) and agouti-related protein (AgRP), and the anorexigens proopiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART), in the hypothalamus and telencephalon at 2 h post-administration. Specifically, treatment with octanoate was observed to significantly downregulate *npy* mRNAs in telencephalon (Fig. 2B) and *agrp* in hypothalamus and telencephalon (Fig. 2C), and to upregulate *cartpt* in hypothalamus (Fig. 2E). Abundance of *agrp* mRNAs decreased in response to intragastrically-administered oleate in both brain tissues analysed (Fig. 2C), while the opposite effect was observed for *pomca1* in hypothalamus (Fig. 2D) and for *cartpt* in telencephalon (Fig. 2E). Administration of ALA resulted in increased telencephalic *npy* (Fig. 2B), hypothalamic *pomca1* (Fig. 2D) and telencephalic *cartpt* (Fig. 2E) mRNAs. Finally, fish treated with butyrate showed higher *npy* levels in the telencephalon, as well as higher *agrp* (Fig. 2C) and *pomca1* (Fig. 2E) in the hypothalamus, while showed lower *agrp* abundance in telencephalon (Fig. 2C).

3.3. The phosphorylation status/levels of transcription factors controlling the expression of appetite-regulating neuropeptides in the hypothalamus is modulated by luminal fatty acids

Available knowledge regarding hypothalamic regulation of feed intake indicates that expression of mRNAs encoding appetite-regulating neuropeptides is under control of forkhead box protein O1 (FOXO1), CAMP responsive element binding protein (CREB) and brain specific homeobox (BSX) transcription factors (Soengas, 2021). Based on this, the phosphorylation status/levels of FoxO1, Creb and Bsx was analysed in the hypothalamus of fish intragastrically administered with fatty acids. Treatment with both octanoate and oleate was observed to decrease levels of hypothalamic Bsx and to increase FoxO1 phosphorylation, whereas the opposite changes were detected upon treatment with butyrate (Fig. 2F, H and I). Butyrate also resulted in increased phosphorylated Creb compared to control (Fig. 2G and I). Intragastric administration of ALA did not produce changes in hypothalamic phosphorylation status and/or levels of FoxO1, Creb or Bsx (Fig. 2F – I).

3.4. The luminal presence of fatty acids results in altered brain levels of mRNAs encoding gastrointestinal hormone receptors

Intragastric administration of fatty acids in rainbow trout caused significant variations in the abundance of mRNAs encoding receptor for Ghrl (growth hormone secretagogue receptor type 1a, Ghsr1a), cholecystokinin (cholecystokinin B receptor, CckBR), Pyy (NPY receptor type 2, Npy2R) and/or Glp-1 (Glp-1 receptor, Glp1r) in the hypothalamus and/or telencephalon at 2 h post-administration. Specifically, expression of *ghsr1a* and *npy2r* mRNAs was found to be lower in the hypothalamus of octanoate-treated fish compared to control (Fig. 2J and L). Administration of oleate reduced *ghsr1a* while induced *cckbr* mRNAs in the hypothalamus, and induced *npy2r* mRNAs in telencephalon (Fig. 2J-L). Treatment with ALA significantly reduced the hypothalamic expression of *ghsr1a*, while significantly increased the hypothalamic and telencephalic expression of *cckbr* and the telencephalic expression of *npy2r* (Fig. 2J-L). Finally, fish treated with butyrate had higher *ghsr1a* and lower *cckbr* mRNA abundance in the hypothalamus compared to control fish (Fig. 2J and K).

