

INFORME PERIODO...2016-2017

1. APELLIDO....REYNALDO.....
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2. OTROS DATOS

INGRESO: Categoría...Asistente.....Mes...Noviembre.....Año...1998
ACTUAL: Categoría...Principal.....Mes...Septiembre.....Año...2011

3. PROYECTOS DE INVESTIGACION EN LOS CUALES COLABORA

a) Estudio del rol modulador del receptor de ghrelina sobre las conductas hiperfágicas agudas. **b)** Estudio del circuito neuronal por el cual la ghrelina coordina acciones orexigénicas y señales de estrés. **c)** Estudios del efecto modulador de ghrelina sobre las neuronas neuropeptidérgicas del hipotálamo. **d)** Estudio multidisciplinario de enfermedades crónicas relacionadas a desarreglos en el peso corporal de origen multifactorial y de relevancia regional y nacional.

4. DIRECTOR

Apellido y Nombre (s)... PERELLÓ, Mario.....
Cargo Institución.... Investigador Independiente del CONICET.....
Dirección: Calle. 526. y.Camino Gral Belgrano...N° .s/n.....Ciudad...La Plata.....
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5. LUGAR DE TRABAJO

Institución..... Instituto Multidisciplinario de Biología Celular (IMBICE).....
Dependencia..... CIC-CONICET-UNLP.....
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Ciudad...La Plata.....C. P..1900.....Prov..Buenos.Aires.....Tel. (0221) 421-0112.....

6. INSTITUCION DONDE DESARROLLA TAREAS DOCENTES U OTRAS

Nombre. **Dedicación Exclusiva con actividad única. No Desarrollo Tareas Docentes ni Otras.**

Dependencia.....

Dirección: Calle.....Nº.....

Ciudad.....C. P.....Prov.....Tel.....

Cargo que ocupa.....

7. RESUMEN DE LA LABOR QUE DESARROLLA (Descripción para el repositorio institucional.

Máximo 150 palabras

8. EXPOSICION SINTETICA DE LA LABOR DESARROLLADA EN EL PERIODO (Debe exponerse la actividad desarrollada, técnicas empleadas, métodos, etc. en dos carillas como máximo, en letra arial 12, a simple espacio)

9. OTRAS ACTIVIDADES

9.1 PUBLICACIONES, COMUNICACIONES, ETC. Debe hacerse referencia, exclusivamente, a aquellas publicaciones en las cuales se ha hecho explícita mención de la calidad de personal de apoyo de la CIC. Toda publicación donde no figure dicha aclaración no debe ser adjuntada. Indicar el nombre de los autores de cada trabajo en el mismo orden en que aparecen en la publicación, informe o memoria técnica, año y, si corresponde, volumen y página, asignándole a cada uno un número.

9.2 CURSOS DE PERFECCIONAMIENTO, VIAJES DE ESTUDIO, ETC. Indicar la denominación del curso, carga horaria, institución que lo dictó y fecha, o motivos del viaje, fecha, duración, instituciones visitadas y actividades realizadas.

9.3 ASISTENCIA A REUNIONES CIENTIFICAS/TECNOLOGICAS o EVENTOS SIMILARES. Indicar la denominación del evento, lugar y fecha de realización, tipo de participación que le cupo y título(s) del(los) trabajo(s) o comunicación(es) presentada(s).

10. TAREAS DOCENTES DESARROLLADAS EN EL PERIODO.

11. OTROS ELEMENTOS DE JUICIO NO CONTEMPLADOS EN LOS TITULOS ANTERIORES. (En este punto se indicará todo lo que se considere de interés para una mejor evaluación de la tarea cumplida en el período).

PAUTAS A SEGUIR EN LA ELABORACIÓN DEL INFORME

Pautas generales

- El informe debe contener los títulos y subtítulos completos que se detallan en hojas adjuntas y un índice
- Se deben anexar al final del informe las copias de las publicaciones, resúmenes de trabajos, informes y memorias técnicas a los que se hace referencia en el desarrollo del mismo, así como cualquier otra documentación que se considere de interés..
- El informe se deberá presentar impreso en hojas **perforadas** A-4. En la etiqueta de mismo se consignará el apellido y nombre del Personal de Apoyo y la leyenda «Informe Científico-tecnológico período
- Incluir en la presentación del informe (en sobre cerrado) la opinión del Director.

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7.- RESUMEN DE LA LABOR DESARROLLADA

La ghrelina es una hormona secretada por células endócrinas de la mucosa gástrica que regula varias funciones neuroendócrinas y la ingesta de alimentos. Sus acciones están mediadas por el receptor secretagogo de la hormona del crecimiento (GHSR). Mostramos que la ghrelina circulante accede principalmente al Área Postrema (AP), que su señalización es necesaria para la escalada en la ingesta de dieta alta en grasa, que las neuronas sensibles a la misma del AP son principalmente GABAérgicas y, que una AP intacta aumenta el vaciado gástrico inducido por ghrelina. Además, hallamos que la des-acil-ghrelina se une y actúa específicamente sobre un subconjunto de células de Núcleo Arcuato (ARC) de una manera independiente de *GHSR* y antagoniza los efectos orexigénicos cuando se la administra periféricamente; también que la F-ghrelina (Ghrelina Fluorescente) se comporta de forma similar a la ghrelina y puede usarse para estudiar tanto la unión como la captación de esta hormona.

8.- EXPOSICIÓN SINTÉTICA DE LA LABOR DESARROLLADA EN EL PERÍODO

Durante el período Agosto 2016 - Agosto 2017, en el Laboratorio de Neurofisiología del IMBICE y con la dirección del Dr. Mario Perelló llevé a cabo para los proyectos en ejecución del laboratorio las siguientes tareas:

I.) perfusión transcárdica de ratones salvajes o transgénicos; II.) disección de cerebros; III.) cortes con crióstato en el plano coronal de cerebros, en series o con técnica de montaje en frío, para animales tratados y no tratados, perfundidos o sin perfundir; IV.) montaje de las fetas de cerebros y deshidratación luego de las técnicas de coloración con diferentes medios de montajes; V) puesta a punto de cortes de cerebros con crióstato en los planos sagital y horizontal para ratones salvajes o transgénicos, sus fetas fueron montadas secuencialmente sobre portaobjetos en frío; VI.) puesta a punto de cortes con crióstato de: a.) estómago y b.) glándula adrenal, en ambos casos el material obtenido fue montado en orden sucesivo sobre portaobjetos en frío; VII.) criopreservación de cerebros; VIII.) determinación de concentraciones de uso para el anticuerpo (Ac) c-Fos (marcador de Actividad Neuronal) tanto policlonal como monoclonal; IX.) inmunohistoquímicas (IHQ) simples y dobles cromogénicas y/o fluorescentes en flotación o montadas en portaobjetos de muestras cortadas a 40 micras para: a.) desenmascaramiento antigénico con y sin citrato para los Ac GFP (Green Fluorescent Proteins) y eGFP (enhanced green fluorescent protein) con secundario anti-goat o anti-conejo; b.) anti-fluoresceína y anti-goat como secundario; c.) C-fos policlonal y monoclonal y anti-conejo o anti-mouse como Ac primario y secundario, respectivamente (**Plate I**); d.) anti-ghrelina con controles negativos y positivos en muestras cortadas a 20 micras y anti-goat como secundario; e.) Factor Liberador de Corticotrofina (CRF-generado por Dra. Castro, Universidad de Michigan, USA) y Ac secundario anti-conejo y, f.) AGRP (amide Agouti-Related Protein) con secundario Alexa 488, entre otros; X.) coloraciones de Nissl (marca ribosomas libres y asociados a membranas de las neuronas) en muestras de cerebros cortados en el plano coronal, sagital y horizontal (**Plate II**); XI.) mapeos de expresión de señales positivas en animales controles y tratados, alimentados o con diferentes tiempos de ayuno; XII.) conteos y registros de expresión de señales positivas individuales y de colocalizaciones para: a.) c-Fos-TH (Tirosina Hidroxilasa); b.) GFP y C-Fos; c.) POMC (Pro-

opio-melano-cortina) y, d.) ISH (Hibridación in situ), entre otros; **XIII.)** análisis de señales positivas, por conteo, en los siguientes núcleos cerebrales: a.) PVN (Paraventricular) para 6 bregmas de -0,58 a -1,22 en 13 animales; b.) ARC en 8 bregmas de -1,22 a -2,06 en 48 animales; c.) ARC y PVN en 4 bregmas de -1,58 a -1,94 y -0,58 a -0,94, respectivamente, en 8 animales para cada uno; d.) ISH en el NTS (del Tracto Solitario) para 18 bregmas de -6,24 a -8,24 en 6 animales; e.) ISH en el LH (Hipotálamo Lateral) para 21 bregmas de -0,34 a -2,80 en 5 animales; f.) NTS para 16 bregmas de -6,24 a -8,00 en 6 animales; g.) VTA (Área Tegmental Ventral) para 8 bregmas de -2,92 a -3,8 en 14 animales y, h.) número de prolongaciones del AP al NTS para 6 bregmas de -7,20 a -7,76 en 32 animales; **XIV.)** análisis cualitativo para C-fos en el 10N para 16 bregmas de -6,36 a -8,12 en 12 animales; **XV.)** control de la ingesta de alimentos, por pesaje de pellet's, a los 30, 60 y 90 ó 120 minutos para 19 animales tratados; **XVI.)** todos los registros fueron volcados en planillas Excel y se utilizó como referencia anatómica primaria para identificar los bregmas el Atlas de Paxinos mientras que para la identificación de señales de la ISH se empleó el Allen; **XVII.)** microfotografías por captura digital a baja, media y alta magnificación en animales Wilde type, GHSR-Null, GAD-GHSR, Dat-Null, GAD-Tom, NPY-GFP o GHSR-GFP para presentaciones a congresos y publicación y, para visualizar: a.) NPY en el PVN y ARC en 14 animales; b.) anti-fluoresceína y AGRP en el PVN, núcleo supra-quiasmático, VTA y ARC en 23 animales; c.) F-ghrelina en NTS y ARC en 6 animales, entre otras; **XVIII.)** análisis de imágenes para localizar y realizar el recuento de núcleos positivos en el hipotálamo lateral (LH) y NTS para ISH en animales transgénicos con FIJI y Trak EM2 tomando como referencia el Atlas Allen; **XIX.)** compra de insumos e informes quincenales al Sedronar del laboratorio; **XX.)** concurrí a seminarios internos del Imbice y realicé tareas de apoyo a pasantes extranjeros, investigadores y becarios del laboratorio; **XXI.)** cabe destacar mi participación como coautora de 1 publicación que fuera ampliamente difundido a nivel local y nacional, coautora en 5 presentaciones a Congresos y 1 asistencia a Congreso (Certificaciones); **XXII.)** finalmente cabe señalar que soy parte del grupo colaborador en 3 subsidios del laboratorio y 1 institucional, integro el Consejo Directivo del IMBICE en representación de la Carrera del Personal de Apoyo a la Investigación y Desarrollo de la CIC y Conicet y, soy miembro de la Comisión de Bioterio del IMBICE.

8.1.1.- Estudios abordados en diferentes ensayos

Todas las evaluaciones de la hormona ghrelina se efectuaron a partir de ratones C57BL6/j o transgénicos generados por el Bioterio del IMBICE con ciclos de luz oscuridad de 12 hs y temperatura constante de 24°C. Esta hormona peptídica secretada por el estómago actúa principalmente en el sistema nervioso central y, ejerce su acción a través del GHSR que se expresa en los núcleos cerebrales que regulan el apetito. También actúa en el AP, un órgano sensorial circunventricular situado en el bulbo raquídeo que regula una variedad de importantes funciones fisiológicas. Exploramos la activación de neuronas del NTS, que forma parte del complejo vagal dorsal y regula tanto la ingesta de alimentos como los reflejos autonómicos que controlan la glucemia, la presión arterial y la motilidad gastrointestinal en ratones GHSR-GFP sometidos a ayuno, dieta alta en grasa (DAG) y a la administración de diferentes sustancias, entre otros. También, evaluamos el potencial rol modulador del sistema por parte de la ghrelina en la hiperfagia

compensatoria en ratones expuestos a un período de 48 horas de ayuno. Por otra parte, como la accesibilidad de la ghrelina plasmática al cerebro es limitada y restringida a pocas áreas específicas del mismo estudiamos cómo se realizaría el transporte de esta hormona desde el plasma hasta el cerebro con un análogo fluorescente. Nuestros resultados muestran que: **I.)** las células endocelulares del plexo coroideo como los tanicitos hipotalámicos mostraron marcación con F-ghrelina **II.)** la accesibilidad de la ghrelina al cerebro depende de un mecanismo saturable, entre otros aspectos; **III.)** la administración de ghrelina activa el eje hipotalámico-hipófisis-suprarrenal (HPA) y el CRF en neuronas del PVN independientemente del Arc. Es decir, su señal es importante para una respuesta normal del eje HPA en ayuno lo cual nos permite sugerir que las neuronas NPY (neuropéptido Y)/GABA participan en esta respuesta; **IV.)** la ghrelina es necesaria para el aumento gradual en la ingesta de DAG y las neuronas GABAérgicas podrían estar involucradas en la escalada de la misma; **V.)** la señal de GHSR es necesaria para desarrollar una hiperfagia compensatoria completa en respuesta a un período de ayuno como también se requiere de su expresión para activar en dichas condiciones a las neuronas del AP; **VI.)** las neuronas sensibles a la ghrelina del AP son principalmente ácido g-aminobutírico GABAérgicas; **VII.)** la ghrelina circulante accede principalmente al AP pero no al núcleo adyacente del NTS y tanto la administración periférica de esta hormona como el ayuno inducen un aumento de c-Fos en neuronas que expresan GHSR en el AP y, **VIII.)** la capacidad de la ghrelina circulante para inducir el vaciamiento gástrico agudo requiere la integridad del AP.

8.1.2.- Perfusiones, Cortes y Preservación de Especímenes

a.) pesaje de los animales

b.) anestesia IP con hidrato de cloral (Biopack, Argentina)

c.) perfusión en el ventrículo izquierdo del corazón, con solución de lavado (PBS 0,01M-PBS con heparina) y, a continuación con solución fijadora de formaldehído al 4% (Biopack, Argentina)

d.) decapitación de los especímenes y extracción de sus cerebros

e.) post-fijación de cerebros en formaldehído al 4% por 120 min a 4°C

f.) criopreservación "overnight" de cerebros en sacarosa al 20% (Biopack, Argentina) a 4°C

g.) congelación en hielo seco y conservación en freezer a -80°C

h.) cortes coronales, sagitales y horizontales de cerebros en 4 ó más series a 40 ó 20 micras de espesor con crióstato (Minotome American Instrument Exchange Inc., USA)

i.) preservación de los cortes en solución criopreservante a -20°C

j.) montaje de los cortes de cerebro sobre portaobjetos gelatinizados en el momento o provenientes de las series criopreservadas. En el caso de los estómagos o adrenales se realizó montaje en frío.

8.1.3. - Técnica de Inmunohistoquímica simple o doble

a.) 3 lavados durante 10 min en cada uno con PBS 0,01M

b.) incubación durante 30 min con H₂O₂ al 0,5% para ratón ó al 0,3% para rata

c.) 3 lavados durante 10 min en cada uno con PBS 0,01M

d.) permeabilización solo para muestras de rata durante 15 min con Dodesyl Sulfato de Sodio SDS (Sigma)

- e.) bloqueo durante 60 min con Suero Normal de Burro –NDS (EquitechBio Inc., USA) en Buffer de Fosfatos Tritón (PBT)
- f.) incubación durante 24 a 4°C ó 48 hs a temperatura ambiente (T^a) según corresponda, con anti C-Fos (Santa Cruz Biotechnology, USA), TH (Santa Cruz Biotechnology), GFP (Molecular Probe, USA), Alexa 488 y 594 (Molecular Probe, USA), AGRP (Phoenix Pharmaceuticals, USA), entre otros.
- g.) 3 lavados durante 10 min en cada uno con PBS 0,01M
- h.) incubación durante 60 min a T^a con anti-rabbit o anti-mouse (Vector Labs, USA), Alexa 488 (Molecular Probe, USA), anti-goat (Vector Labs, USA), entre otros
- i.) 3 lavados durante 10 min en cada uno con PBS 0,01M
- j.) incubación durante 60 min a T^a con solución peroxidasa biotinilada-avidina (ABC, Vector Kit PK6200, USA)
- k.) 3 lavados durante 10 min en cada uno con PBS 0,01M
- l.) revelado en agitación entre 3-10 min con DAB (3,3'-diaminobenzidine, Vector, SK4100, USA) con o sin cloruro de níquel (Biopack, Argentina).
- ll.) 3 lavados durante 10 min en cada uno con PBS 0,01M
- m.) deshidratación durante 2 min en alcoholes 70°, 96° y 99,5° (Biopack, Argentina) y por último aclaración durante 30 min en xilol (Biopack, Argentina)
- n.) montaje con Bálsamo de Canadá (Biopack, Argentina) o anti-fading con o sin H₂O₂ (B2883, Sigma), según correspondiera

8.1.4.- Tinción de Nissl

- a.) 1 min en H₂O destilada
- b.) 20 min en Solución de Tionina (Certistain®, Merck)
- c.) 1 min en H₂O destilada
- d.) 2 pasajes de 1 min de duración, cada uno, en alcohol 96°
- e.) 2 pasajes de 1 min de duración, cada uno, en alcohol 100°
- f.) 5 min en xilol
- g.) montaje con Bálsamo de Canadá.