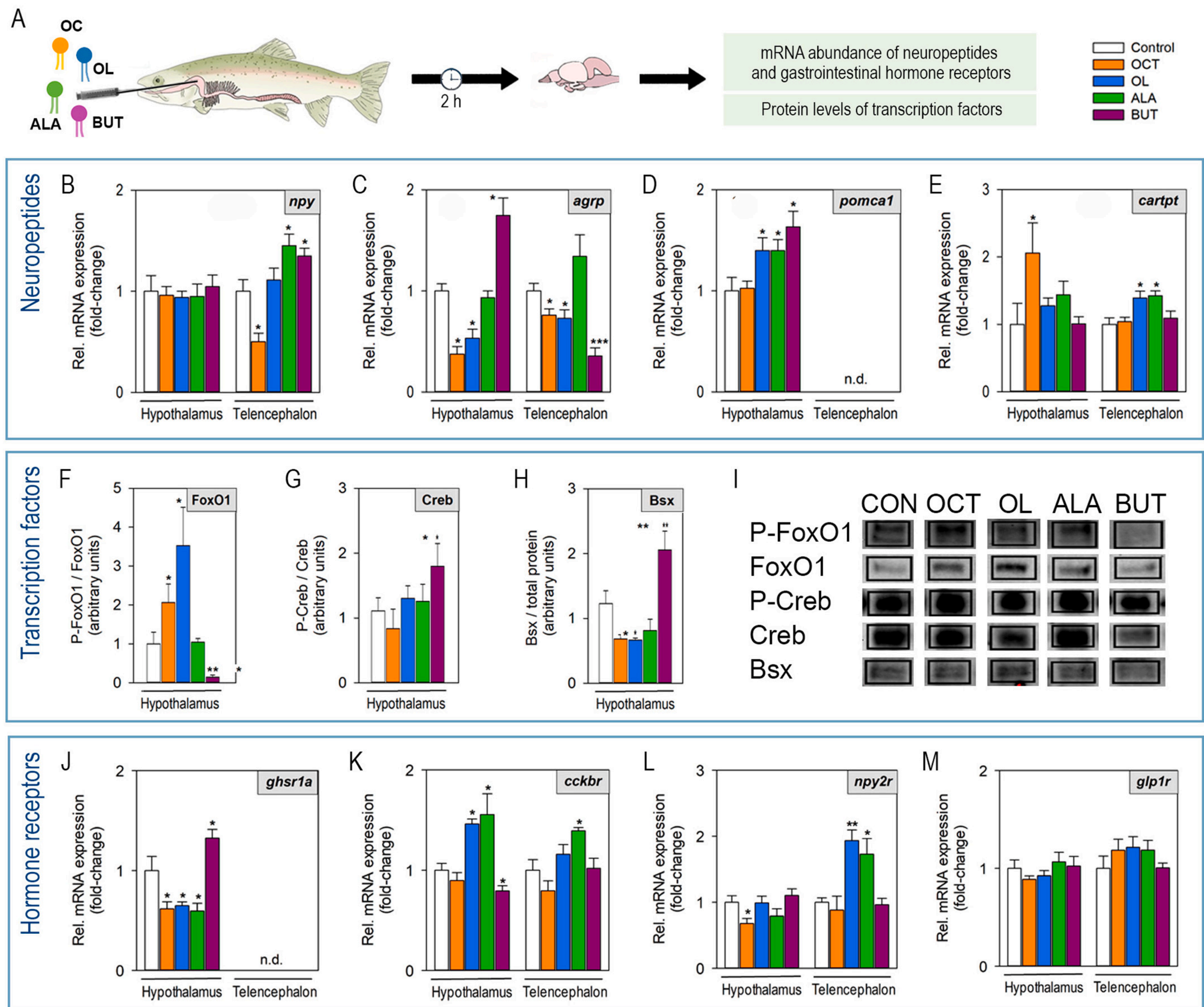


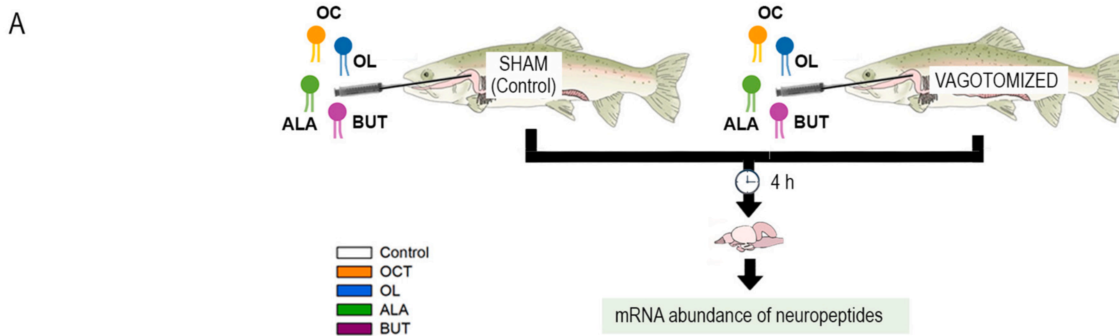
Fig. 2. Impact of the intragastric administration of octanoate, oleate, ALA and butyrate in brain circuits involved in the control of feeding in rainbow trout. (A) Rainbow trout were intragastrically offered 1 mL per 100 g^{-1} bw of vehicle (distilled water containing 5% EtOH) alone (control) or containing 50 μmol per mL^{-1} of octanoate, oleate, ALA or sodium butyrate. Samples of hypothalamus and telencephalon were collected at 2 h post-treatment, and several parameters related to appetite regulation were assessed. (B – E, J – M) Abundance of mRNAs encoding key appetite-regulating neuropeptides and gastrointestinal hormone receptors in rainbow trout hypothalamus and telencephalon 2 h after intragastric administration of vehicle alone or containing octanoate, oleate, ALA or butyrate. Data were normalized to *actb* and *ee1af1α* expression, and are shown as mean + SEM ($n = 6$), relative to the control group. Statistical differences between treatment and control groups were assessed by *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (F – I) Abundance and phosphorylation status of appetite-related transcription factors in the hypothalamus of rainbow trout 2 h after intragastric administration of vehicle alone or containing octanoate, oleate, ALA or butyrate. Data is expressed as the ratio between phosphorylated protein and total amount of the target protein, except for Bsx that is expressed as the ratio of target protein to amount of total protein, relative to the control group. Western blots were carried out on six samples per treatment; one representative blot per treatment is included here (Fig. I). Statistical differences between treatment and control groups were assessed by *t*-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ALA, α-linolenate; *agrp*, agouti-related protein; Bsx, brain specific homeobox; BUT, butyrate; *cartpt*, cocaine- and amphetamine-regulated transcript prepropeptide (gene encoding Cart); *cckbr*, cholecystokinin B receptor; Creb, cAMP responsive element binding protein; FoxO1, forkhead box protein O1; *ghsr1a*, growth hormone secretagogue receptor type 1a (Ghrl receptor); *glp1r*, Glp-1 receptor; mTor, mammalian target of rapamycin; *npy*, neuropeptide Y; *npy2r*, NPY receptor type 2 (Pyy receptor); OC, octanoate; OL, oleate; *pomca1*, proopiomelanocortin a1.

3.5. Brain expression of appetite-regulating neuropeptides mRNAs is not affected (or is slightly affected) by luminal fatty acids in vagotomized rainbow trout

To analyse the putative involvement of the vagus nerve in the gut-to-brain communication in rainbow trout, we assessed whether variations in the hypothalamic and telencephalic abundance of neuropeptides in response of intragastrically-administered fatty acids (described above) are observed in vagotomized individuals (Fig. 3A). Vagotomy procedures used here had been previously used in the same species to study the involvement of hypothalamus in the control of peripheral metabolism (Conde-Sieira et al., 2020). Results showed that all changes in the hypothalamic and telencephalic mRNA abundance of neuropeptides in response to intragastrically-administered octanoate (i.e., decreased telencephalic *npy* and hypothalamic and telencephalic *agrp*, and increased hypothalamic *cartpt*) were observed in sham individuals, and

(except for the decrease in hypothalamic *agrp*) reverted (at least partially) in vagotomized trout (Fig. 2B, C, E and F). Oleate-evoked decrease in *agrp* mRNAs in the hypothalamus and telencephalon observed in the first intragastric experiment was also observed in this experiment in sham individuals, and, again, reverted (at least partially) upon vagotomy (Fig. 2C and F). However, sham trout failed to show the oleate-evoked increase in hypothalamic *pomca1* and telencephalic *cartpt* observed in response to the intragastric administration of fatty acid in

A



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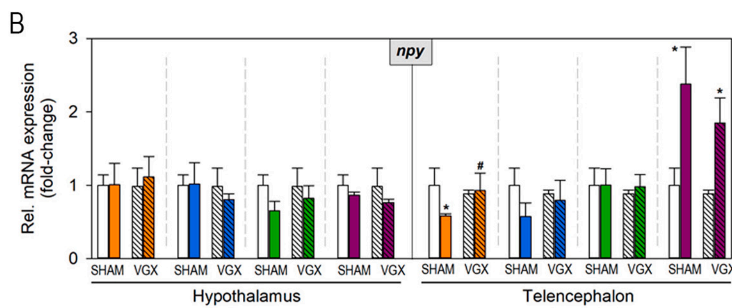

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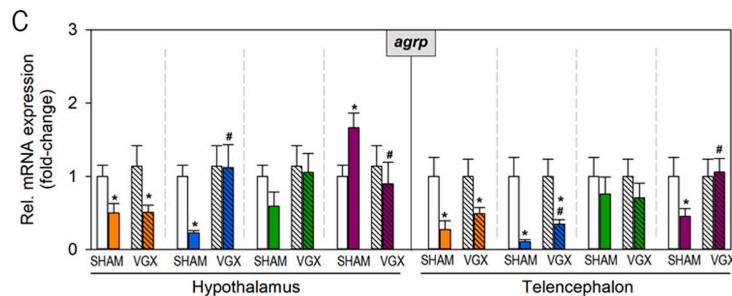

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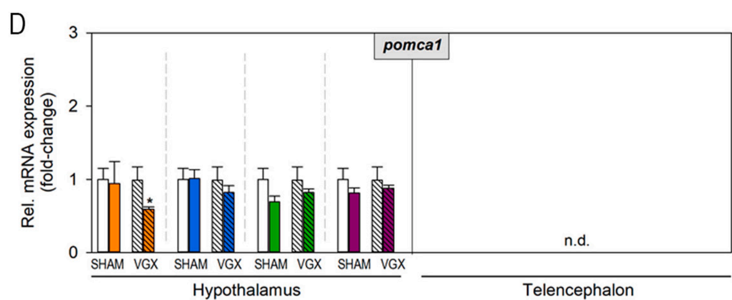

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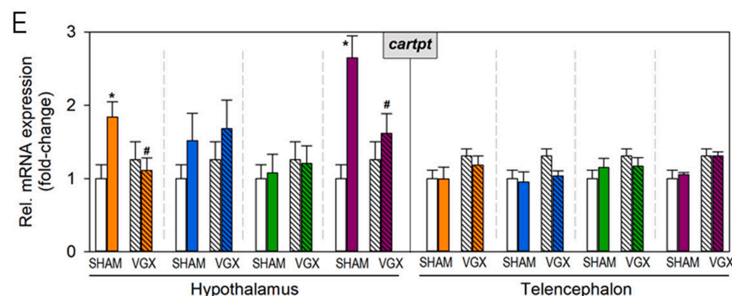

F

		Effects of intragastrically-administered fatty acids on appetite-regulating neuropeptide mRNAs in intact rainbow trout	Equivalent effects of intragastrically-administered fatty acids observed in sham vagotomized rainbow trout?	Effects observed in sham rainbow trout reverted in vagotomized individuals?
OCT	**Hypothalamus**:			
		▼ *agrp*	Yes	No
		▲ cartpt	Yes	Yes
	Telencephalon:			
		▼ *npy*	Yes	Yes
		▼ *agrp*	Yes	Partially
OL	**Hypothalamus**:			
		▼ *agrp*	Yes	Yes
		▲ *pomca1*	No, lack of effect	-
	Telencephalon:			
		▼ *agrp*	Yes	Partially
		▲ *cartpt*	No, lack of effect	-
ALA	**Hypothalamus**:			
		▲ *pomca1*	No	-
	Telencephalon:			
		▲ *npy*	No, lack of effect	-
		▲ *cartpt*	No, lack of effect	-
BUT	**Hypothalamus**:			
		▲ *agrp*	Yes	Yes
		▲ *pomca1*	No, lack of effect	-
	Telencephalon:			
		▲ *npy*	Yes	Partially
		▼ *agrp*	Yes	Yes

(caption on next page)