Nota: en Anexo I Preparación de Soluciones

8.1.5.- Recuento de señales, análisis microscópico y microfotografías

Todo el material fue analizado con microscopio Nikon (Nikon Corporation, Japan), lentes múltiples neofluarPH y óptica de campo claro. Por su parte, las microfotografías fueron captadas con cámara Nikon Digital Sight D5U3 y software NISElements, Versión 3.22.

8.1.6.- Procesamientos digitales de imágenes

Las imágenes microscópicas obtenidas fueron analizadas con los software's Adobe Photoshop CS2 9.0.2 y Focus Magic 3.02, en tanto las mediciones celulares o nucleares y las marcaciones celulares fueron realizadas con los software's ImagePro Plus 6.0 e ImageJ versión 1.49a/ FIJI versión 2.0 usando Java 2.8. Según lo señalado en el punto 8 (XVIII) las imágenes del NTS se analizaron con el Allen, empleando el software FIJI. Para ello, con la herramienta lapicera se marcaron todas aquellas neuronas que mostraron expresión positiva para ISH en 19 microfotografías.

9.- OTRAS ACTIVIDADES

9.1.- PUBLICACIONES, COMUNICACIONES, ETC.

9.1.1.- Publicaciones

Año 2017.-

- l.) Agustina Cabral, María P. Cornejo, Gimena Fernández, Pablo N. De Francesco, Guadalupe Garcia-Romero, Maia Uriarte, Jeffrey M. Zigman,

Enrique Portiansky, **Mirta Reynaldo**, y Mario Perelló. *Circulating Ghrelin Acts on GABA Neurons of the Area Postrema and Mediates Gastric Emptying in Male Mice*. *Endocrinology*, May 2017, 158(5):1436–1449. ISSN 0013-7227.

9.1.2.- Congresos

Año 2016.-

*I.) 2nd Congress of the Federation of Latin-American and Caribbean Societies for Neuroscience (FALAN), 17 al 20 de Octubre de 2016, Buenos Aires. Cornejo MP, Valdivia S, García-Romero G, De Francesco PN, Andreoli MF, Lazzarino G, **Reynaldo Mirta**, Ramos G, Perelló M. *High fat intake in a mouse binge eating model may involve constitutive ghrelin receptor signaling.**

*II.) 2nd Congress of the Federation of Latin-American and Caribbean Societies for Neuroscience (FALAN), 17 al 20 de Octubre de 2016, Buenos Aires. De Francesco, Pablo Nicolás; Cornejo, Paula; Koç, Volkan; Padín, María Eugenia; García Romero, Guadalupe; **Reynaldo, Mirta**; Perelló, Mario. *Neuroanatomical and functional characterization of ghrelin receptor-expressing neurons of the nucleus of the solitary tract in a transgenic mouse model.**

*III.) 2nd Congress of the Federation of Latin-American and Caribbean Societies for Neuroscience (FALAN), 17 al 20 de Octubre de 2016, Buenos Aires. Uriarte M, De Francesco N, Fernández G, Cabral A, García Romero G, **Reynaldo Mirta**, Perelló M. *Potential role of hypothalamic tanycytes mediating the blood to brain transport of ghrelin.**

*IV.) 2nd Congress of the Federation of Latin-American and Caribbean Societies for Neuroscience (FALAN), 17 al 20 de Octubre de 2016, Buenos Aires. Gimena Fernández, Agustina Cabral, Alexandra Labarthe, Guadalupe García-Romero, M. Florencia Andreoli, Florencia Acutain, **Mirta Reynaldo**, Guillermo Ramos, Virginie Tolle, Epelbaum Jacques, Mario Perelló. *Impact of the Ghrelin Signaling on Food Intake after a Fasting Event.**

*V.) 2nd Congress of the Federation of Latin-American and Caribbean Societies for Neuroscience (FALAN), 17 al 20 de Octubre de 2016, Buenos Aires. Agustina Cabral, Gimena Fernández, Guillermina Zubiría, **Mirta Reynaldo**, Andrés Giovambattista, Mario Perelló. *Impact of the Ghrelin Signaling in the Response of the Hypothalamic – Pituitary–Adrenal Axis to Fasting.**

9.3.- ASISTENCIA A REUNIONES CIENTÍFICAS/TECNOLÓGICAS o EVENTOS SIMILARES.

Año 2016.-

1.) Tercer Congreso Internacional Científico y Tecnológico de la Comisión de Investigaciones Científicas de la Provincia de Buenos Aires. 1º de Septiembre. La Plata.

11.- OTROS ELEMENTOS DE JUICIO NO CONTEMPLADOS EN LOS TITULOS ANTERIORES.

11.1.- Difusión en los medios de la publicación en Endocrinology

1.) Institucionales

2.) Gráficos

3.) Televisivos

11.2.- Tareas de Apoyo

1.) Asistencia a Seminarios organizados en el IMBICE

2.) Tareas de apoyo a investigadores y becarios del Laboratorio

3.) Tareas de apoyo a 3 pasantes extranjeros del Laboratorio, provenientes Méjico, Turquía y Alemania. Las mismas consistieron en técnicas de corte de cerebros, IHQ y captura digital de imágenes.

4.) Preparación de soluciones para la realización de diferentes ensayos

ANEXO I: Preparación de Soluciones de Trabajo

- 1.) ABC 1: 500: 10 µl reactivo A, 10 µl reactivo B y 5 ml PBS pH7.4
- 2.) AntiFading: 5 ml PBS 0.01 M, 0.1g de 1,4 Diazabicyclo [2.2.2]octane y 5 ml glicerol.
- 3.) AntiFading con H_öchst: 1 ml solución antifading y 10 ul bisBenzimide H33258
- 4.) Buffer Fosfato Tritón (PBT): 500 µl tritón X en 200 ml de PBS 0.01M
- 5.) Cloruro de Níquel al 8%: H₂O destilada 10 ml y 0.8 g cloruro de níquel
- 6.) DAB: Solución Stock al 1%: 10 ml H₂O destilada, 0.1 g DAB y 1ml HCl 1N.
- 7.) DAB Solución de Trabajo: 5 ml PBS, 250 µl DAB al 1%, 250 µl cloruro de níquel al 8% y 250 µl H₂O₂ al 0.3%
- 8.) Dodesyl Sulfato de Sodio (SDS): 10 ml PBS 0,01M y 300 µl SDS
- 9.) Formaldehído al 4%: 450 ml PBS 0.01M y 50 ml formaldehído
- 10.) Preparación de Gelatina para portaobjetos: 500 ml H₂O desionizada, 0.25g alúmina y 1.5 g gelatina.
- 11.) Proceso de Gelatinización de portaobjetos: inmersión por 5 min a 60°C en racks, escurrido y 48 hs de secado a 60°C.
- 12.) Hidróxido de Sodio:
- 13.) Proceso de limpieza de cubreobjetos con Hidróxido de Sodio:
- 14.) Hidrato de Cloral: 5 ml H₂O destilada y 3 g hidrato de cloral
- 15.) H₂O₂ al 0,5%: 10 ml PBS 0.01M y 166 µl H₂O₂ al 30%.
- 16.) H₂O₂ al 0,3%: 100 µl H₂O₂ al 30% y 10 ml H₂O destilada pH 7.2
- 17.) PBS 0.1 M 10x pH 7.4: 1000 ml H₂O destilada, 10.9 g Na₂HPO₄, 3.2 g NaH₂PO₄ y 81.6 g NaCl. Ajuste a pH con NaOH 10M
- 18.) Solución Criopreservante: 50 ml PBS 0.1 M, 30 ml etilenglicol y 20 ml glicerol
- 19.) Solución Bloqueante: 5000 µl PBT y 150 µl suero normal de burro (NDS)
- 20.) Solución de Lavado: 500 ml PBS 0.01M y 200 µl heparina cálcica
- 21.) Solución de Sacarosa al 20%: 40 ml PBS 0.01M y 8 g sacarosa

Plate I

IHQ en ratones Wilde Type ayunados durante 48hs. Se muestra a 40x la activación de C-fos en el PVN y ARC en respuesta a ésta situación experimental, respectivamente.