Fig. 3. Putative involvement of the vagus nerve in the afferent transmission from gut-derived information to the brain. (A) Schematic representation of the experiment carried out to study the putative involvement of the vagal transmission on the afferent signaling from the GIT. Vagotomized (VGX) and sham (control) rainbow trout were intragastrically administered with 1 mL per 100 g^{-1} bw of vehicle (distilled water containing 5% EtOH) alone (control) or containing 50 μmol per mL^{-1} of octanoate, oleate, ALA or sodium butyrate, and, following 4 h, samples of hypothalamus and telencephalon were collected to assess the mRNA abundance of appetite-regulating neuropeptides. (B – E) mRNA abundance of appetite-regulating neuropeptide in the hypothalamus and telencephalon of sham or VGX rainbow trout 4 h after intragastric administration of vehicle alone or containing octanoate, oleate, ALA or butyrate. Data obtained by RT-qPCR were normalized to the expression of *actb* and *ee1af1α*, standardized to the sham-control group, and are shown as mean + SEM (n = 6). Asterisks (*) denote statistical differences ($p < 0.05$) between control (no fatty acid) and fatty acid (OCT, OL, ALA or BUT) treatment within sham or VGX fish; hash signs (#) denote statistical differences ($p < 0.05$) between sham and VGX fish in the absence (control) or presence (OCT, OL, ALA or BUT) of fatty acid treatment, as assessed assessed by two-way ANOVA followed by Holm-Sidak multiple comparison test. (F) Table summerizing the effects of intragastrically-administered fatty acids on the mRNA abundance of appetite-regulating neuropeptides observed in intact (not subjected to surgery) rainbow trout (experiment 1, Fig. 4B-E) and whether such effects were reverted by vagotomy. ALA, α-linolenate; *agrp*, agouti-related protein; BUT, butyrate; *cartpt*, cocaine- and amphetamine-regulated transcript prepropeptide (gene encoding Cart); *npy*, neuropeptide Y; OC, octanoate; OL, oleate; *pomca1*, proopiomelanocortin a1.

intact (not subjected to surgery) rainbow trout (Fig. 2D, E and F). A similar lack of effects of fatty acid treatment in sham trouts was observed in the case of ALA (Fig. 2B-F). Finally, intragastrically-administered butyrate led in general to changes in the mRNA expression of brain neuropeptides in sham trout similar to those observed in intact fish, most of which were not observed in vagotomized fish (Fig. 2B, C and F).

4. Discussion

Nutrients are one of the major factors influencing feed intake. Over the last years, it has been a great interest for studying the mechanisms by which the GIT detects luminal nutrients and how this sensing influences the homeostatic control of appetite. In mammals, it is well known that the GIT contains specific receptors and transporters able to respond to decreased or increased nutrient levels in the lumen by releasing signaling molecules that convey information on nutritional status to the CNS, ultimately modulating feed intake (Janssen and Depoortere, 2013; Raka et al., 2019; Rasoamanana et al., 2012). In fish, knowledge on this topic is very scarce (Blanco et al., 2021). Among the different macronutrients, lipids stand as key nutrients for fish because of three reasons: i) they constitute the main aerobic fuel source for energy metabolism of fish muscle (Tacon, 1987), ii) they are the major energy source in aquaculture nutrition (Bell and Koppe, 2010), and iii) there are important differences in lipid metabolism between mammals and fish (Kuah et al., 2015). In recent research from our group, we described that the luminal presence of fatty acids of different length and degree of unsaturation (MCFA: octanoate, LCFA: oleate, PUFA: ALA, and SCFA: butyrate) differentially modulate the expression of several fatty acid receptors and transporters, as well as mRNA/protein levels of major appetite-regulating hormones, in the rainbow trout GIT (Calo et al., *under review*), suggesting that the rainbow trout gut is able to respond to luminal fatty acids in a way comparable to that in mammals. However, the putative impact of luminal fatty acids in central circuits governing feed intake remains unknown. The present research aims to address this gap of knowledge in the fish literature and offers the first report on the impact of gastrointestinal sensing of fatty acids on brain parameters governing feed intake in a carnivore fish species, the rainbow trout.

As mentioned in earlier sections, the impact of gastrointestinal nutrient sensing in the brain would be mediated by the gut-to-brain signaling exerted by signaling molecules (i.e., hormones) secreted by the GIT. Thus, apart from exerting local actions, hormones released by the GIT reach the CNS, particularly regions involved in the control of feeding. The most important of such regions is (in both mammals and fish) the hypothalamus, which functions to integrate biologically relevant information related to feeding and energy balance to achieve appropriate behavioral output (Delgado et al., 2017; Schwartz et al., 2000). In this region, gastrointestinal hormones would activate a network starting with the modulation of intracellular signaling elements (e.g., AMP-activated protein kinase -AMPK- and mammalian target of rapamycin -mTOR-) which in turn regulate the phosphorylation status of transcription factors (e.g., FOXO1, CREB, BSX) controlling the expression of several appetite-regulating neuropeptides, mainly the orexigens NPY and AgRP, and the anorexigens POMC and CART. Changes in the amount of these neuropeptides finally result in changes in feed intake (Delgado et al., 2017; Schwartz et al., 2000; Soengas, 2021). Besides the hypothalamus, the fish telencephalon (equivalent to the mammalian forebrain) has lately gained attention as an important modulator of feeding behavior (Soengas et al., 2018). The telencephalon has been mainly related to hedonic circuits underlying feeding (Soengas et al., 2018), but expression of Npy, AgRP, Pomc and Cart has been detected in this area in several fish species (Burren and Pietsch, 2021; Cerdá-Reverter et al., 2000; García-Fernández et al., 1992; Subhedar et al., 2011; Vallarino et al., 1989), supporting also a role in the homeostatic control of feed intake.

Major results from the present study demonstrated important changes in the levels of mRNAs encoding Npy, Agrp, Pomc and/or Cart, accompanied with changes in the phosphorylation status/levels of FoxO1, Creb and/or Bsx, in the hypothalamus and/or telencephalon of rainbow trout at 2 h post-intragastric administration of fatty acids. Observed changes in the activity of transcription factors and neuropeptide expression were different depending on the fatty acid tested, therefore resulting in a different effect on feed intake levels. In this regard, octanoate intragastric treatment was found to downregulate *npy* mRNAs in telencephalon and *agrp* mRNAs in both hypothalamus and telencephalon, while it upregulated *cartpt* mRNAs in hypothalamus. This occurred in parallel with a reduction in Bsx levels and an increase in the phosphorylation status of FoxO1 in the hypothalamus, changes that are consistent with those previously described for these parameters after octanoate injection in rainbow trout (Soengas, 2021). All these observations, together with the octanoate-evoked increase in Cck, Pyy and Glp-1 levels, agree with an enhanced anorexigenic potential. Indeed, in the present study, reduced feed intake levels were detected in rainbow trout 2 h after octanoate administration. This is consistent with previous reports from our research group showing a reduction in feeding levels in response to intracerebroventricular (Librán-Pérez et al., 2014) or intraperitoneal (Librán-Pérez et al., 2015b, 2012) administration of octanoate in the same fish species. The anorexigenic nature of octanoate appears to be specific of fish (at least rainbow trout), given that treatment with this fatty acid in mammals did not affect feed intake (Obici et al., 2002).

As for oleate, treatment-derived changes in central neuropeptide expression (decreased *agrp* mRNAs in hypothalamus and telencephalon, and increased hypothalamic *pomca1* and telencephalic *cartpt* mRNAs) and in the activity of transcription factors (reduced Bsx levels and increased phosphorylation status of FoxO1 in hypothalamus) also enhanced anorexigenic potential, supported as well by the observed increased abundance of Cck, Pyy and Glp-1 in response to this fatty acid. However, feed intake levels were not significantly altered upon administration of oleate at any of the times analysed. It might be possible that a different dose than the one used in the present experiment is required to modify feeding levels, or that such a response occurs at a shorter/longer time than the ones analysed. As described for octanoate, both central (Librán-Pérez et al., 2014) and peripheral (Librán-Pérez et al., 2015b, 2012) administration of oleate were reported to

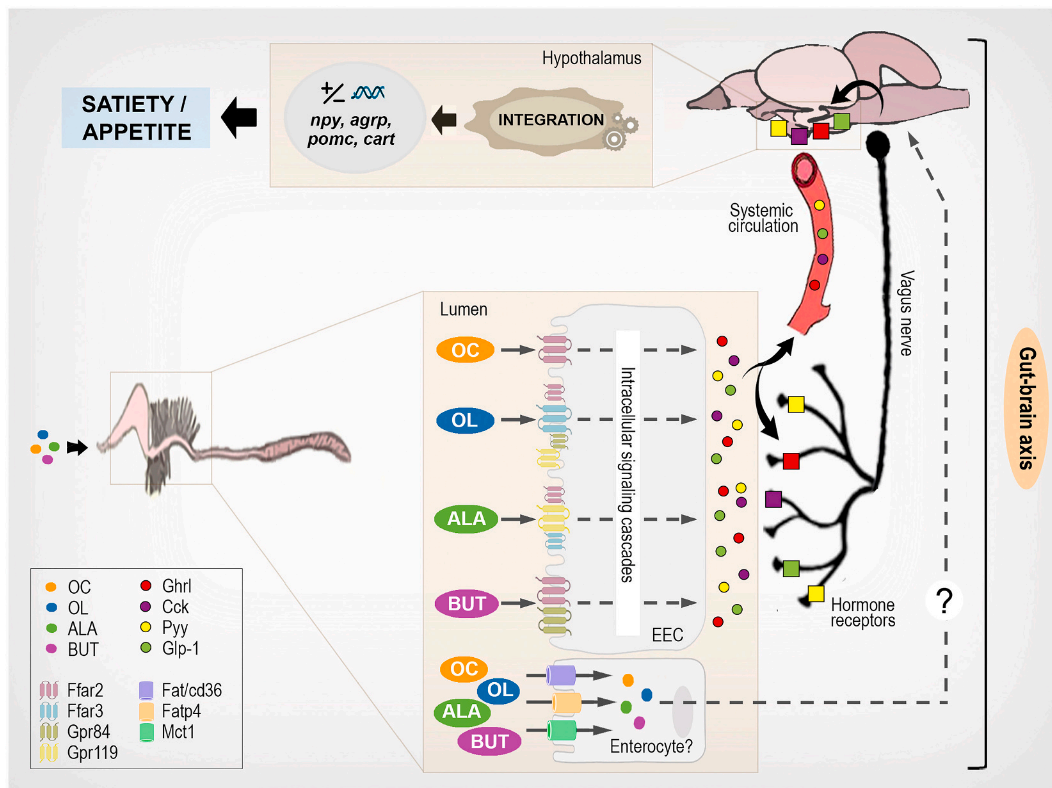

Fig. 4. Schematic representation of the proposed role of gut fatty acid sensing in the control of feed intake in fish. The rainbow trout intestine expresses mRNAs encoding several fatty acid transporters (Fat/cd36, Fatp4 and Mct-1) and receptors (Ffar2, Ffar3, Gpr84 and Gpr119). These receptors, likely located in the apical face of enteroendocrine cells, would selectively detect the luminal presence of fatty acids, and subsequently trigger intracellular signaling cascades resulting in an increase in intracellular [Ca^{2+}]. This would lead to the release of peptide hormones, mainly ghrelin, CCK, PYY and GLP-1, in the lamina propria (Calo et al., *under review*). These peptides would activate respective receptors located in vagal afferent terminals innervating the GIT or enter into systemic circulation, thus (either through vagal transmission or bloodstream, respectively) transmitting information of nutrient availability to the CNS. The CNS (particularly the hypothalamus) would then integrate gut-derived information and respond with changes in the expression of key appetite-regulating neuropeptides (mainly Npy, Agrp, Pomc and Cart), ultimately controlling feeding levels. *Agrp*, agouti-related protein; ALA, α-linolenate; BUT, butyrate; *cart*, cocaine and amphetamine regulated transcript; Cck, cholecystokinin; EEC, enteroendocrine cell; Fat/cd36, fatty acid transporter Cd36; Fatp4, fatty acid transporter 4; Ffar2/3, free fatty acid receptor 2/3; Ghrl, ghrelin; Glp-1, glucagon-like peptide-1; Gpr84/119, G protein coupled-receptor 84/119; Mct1, monocarboxylate transporter isoform 1; *npy*, neuropeptide Y; OC, octanoate; OL, oleate; *pomc*, proopiomelanocortin; Pyy, peptide tyrosine-tyrosine.

supress feed intake in trout. Additionally, reduced feeding in response to intracerebroventricularly injected oleate was described in rats (Morgan et al., 2004; Obici et al., 2002).