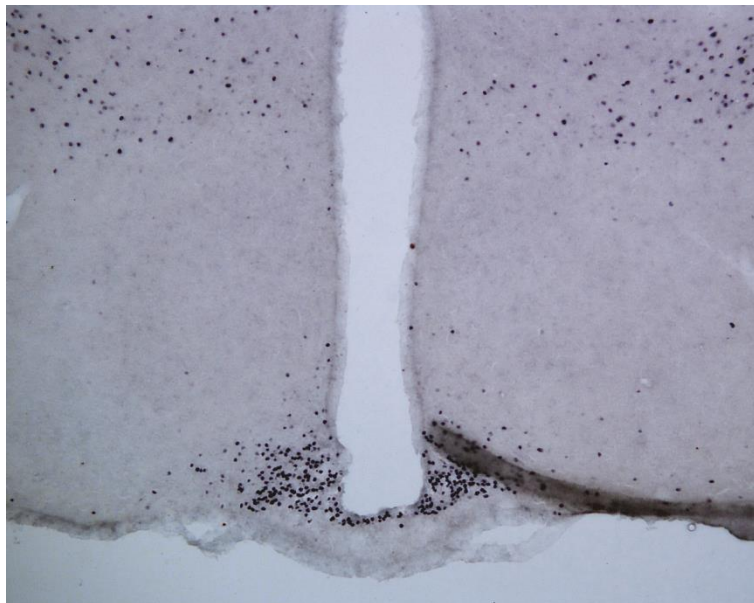
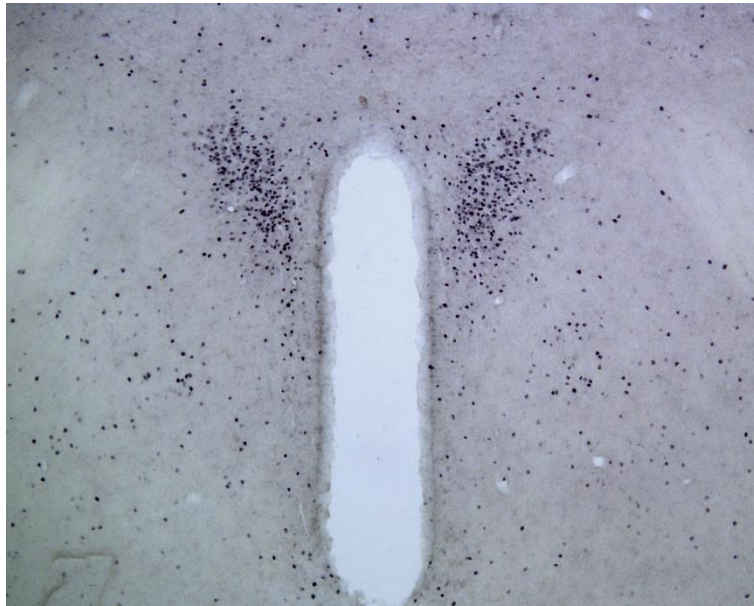
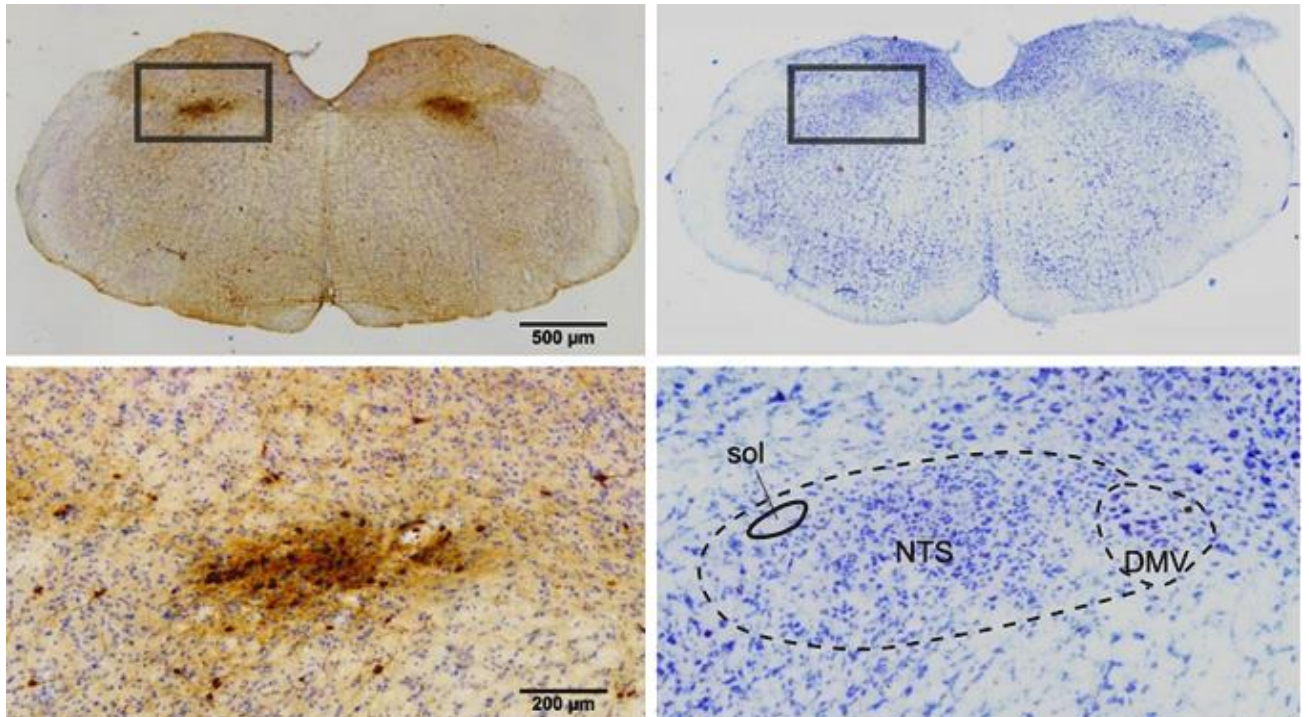


Plate II

Microfotografías a 10 y 60x del NTS donde se observan los resultados obtenidos con IHC y tinción de Nissl. En marrón señal positiva para GFP y en azul coloración según Nissl. Sol: núcleo del tracto solitario y DMV: núcleo dorso medial ventral.



CERTIFICACIONES

2nd FALAN Congress

XII Reunión Anual de la Sociedad Chilena de Neurociencias
XV Jornadas de la Sociedad de Neurociencias del Uruguay
XXXI Congreso Anual de la Sociedad Argentina de Investigación en Neurociencias
XXXIX Reunião Anual da Sociedade Brasileira de Neurociências e Comportamento

October 17-20, 2016

Buenos Aires

Argentina



Puente de la mujer, Puerto Madero (Arg. Santiago Calatrava)



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High fat intake in a mouse binge eating model may involve constitutive ghrelin receptor signaling

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A variety of human eating disorders display binge eating events, which involve the consumption of large amounts of food in a discrete period of time. Ghrelin is the only peptide hormone known to increase food intake, and its receptor (GHSR) is a G-protein coupled receptor capable of signal in a ghrelin-independent manner. The central distribution of GHSR indicates that ghrelin system regulates both homeostatic and hedonic aspects of feeding. Using a simple model of binge eating, in which ad libitum fed mice are exposed to high fat diet (HFD) 4 consecutive days for 2 h/day, we have shown that mice develop a HFD intake escalation over the successive events that involve the activation of the mesolimbic system and ghrelin signaling. Here, we tested if ghrelin-evoked GHSR signaling is required for the HFD intake escalation and we found that the pharmacologic blockage of ghrelin signaling failed to affect HFD intake escalation. Interestingly, mice eating HFD 2 h/day for 4 successive days display an increase in GHSR levels in the ventral tegmental area (VTA), as well as an increase in c-Fos levels in the dopaminergic neurons of the VTA and in the nucleus accumbens. Unexpectedly, we found that mice with GHSR expression limited to the dopaminergic neurons failed to show HFD intake escalation. Thus, we conclude that food intake escalation in mice daily exposed to HFD involves ghrelin-independent GHSR signaling in a subset of neurons different from the dopaminergic neurons of the VTA.

Supported by PICTO 2013-0065.

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INTRODUCTION

Binge eating is characterized by the consumption of large amounts of food in a discrete period of time, while feeling a sense of loss of control over eating. The etiology of the human binge eating is currently unknown. *Ad lib* fed rodents exposed to a palatable food display events of hyperphagia, which gradually escalate over the initial access until they finally stabilize. Intake escalation has been proposed to mediate the transition from a controlled to a compulsive behavior.

Ghrelin is the only peptidic hormone known to increase food intake and its receptor (GHSR) is a G-protein coupled receptor capable of signal in the absence of ghrelin. GHSR shows a central pattern of expression that indicates that ghrelin system regulates both homeostatic and hedonic aspects of eating.

Here, we use a simple binge eating protocol in which animals are exposed to high fat diet (HFD) 2h/day for 4 days to show that mice with intermittent access to HFD display an escalating profile in HFD intake. This escalation involves the activation of the mesolimbic pathway, which includes the ventral tegmental area (VTA) and the nucleus accumbens (Acb). We also show that ghrelin signaling is required for the escalation in HFD intake.

We also incorporate the use of a pharmacological approach to test if ghrelin-evoked GHSR signaling is required for the escalation in HFD intake. Finally, we use genetically modified animals with expression of the GHSR limited to specific subpopulations of neurons to determine if GHSR expression in GABAergic or catecholaminergic neurons is involved in the regulation of HFD intake escalation.