Regarding ALA, present results reported a time-dependent effect of this fatty acid on feed intake, characterized by an anorexigenic action at 2 h, but an orexigenic response at 24 h. Molecular mechanisms underlying this bimodal response need further exploration, as, while the herein observed ALA-evoked increase in *cartpt* and *npy* mRNAs in the telecephalon might respectively explain its effects on feed intake, almost no changes in neuropeptide expression and transcription factor activity were detected in hypothalamus besides a slight induction of *pomca1* expression. In addition, at the GIT level, ALA was observed to induce the expression of both orexigenic gastrointestinal hormones (Ghrl) and anorexigenic (Cck, Pyy and Glp-1). The involvement of ALA in the regulation of feed intake is poorly known. In a previous study from our group, intraperitoneal administration of this fatty acid, while led to unaltered feeding levels, was reported to reduce both *agrp2* and *cart4* mRNAs in the Senegalese sole hypothalamus, observations that also argues in favor of a bimodal nature of ALA in regulating feed intake (Conde-Sieira et al., 2015).

Finally, unlike the rest of fatty acids tested, intragastrically administered butyrate led to changes in central neuropeptide expression (increased hypothalamic *agrp* and telencephalic *npy* mRNAs) and in the activity of transcription factors (increased Creb phosphorylation and Bsx levels, and reduced FoxO1 phosphorylation) resulting in enhanced orexigenic potential. Such an orexigenic trend is the opposite of the anorexigenic effect elicited by all other FAs assessed, and was also evidenced at the GIT level, as represented by increased butyrate-evoked Ghrl levels and decreased Cck and Pyy. The orexigenic potential observed for butyrate matches with the observed increased levels of feeding in rainbow trout treated with this fatty acid. As far as we are aware, this is the first report on the effects of butyrate or any SCFA in feed intake levels in fish. We currently cannot offer a certain explanation on why butyrate exerts an opposite effect on feed intake in rainbow trout than that of the rest of fatty acid tested. It might be possible that the gut microbiota (main producer of endogenous SCFAs in the body) may be influencing such a response. However, future studies are needed to elucidate the physiological significance of butyrate's orexigenic response in rainbow trout. In any case, this result agrees with previous studies in mammals reporting increased feed consumption in pigs fed a diet with sodium n-butyrate (Gálfi and Bokori, 1990). Together, these set of results demonstrate that hormones released in the rainbow trout GIT impact hypothalamic and telencephalic circuits governing feed intake, thus indicating the existence of a gut-brain axis in fish, as is the case in mammals (Blanco et al., 2021).

How do the signals from the GIT reach the CNS? According to the mammalian model, gastrointestinal hormones can either enter the systemic circulation and bind to corresponding receptors located in appetite-regulating brain centers (i.e., hypothalamus, telencephalon) or they can activate respective receptors on vagal afferent terminals

innervating the GIT and thus signal to the brain via the vagus nerve (Janssen and Depoortere, 2013; Raka et al., 2019; Rasoamanana et al., 2012). Because of rapid break down by proteases, signaling via the vagus nerve is probably the most potent signal transmitter to the brain (Moran and Dailey, 2011). Therefore, in the present study we focused primarily on the study of a putative vagal transmission. Nevertheless, the observed changes in the expression of mRNAs encoding receptor for Ghrl (Ghsr1a), cholecystokinin (CckBR), Pyy (Npy2R) and/or Glp-1 (Glp1r) in the hypothalamus and/or telencephalon of rainbow trout in response to the intragastric administration of fatty acids argue in favor of (at least part of) the signaling from the GIT to the brain in rainbow trout taking place via gastrointestinal hormone transport through systemic circulation. To study the possible implication of the vagus nerve in the afferent signaling of gastrointestinal hormones, we assessed whether changes in the hypothalamic and telencephalic abundance of appetite-regulating neuropeptides in response of intragastrically-administered fatty acids (described in the first experiment) are also observed in vagotomized fish. Results from this experiment only considering sham individuals were in general (except for treatment with ALA) equivalent to those observed in the first experiment (summarized in Fig. 3F), corroborating the modulatory action of luminal fatty acids on brain circuits governing appetite. Almost all of such luminal fatty acid-induced changes in the levels of *npy*, *agrp*, *pomca1* and/or *cartpt* mRNAs in the hypothalamus and/or telencephalon were not observed, or were lessened, in vagomotomized rainbow trout. These include octanoate-induced changes in hypothalamic *cartpt* and telencephalic *npy* and *agrp*, oleate-induced changes in hypothalamic and telencephalic *agrp*, and butyrate-induced changes in hypothalamic *agrp* and telencephalic *npy* and *agrp*. These results support, for the first time in fish, that vagal transmission is an important element of the gut-to-brain communication in rainbow trout, importantly mediating the gastrointestinal input to central circuits governing feed intake.

In summary, the present study demonstrates for the first time in fish that the luminal presence of fatty acids has an important impact on central appetite-regulating circuits, ultimately affecting feed intake levels, and offers important evidence supporting that such a connection between the gut and brain would be mediated in fish by the transmission of information by gastrointestinal hormones via both the vagus nerve and systemic circulation. This provides consistent evidence supporting the existence of a gut-brain axis in fish, as it exists in mammals (Fig. 4). It is of relevance to point out that the present study demonstrates for the first time in fish that butyrate has a modulatory role on feed intake, suggesting that the fish (at least rainbow trout) gut microbiota may influence feeding behavior, as described in mammals. This observation sets the basis for a yet unexplored field of study in fish, which would allow us to have a wider point of view of the gastrointestinal regulation of appetite in fish. Considering differences in fatty acid composition in aquafeeds, their differencital impact on gastrointestional regulation of feed intake in fish is very relevant for aquaculture.

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Authors' contributions

J.C., J.L.S. and A.M.B. designed the experiments, J.C., M.C-S., S.C. and A.M.B. performed the experiments, J.C. and A.M.B. analysed samples, J.C., J.L.S. and A.M.B. interpreted results and wrote the manuscripts, all authors approved final version of the manuscript, J.L.S. secured funding for the study.

Declaration of Competing Interest

No competing interests are declared by authors.

Data availability

Data will be made available on request.

References

Bell, J., Koppe, W., 2010. Lipids in aquafeeds. In: Turchini, G., Ng, W.-K., Tocher, D. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, pp. 21–59. https://doi.org/10.1201/9781439808634-c2.

Blanco, A.M., Calo, J., Soengas, J.L., 2021. The gut-brain axis in vertebrates: implications in food intake regulation. J. Exp. Biol. 224, jeb231571. https://doi.org/10.1242/jeb.231571.

Burren, A., Pietsch, C., 2021. Distress regulates different pathways in the brain of common carp: a preliminary study. Animals. 11, 585. https://doi.org/10.3390/ani11020585.

Calo, J., Blanco, A.M., Comesaña, S., Conde-Sieira, M., Morais, S., Soengas, J.L., 2021. First evidence for the presence of amino acid sensing mechanisms in the fish gastrointestinal tract. Sci. Rep. 11, 4933. https://doi.org/10.1038/s41598-021-84303-9.

Cerdá-Reverter, J.M., Anglade, I., Martínez-Rodríguez, G., Mazurais, D., Muñoz-Cueto, J. A., Carrillo, M., Kah, O., Zanuy, S., 2000. Characterization of neuropeptide Y expression in the brain of a perciform fish, the sea bass (*Dicentrarchus labrax*). J. Chem. Neuroanat. 19, 197–210. https://doi.org/10.1016/s0891-0618(00)00063-6.

Chambers, A.P., Sandoval, D.A., Seeley, R.J., 2013. Integration of satiety signals by the central nervous system. Curr. Biol. 23, R379–R388. https://doi.org/10.1016/j.cub.2013.03.020.

Conde-Sieira, M., Bonacic, K., Velasco, C., Valente, L.M.P., Morais, S., Soengas, J.L., 2015. Hypothalamic fatty acid sensing in Senegalese sole (*Solea senegalensis*): response to long-chain saturated, monounsaturated, and polyunsaturated (n-3) fatty acids. Am. J. Phys. Regul. Integr. Comp. Phys. 309, R1521–R1531. https://doi.org/10.1152/ajpregu.00386.2015.

Conde-Sieira, M., Ceinos, R.M., Velasco, C., Comesaña, S., López-Patiño, M.A., Míguez, J. M., Soengas, J.L., 2018. Response of rainbow trout's (*Oncorhynchus mykiss*) hypothalamus to glucose and oleate assessed through transcription factors BSX, ChREBP, CREB, and FoxO1. J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol. 204, 893–904. https://doi.org/10.1007/s00359-018-1288-7.

Conde-Sieira, M., Capelli, V., Álvarez-Otero, R., Díaz-Rúa, A., Velasco, C., Comesaña, S., López, M., Soengas, J.L., 2020. Hypothalamic AMPKα2 regulates liver energy metabolism in rainbow trout through vagal innervation. Am. J. Phys. Regul. Integr. Comp. Phys. 318, R122–R134. https://doi.org/10.1152/ajpregu.00264.2019.

Cummings, D.E., Overduin, J., 2007. Gastrointestinal regulation of food intake. J. Clin. Invest. 117, 13–23. https://doi.org/10.1172/JCI30227.

De Pedro, N., Pinillos, M.L., Valenciano, A.I., Alonso-Bedate, M., Delgado, M.J., 1998. Inhibitory effect of serotonin on feeding behavior in goldfish: involvement of CRF. Peptides 19, 505–511. https://doi.org/10.1016/s0196-9781(97)00469-5.

Delgado, M.J., Cerdá-Reverter, J.M., Soengas, J.L., 2017. Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. Front. Neurosci. 11, 354. https://doi.org/10.3389/fnins.2017.00354.

Gálfi, P., Bokori, J., 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Vet. Hung. 38, 3–17.

García-Fernández, J.M., del Brío, M.A., Cernuda, R., Coto, A., Riera, P., 1992. Distribution of neuropeptide Y-like immunoreactivity in the brain of *Salmo salar* and Gambusia affinis. Histol. Histopathol. 7, 385–392.

Guyenet, S.J., Schwartz, M.W., 2012. Clinical review: regulation of food intake, energy balance, and body fat mass: implications for the pathogenesis and treatment of obesity. J. Clin. Endocrinol. Metab. 97, 745–755. https://doi.org/10.1210/jc.2011-2525.

Janssen, S., Depoortere, I., 2013. Nutrient sensing in the gut: new roads to therapeutics? Trends Endocrinol. Metab. 24, 92–100. https://doi.org/10.1016/j.tem.2012.11.006.

Kuah, M.-K., Jaya-Ram, A., Shu-Chien, A.C., 2015. The capacity for long-chain polyunsaturated fatty acid synthesis in a carnivorous vertebrate: functional characterisation and nutritional regulation of a Fads2 fatty acyl desaturase with Δ4 activity and an Elovl5 elongase in striped snakehead (*Channa striata*). Biochim. Biophys. Acta 1851, 248–260. https://doi.org/10.1016/j.bbalip.2014.12.012.