RESULTS

1) Intermittent and limited access to HFD induces events of hyperphagia with an escalating profile and activates the mesolimbic pathway

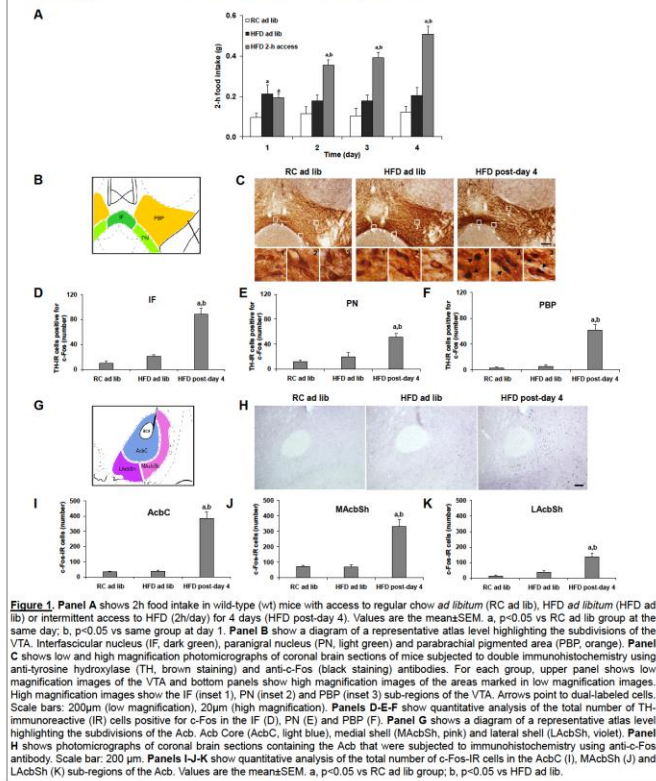


Figure 1. Panel A shows 2h food intake in wild-type (wt) mice with access to regular chow ad libitum (RC ad lib), HFD ad libitum (HFD ad lib) or intermittent access to HFD (2h/day) for 4 days (HFD post-day 4). Values are the means±SEM. a, p<0.05 vs RC ad lib group at the same day; b, p<0.05 vs same group at day 1. Panel B shows a diagram of a representative atlas level highlighting the subdivisions of the VTA. Interfascicular nucleus (IF, dark green), paraventricular nucleus (PN, light green) and parabrachial pigmented area (PBP, orange). Panel C shows low and high magnification photomicrographs of coronal brain sections of mice subjected to double immunohistochemistry using anti-tyrosine hydroxylase (TH, brown staining) and anti-c-Fos (black staining) antibodies. For each group, upper panel shows low magnification images of the VTA and bottom panels show high magnification images of the areas marked in low magnification images. High magnification images show the IF (inset 1), PN (inset 2) and PBP (inset 3) sub-regions of the VTA. Arrows point to dual-labeled cells. Scale bars: 200µm (low magnification), 20µm (high magnification). Panels D-F show quantitative analysis of the total number of TH-immunoreactive (IR) cells positive for c-Fos in the IF (D), PN (E) and PBP (F). Panel G shows a diagram of a representative atlas level highlighting the subdivisions of the Acb: Acb Core (AcbC, light blue), medial shell (MAcbSh, pink) and lateral shell (LAcbSh, violet). Panel H shows photomicrographs of coronal brain sections containing the Acb that were subjected to immunohistochemistry using anti-c-Fos antibody. Scale bar: 200 µm. Panels I-K show quantitative analysis of the total number of c-Fos-IR cells in the AcbC (I), MAcbSh (J) and LAcbSh (K) sub-regions of the Acb. Values are the means±SEM. a, p<0.05 vs RC ad lib group; b, p<0.05 vs HFD ad lib.

2) Ghrelin signaling is required for the escalation in HFD intake and full c-Fos induction in the mesolimbic pathway during repeated events of hyperphagia

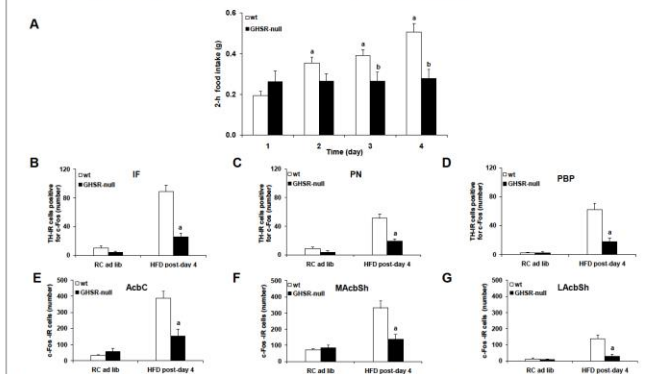


Figure 2. Panel A shows 2h food intake of wt and mice lacking the GHSR (GHSR-null) with intermittent access (2h/day) to HFD for 4 days. Values are the means±SEM. a, p<0.05 vs same group at day 1; b, p<0.05 vs wt at same day. Panel B-C-D show quantitative analysis of the total number of TH-IR cells positive for c-Fos in the IF (B), PN (C) and PBP (D) sub-regions of the VTA of wt and GHSR-null mice with intermittent access to HFD for 4 days and perfused 2h after last HFD consumption event. Panel E-G show quantitative analysis of the total number of c-Fos-IR neurons in the AcbC (E), MAcbSh (F) and LAcbSh (G) sub-regions of the Acb of wt and GHSR-null mice with intermittent access to HFD for 4 days and perfused 2h after last HFD consumption event. Values are the means±SEM. a, p<0.05 vs wt mice in the same experimental condition.

3) Pharmacologic blockade of ghrelin evoked GHSR signaling fails to affect the escalation in HFD intake and c-Fos induction in the mesolimbic pathway

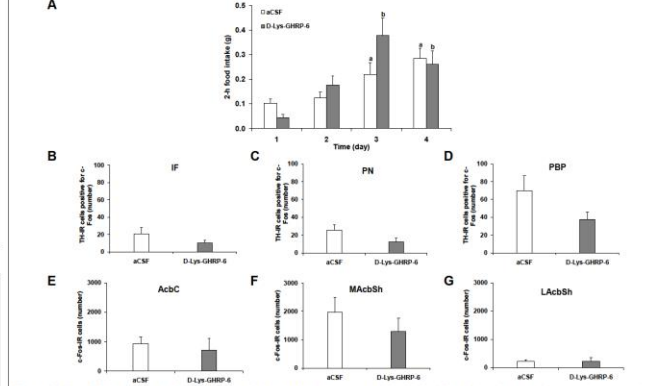


Figure 3. Panel A shows 2h intake in animals with intermittent access to HFD (2h/day) for 4 days that were intra-cerebro-ventricularly injected with either artificial cerebrospinal fluid alone (aCSF) or containing 2 nmol of the GHSR antagonist D-Lys-GHRP-6 20 min before exposure to HFD each day. Values are the means±SEM. a, p<0.05 vs aCSF day 1; b, p<0.05 vs D-Lys-GHRP-6 day 1. Panels B-C-D show quantitative analysis of the total number of TH-IR cells positive for c-Fos-IR in the IF (B), PN (C) and PBP (D) sub-regions of the VTA of aCSF- and D-Lys-GHRP-6-treated mice with intermittent access to HFD for 4 days and perfused 2h after last HFD consumption event. Panels E-F-G show quantitative analysis of the total number of c-Fos-IR cells in the AcbC (E), MAcbSh (F) and LAcbSh (G) sub-regions of the Acb of aCSF- and D-Lys-GHRP-6-treated mice with intermittent access to HFD for 4 days and perfused 2h after last HFD consumption event. Values are the means±SEM.

4) Reactivation of GHSR expression in GABAergic neurons is sufficient to recapitulate the escalating profile in HFD intake

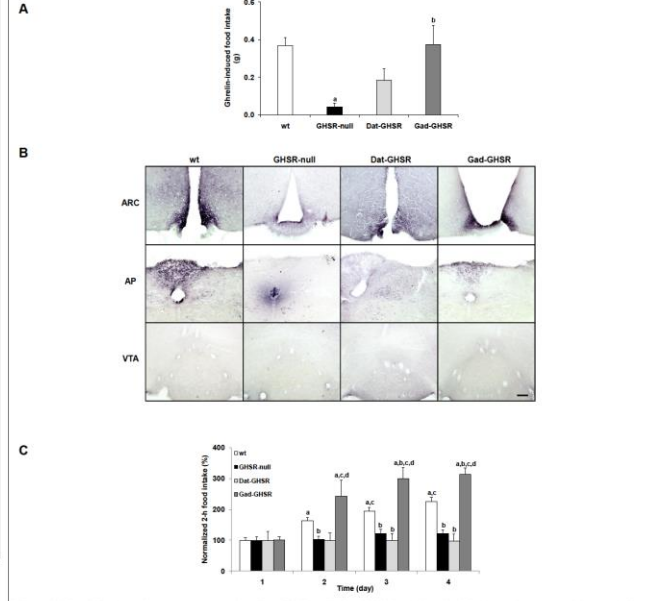


Figure 4. Panel A shows 2h regular chow intake of wt, GHSR-null, Gad-GHSR and Dat-GHSR mice subcutaneously injected with either saline alone (vehicle) or containing 60 pmol/gBW ghrelin. Gad-GHSR mice have GHSR expression limited to GABAergic neurons and were derived from crosses between *gad2-CreER* and GHSR-null mice. Dat-GHSR mice have GHSR expression limited to catecholaminergic neurons and were derived from crosses between *dat-Cre* and GHSR-null animals. Values are the means±SEM. a, p<0.05 vs wt; b, p<0.05 vs GHSR-null. Panel B shows representative photomicrographs of coronal brain sections of wt, GHSR-null, Gad-GHSR and Dat-GHSR mice ICV-injected with 60 pmol of fluorescein-ghrelin and perfused 30 min after. Images depict coronal sections containing the arcuate nucleus (ARC), VTA and area postrema (AP) that were subjected to immunohistochemistry using an anti-fluorescein antibody. Scale bar: 100µm. Panel C shows normalized 2h food intake of wt, GHSR-null, Dat-GHSR and Gad-GHSR mice with intermittent access (2h/day) to HFD for 4 days. Data of each genotype is presented as the percentage of HFD intake normalized to their own intake at day 1. Values are the means±SEM. a, p<0.05 vs same group at day 1; b, p<0.05 vs wt at same day; c, p<0.05 vs GHSR-null at same day; d, p<0.05 vs Dat-GHSR at same day.

CONCLUSIONS

- Intermittent and limited access to HFD induce events of hyperphagia with an escalating profile and activates the mesolimbic pathway.
- Ghrelin signaling is required for the escalation in HFD intake and full c-Fos induction in the mesolimbic pathway.
- Ghrelin-evoked GHSR signaling is not involved in the escalation in HFD intake.
- GHSR expression limited to GABAergic neurons restores the escalating profile in HFD intake.

Thus, ghrelin signaling is required for the escalation in HFD intake and ghrelin independent GHSR signaling in GABAergic neurons may be involved in the escalation in HFD intake.

Acknowledgements
This study was supported by PICT2011-2142 and PICT2013-0065 grants. SV and MR were supported by the Commission of Scientific Investigations of the Province of Buenos Aires.

Neuroanatomical and functional characterization of ghrelin receptor-expressing neurons of the nucleus of the solitary tract in a transgenic mouse model

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The nucleus of the solitary tract (NST) is a sensory nucleus in the dorsal medulla that receives visceral, gustatory, and orotactile information, amongst others. It is part of the dorsal vagal complex, which regulates food intake and relays autonomic reflexes controlling glucaemia, blood pressure and gastro-intestinal motility. Some NST neurons express the growth hormone secretagogue receptor (GHSR), the target of the hormone ghrelin. Here, we used a transgenic mouse line in which green fluorescent protein (GFP) is under the control of the GHSR promoter (GHSR-GFP mice) to perform a detailed neuroanatomical description and a functional exploration of the GHSR-expressing neurons of the NST. GFP+ neurons were distributed throughout the NST, but preferentially formed two clusters within its rostral and caudal ends. These neurons were negative for M-enk, TH, NPY, TRH, ChAT and GAD-67, as shown by immunophenotypic analysis. We explored the activation of these neurons by exposing GHSR-GFP mice to experimental paradigms known to activate the NST (48h fasting, gastric distention, 24h water deprivation, i.p. LiCl administration, i.p. hypertonic NaCl administration, oral sucrose administration, and high-fat diet binge eating), and mapping the induction of c-Fos. We found that only high-fat diet binge eating group showed a significant increase of c-Fos expression in NST GFP+ neurons. Thus, we conclude that GHSR-expressing neurons of the NST are activated by acute intake of high fat diet.

Neuroanatomical and functional characterization of ghrelin receptor-expressing neurons of the nucleus of the solitary tract in a transgenic mouse model

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INTRODUCTION

The nucleus of the solitary tract (NST) is a sensory nucleus in the dorsal medulla that receives visceral, gustatory, and orotactile information, amongst others. It is part of the dorsal vagal complex, which regulates food intake and relays autonomic reflexes controlling glucosaemia, blood pressure and gastro-intestinal motility. Some NST neurons express the growth hormone secretagogue receptor (GHSR), the target of the hormone ghrelin. The identification of GHSR-expressing neurons by traditional techniques is challenging. To overcome this issue we take advantage of a transgenic mouse line in which green fluorescent protein (GFP) is under the control of the GHSR promoter (GHSR-GFP mice).

AIMS

The aim of the present work is to perform a detailed neuroanatomical description as well as a functional exploration of the GHSR-expressing neurons within the NST by means of the GHSR-GFP mouse line.

RESULTS

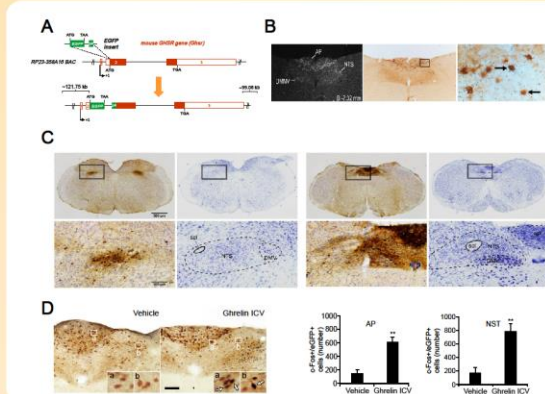


Figure 1. GHSR-GFP mice: Panel A shows a schematic diagram of the genetic construct of GHSR-GFP mice. In this specific transgenic line an EGFP reporter gene, followed by a polyadenylation sequence, was inserted into BAC clone RP23-358A16 at the initiating ATG codon of the first coding exon of the Ghsr gene. Panel B shows in-situ hybridization for GHSR and IHC staining for GFP (brown) on sections of the brain at the level of the NST, along with a higher magnification detail (taken from Mani et al. J Comp Neurol. 2014 Nov 1;522(16):3544-56). Panel C shows representative sections of rostral and caudal portions of the NST stained with IHC for GFP (brown) or with Nissl stain. Panel D illustrates that GFP-IR cell respond to ghrelin delivered ICV. To the left, sections with a double IHC for GFP (brown) and c-Fos (black). Arrow show double positive cells. On the right are shown the corresponding quantifications for the area postrema (AP) and NST.

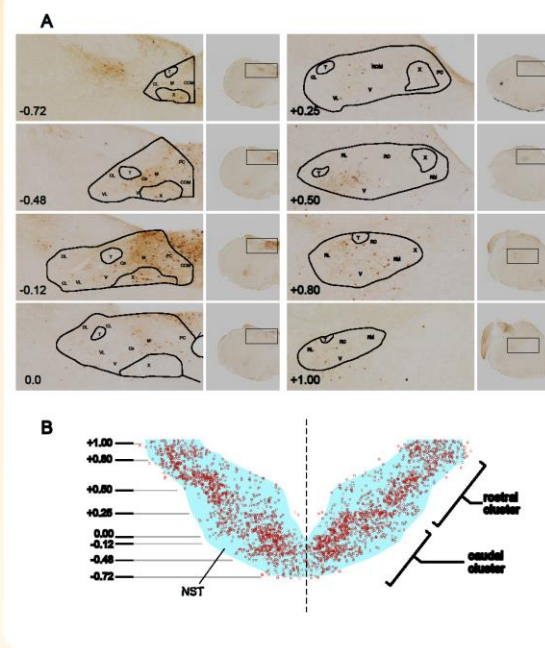


Figure 3. Detailed pattern distribution of GFP-positive neurons within NST of GHSR-GFP mice: Panel A depicts a set of representative coronal images from caudal to rostral levels of the NST containing GFP-positive neurons, demonstrated using IHC (brown). For each level, a diagram of the NST is overlaid following the nomenclature and delineation described in Gancharov et al. J Comp Neurol. 2014 May 1;522(7):1562-66. On the right, a rectangle indicates the approximate region from where the higher magnification images were obtained. Rostrocaudal coordinates are indicated in reference to the obex (0.0). Panel B shows a dorsal projection of all the GFP positive neurons (shown as red circles) found in 55 serial sections of a GHSR-GFP mouse, along with a projection of the NST for reference. The levels shown in A are also indicated. In this projection, a rostral and a caudal cluster of cells are clearly distinguished. Nomenclature: Ce: central subnucleus, CL: caudal lateral subnucleus, COM: commissural subnucleus, DL: dorsal lateral subnucleus, M: medial subnucleus, PC: parvocellular subnucleus, RC: rostral central subnucleus, RL: rostral lateral subnucleus, RM: rostral medial subnucleus, V: ventral subnucleus, VL: ventral lateral subnucleus, X: vagal motor nucleus, T: solitary tract.