Librán-Pérez, M., Polakof, S., López-Patiño, M.A., Míguez, J.M., Soengas, J.L., 2012. Evidence of a metabolic fatty acid-sensing system in the hypothalamus and Brockmann bodies of rainbow trout: implications in food intake regulation. Am. J. Phys. Regul. Integr. Comp. Phys. 302, R1340–R1350. https://doi.org/10.1152/ajpregu.00070.2012.

Librán-Pérez, M., Otero-Rodiño, C., López-Patiño, M.A., Míguez, J.M., Soengas, J.L., 2014. Central administration of oleate or octanoate activates hypothalamic fatty acid sensing and inhibits food intake in rainbow trout. Physiol. Behav. 129, 272–279. https://doi.org/10.1016/j.physbeh.2014.02.061.

Librán-Pérez, M., Geurden, I., Dias, K., Corraze, G., Panserat, S., Soengas, J.L., 2015a. Feeding rainbow trout with a lipid-enriched diet: effects on fatty acid sensing, regulation of food intake and cellular signaling pathways. J. Exp. Biol. 218, 2610–2619. https://doi.org/10.1242/jeb.123802.

Librán-Pérez, M., Velasco, C., Otero-Rodiño, C., López-Patiño, M.A., Míguez, J.M., Soengas, J.L., 2015b. Effects of insulin treatment on the response to oleate and octanoate of food intake and fatty acid-sensing systems in rainbow trout. Domest. Anim. Endocrinol. 53, 124–135. https://doi.org/10.1016/j.domaniend.2015.06.004.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Moran, T.H., Dailey, M.J., 2011. Intestinal feedback signaling and satiety. Physiol. Behav. 105, 77–81. https://doi.org/10.1016/j.physbeh.2011.02.005.

Morgan, K., Obici, S., Rossetti, L., 2004. Hypothalamic responses to long-chain fatty acids are nutritionally regulated. J. Biol. Chem. 279, 31139–31148. https://doi.org/10.1074/jbc.M400458200.

Obici, S., Feng, Z., Morgan, K., Stein, D., Karkanias, G., Rossetti, L., 2002. Central administration of oleic acid inhibits glucose production and food intake. Diabetes 51, 271–275. https://doi.org/10.2337/diabetes.51.2.271.

Polakof, S., Soengas, J.L., 2013. Evidence of sugar sensitive genes in the gut of a carnivorous fish species. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 166, 58–64. https://doi.org/10.1016/j.cbpb.2013.07.003.

Polakof, S., Alvarez, R., Soengas, J.L., 2010. Gut glucose metabolism in rainbow trout: implications in glucose homeostasis and glucosensing capacity. Am. J. Phys. Regul. Integr. Comp. Phys. 299, R19–R32. https://doi.org/10.1152/ajpregu.00005.2010.

Raka, F., Farr, S., Kelly, J., Stoianov, A., Adeli, K., 2019. Metabolic control via nutrient-sensing mechanisms: role of taste receptors and the gut-brain neuroendocrine axis. Am. J. Physiol. Endocrinol. Metab. 317, E559–E572. https://doi.org/10.1152/ajpendo.00036.2019.

Rasoamanana, R., Darcel, N., Fromentin, G., Tomé, D., 2012. Nutrient sensing and signalling by the gut. Proc. Nutr. Soc. 71, 446–455. https://doi.org/10.1017/S0029665112000110.

Santos, A.L., Preta, G., 2018. Lipids in the cell: organisation regulates function. Cell. Mol. Life Sci. 75, 1909–1927. https://doi.org/10.1007/s00018-018-2765-4.

Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. Nature 404, 661–671. https://doi.org/10.1038/35007534.

Seth, H., Axelsson, M., 2010. Sympathetic, parasympathetic and enteric regulation of the gastrointestinal vasculature in rainbow trout (*Oncorhynchus mykiss*) under normal and postprandial conditions. J. Exp. Biol. 213, 3118–3126. https://doi.org/10.1242/jeb.043612.

Soengas, J.L., 2021. Integration of nutrient sensing in fish hypothalamus. Front. Neurosci. 15, 653928 https://doi.org/10.3389/fnins.2021.653928.

Soengas, J.L., Cerdá-Reverter, J.M., Delgado, M.J., 2018. Central regulation of food intake in fish: an evolutionary perspective. J. Mol. Endocrinol. 60, R171–R199. https://doi.org/10.1530/JME-17-0320.

Subhedar, N., Barsagade, V.G., Singru, P.S., Thim, L., Clausen, J.T., 2011. Cocaine- and amphetamine-regulated transcript peptide (CART) in the telencephalon of the catfish, *Clarias gariepinus*: distribution and response to fasting, 2-deoxy-D-glucose, glucose, insulin, and leptin treatments. J. Comp. Neurol. 519, 1281–1300. https://doi.org/10.1002/cne.22569.

Tacon, A.G.J., 1987. The Essential Nutrients, in: the Nutrition and Feeding of Farmed Fish and Shrimp - a Training Manual. FAO, Brasilia, Brazil. GCP/RLA/075/ITA.

Vallarino, M., Delbende, C., Bunel, D.T., Ottonello, I., Vaudry, H., 1989. Proopiomelanocortin (POMC)-related peptides in the brain of the rainbow trout, Salmo gairdneri. Peptides. 10, 1223–1230. https://doi.org/10.1016/0196-9781(89)90016-8.

Velasco, C., Bonacic, K., Soengas, J.L., Morais, S., 2017. Orally administered fatty acids enhance anorectic potential but do not activate central fatty acid sensing in Senegalese sole post-larvae. J. Exp. Biol. 220, 677–685. https://doi.org/10.1242/jeb.150979.

Ye, L., Mueller, O., Bagwell, J., Bagnat, M., Liddle, R.A., Rawls, J.F., 2019. High fat diet induces microbiota-dependent silencing of enteroendocrine cells. eLife 8, e48479. https://doi.org/10.7554/eLife.48479.