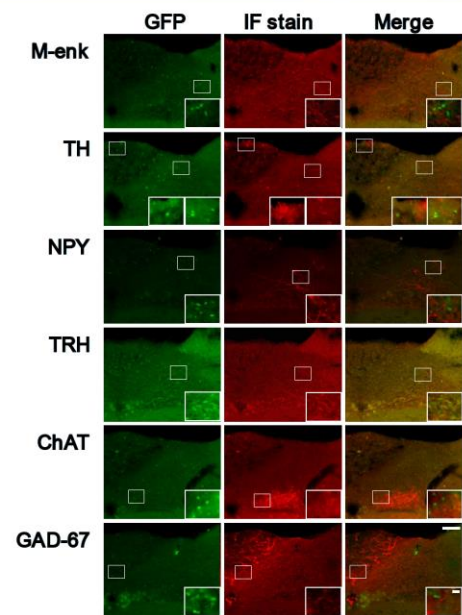


Figure 2. Immunophenotype of GFP positive cells in GHSR-EGFP mice. Panels show representative photomicrographs of coronal brain sections of GHSR-GFP mice subjected to IHC using different markers of specific neuronal populations. The first column shows the GFP autofluorescence. The second column shows, from top to bottom, the IHC using either anti: Mel-enkephalin (M-enk), anti-proline hydroxylase (TH), anti-thyrotropin releasing hormone (TRH), anti-tyrosine hydroxylase (TH), anti-neuropeptide Y (NPY), anti-glutamate decarboxylase 67 (GAD-67) antibodies (re-staining). The third column shows the merge of the respective green and red images. Inserts in each panel shows in high magnification the areas marked in low magnification photomicrographs. Arrows point to GFP cells and arrowheads point to IR cells. No GFP cells were found positive for the markers used in the current study. Scalebars: 100µm (low magnification) 10µm (high magnification).

Experimental Condition	Brief protocol description	Rostral		Caudal	
		c-Fos	% neurons c-Fos ⁺ /GFP ⁺ (vs. GFP ⁺)	c-Fos	% neurons c-Fos ⁺ /GFP ⁺ (vs. GFP ⁺)
Control	Mice used as control for the experiments (each one mouse with normal access to food and water, mice 1x treated with vehicle, mice, etc) were pooled and termed the control group, as no c-Fos signal was observed in GFP-IR neurons of the NST.	+	3.1±0.7	+	4.8±1.2
Shape fasting	In the morning, mice were given IPTD and Bitter for 2 h during 4 consecutive days. Their IPTD intake was recorded and verified to 0. Animals were perfused 2 h post last IPTD consumption.	++	5.8±1.6	++	5.8±1.4
Gastro distension	Mice were given 500µl vehicle via a gastro-intestinal ball-tipped feeding needle. After 2h, mice were perfused.	+++	2.3±0.9	+++	1.0±1.0
Oral sucrose	Mice were orally given 100µl of a 0.1M sucrose solution. After 2h mice were perfused.	++	5.6±0.7	++	3.6±0.7
4th fasting	Mice were fasted by removing their food from the home cages at 10:00 am. After 48 h, mice were perfused.	++	5.1±3.3	++	17.5±5.5
2h water deprivation	Water access was restricted to mice at 10:00 am. Access to regular chow was fully available. After 24 h, mice were perfused.	+	0.0±0.0	+	0.0±0.0
LXCL I.p.	Mice were injected with LXCL (100 µg/kg, 2x/day, I.p) or saline. After 2h mice were perfused.	+	0.0±0.0	+	17.8±13.0
Ghrelin s.c.	Mice were injected ghrelin (2 µg/kg, subcutaneous), and perfused 2 h later.	+	3.3±2.8	+	1.7±1.7

Table 1. Activation of GFP cells in the NST by different specific experimental conditions: Several protocols known to activate neurons in the NST, listed in this table, were performed in GHSR-GFP mice, and evaluated using c-Fos as a marker for neuronal activation. IHC staining of GFP and c-Fos positive nuclei was performed on coronal brain sections of each experimental mice, and colocalization for GFP and c-Fos was evaluated, both rostrally and caudally at the NST. None of the currently tested conditions significantly induced c-Fos in NST GFP neurons. For each condition, a brief description of the followed experimental protocol is included, along a qualitative estimate of overall c-Fos-IR (++++, very high density; ++, high density; +, moderate density) and the percentage of GFP-IR neurons with nuclei positive for c-Fos with respect to total GFP-IR cells.

CONCLUSIONS

- GFP cells of GHSR-GFP mice are located throughout the extension of the NST, but preferentially forming two clusters, one rostral, mostly comprising the ventral subnucleus, and one caudal, mostly involving the parvocellular subnucleus.
- These cells are not reactive to immunostaining against M-enk, TH, NPY, TRH, of GAD 67. Further studies will be needed to elucidate their immunophenotypic identity.
- Currently, the set of experimental protocols failed to show a statistically significant differential activation of GFP cells in the transgenic mouse. Further investigation of other experimental conditions will be required.
- In summary, this transgenic GHSR-GFP line was shown to be a promising tool to gain insights into the localization, phenotype and possible functional role of this particular neuronal subset within the NST.

Acknowledgements: This work was supported by grants of the National Agency of Scientific and Technological Promotion of Argentina (PICT2010-1954, PICT2011-2142 and PICT2013-0065 to MP). MR was supported by the Commission of Scientific Research of the Province of Buenos Aires.

Potential role of hypothalamic tanycytes mediating the blood to brain transport of ghrelin

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Ghrelin is a peptide hormone secreted from the stomach that mainly acts in the central nervous system. However, the accessibility of plasma ghrelin to the brain is limited and restricted to few specific brain areas. The goal of this study was to get insights into the system mediating the transport of ghrelin from the plasma to the brain in mice. First, we centrally infused a fluorescent ghrelin analog to mice and look for brain cells labeled by the tracer. We found that both the ependymal cells of the choroids plexus and the hypothalamic tanycytes display tracer labeling. Then, we peripherally infused the fluorescent ghrelin analog to mice and look for brain cells labeled at different time points. We found that the hypothalamic tanycytes were early labeled by the probe, and that the periventricular brain parenchyma was also labeled after 15 min of treatment. In order to test if the accessibility of ghrelin to the brain depends on a saturable mechanism, we centrally infused mice with a scrambled fluorescent ghrelin peptide and then peripherally injected ghrelin. We found that the centrally-administered scrambled peptide labeled tanycytes and failed to affect food intake; however, it impaired the orexigenic activity as well as the increase of the marker of cellular activation c-Fos induced by peripherally administered ghrelin. Thus, we conclude that hypothalamic tanycytes may be involved in the transport of ghrelin in a blood-to-brain direction.

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INTRODUCTION

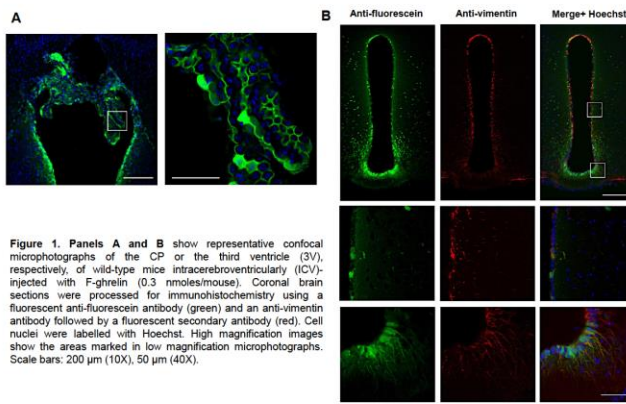
Ghrelin is a 28-amino acid octanoylated peptide hormone secreted from the stomach that mainly acts in the central nervous system to regulate food intake and neuroendocrine axis. However, the accessibility of plasma ghrelin to the brain is limited and restricted to few specific brain areas.

The goal of this study was to gain insights into the system mediating the transport of circulating ghrelin to the brain in mice.

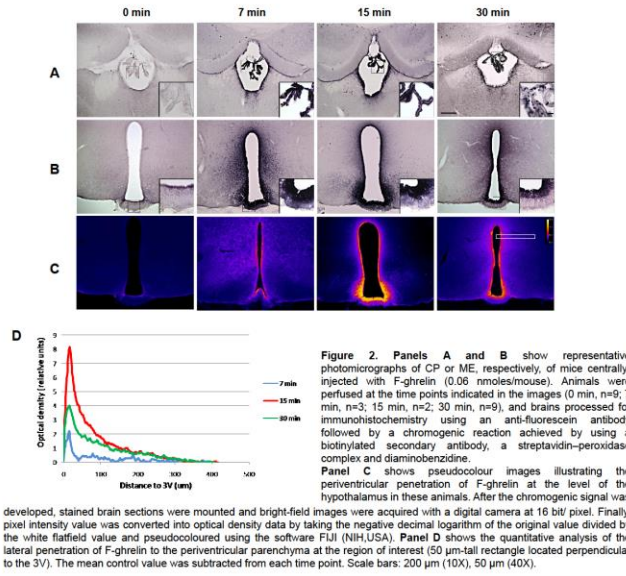
For this purpose, we used a fluorescent ghrelin tracer (F-ghrelin) that was centrally or peripherally injected to mice and mapped its distribution within the blood-cerebrospinal fluid barrier (the choroid plexus, CP, and the tanycytes of the median eminence, ME)

RESULTS

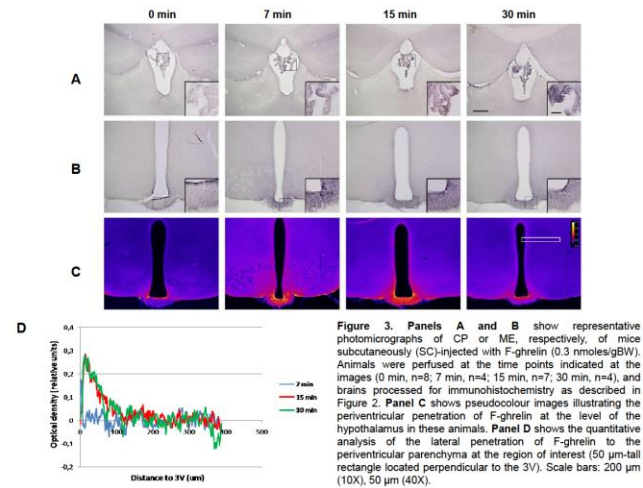
1) Centrally administered F-ghrelin labels cells of the blood-cerebrospinal fluid barrier



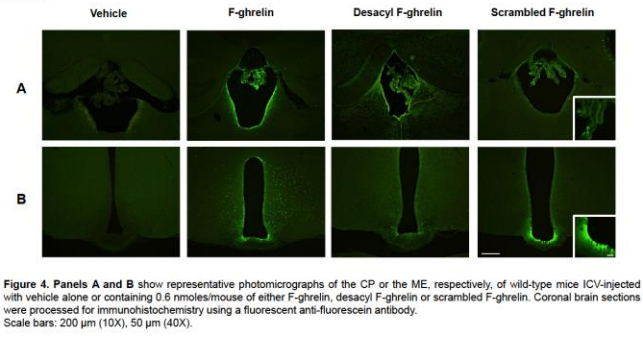
2) Analysis of F-ghrelin labeling after central administration of the tracer



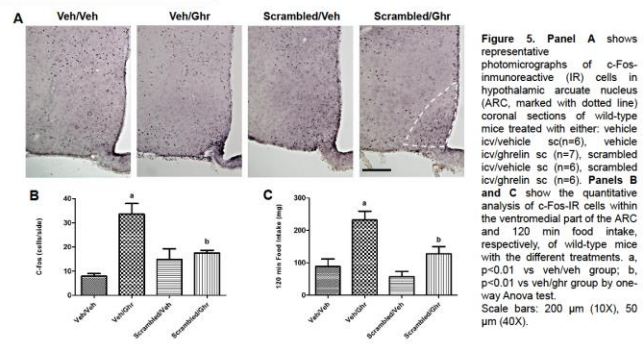
3) Analysis of F-ghrelin labeling after peripheral administration of the tracer



4) Centrally administered F-ghrelin analogs label cells of the blood-cerebrospinal fluid barrier



5) Centrally administered scrambled F-ghrelin inhibits peripheral ghrelin effects on food intake and neuronal activation



CONCLUSIONS

- The tanycytes of the median eminence and the ependymal cells of the choroid plexus display F-ghrelin labeling when the tracer is either centrally or peripherally injected.
- F-ghrelin labeling was found in the hypothalamus brain parenchyma at 7 minutes after icv-administration.
- F-ghrelin labeling was found in the hypothalamus brain parenchyma at 15 min after subcutaneously-administration.
- All F-ghrelin analogs tested labeled the cells of the blood-cerebrospinal fluid barrier, suggesting that the process occurs in a ghrelin receptor-independent manner.
- Centrally-administered scrambled F-ghrelin failed to affect food intake; however, it impaired the orexigenic activity as well as the increase of the marker of cellular activation c-Fos induced by peripherally administered ghrelin. Thus, these data suggest that the accessibility of ghrelin to the brain depends on a saturable mechanism.

Acknowledgements
This work was supported by grants of the National Agency of Scientific and Technological Promotion of Argentina PICTO2013-0065 to MP.

IMPACT OF THE GHRELIN SIGNALING ON FOOD INTAKE AFTER A FASTING EVENT

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Refeeding after a period of severe fasting triggers a robust hyperphagia, which persists even after animals have reached their energy needs. Plasma ghrelin levels and the hypothalamic gene expression of the ghrelin receptor (or growth hormone secretagogue receptor, GHSR) increase during fasting in order to cope with the negative energy balance condition. Here, we investigated the potential modulatory role of the ghrelin system on the compensatory hyperphagia that display mice that have been exposed to a 48h fasting period. Using automated feeding/activity stations in mice exposed to a fast-refeeding paradigm, we found that refed wild-type mice display a significant increase of the total food intake that continues for 6 days after refeeding. GHSR-deficient mice also show a compensatory hyperphagia, which was significantly smaller than observed in wild-type mice. Fasted wild-type mice show an increase of plasma ghrelin levels as well as an increase of the GHSR levels in the hypothalamic arcuate nucleus, as indicated by both a ghrelin binding assay and gene expression analysis. Notably, plasma ghrelin levels return to basal levels 1 day after refeeding while hypothalamic GHSR levels seems to remain increased even after 4 days of refeeding. Thus, we conclude that the ghrelin system plays a modulatory role on the magnitude of the hyperphagia observed after a severe fasting period. Supported by PICTO 2013-0065.

INTRODUCTION

Refeeding after a period of severe fasting triggers a robust hyperphagia, which persists even after animals have reached their energy needs. Plasma ghrelin levels and the hypothalamic gene expression of the ghrelin receptor (or growth hormone secretagogue receptor, GHSR) increase during fasting in order to cope with the negative energy balance condition. Here, we investigated the potential modulatory role of the ghrelin system on the compensatory hyperphagia that display mice that have been exposed to a 48h fasting period.

RESULTS

1) Refed mice after 2 days of fasting display a significant hyperphagia that last 6 days after refeeding and show an important disruption of the daily feeding and activity patterns

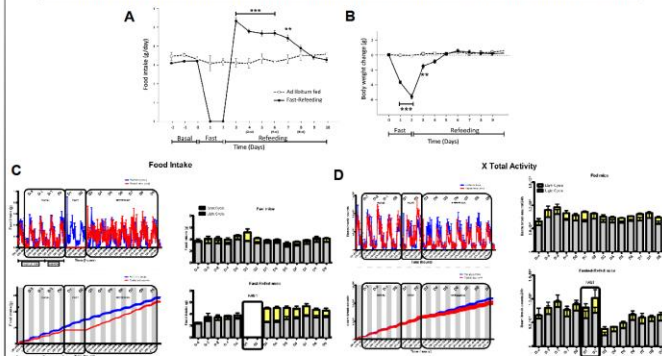
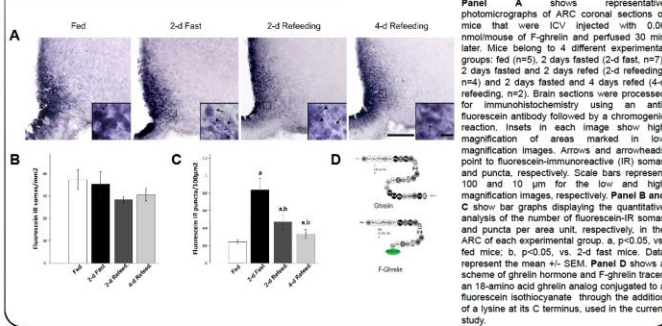


Figure 1. Panels A and B show the changes in food intake and body weight, respectively, of 2-3 month old male wild-type mice that were maintained with *ad libitum* access to regular chow (n=11) or fasted for 2 days and then allowed free access to food at 10:00 am (n=24). ***p<0.001 vs. *ad libitum* fed mice at the same day and its same group at the days before fasting (Two-way ANOVA test). Data represent the mean \pm SEM. Panel C and D show the daily feeding patterns and the circadian activity, respectively, monitored simultaneously in the same animals using an automated feeding-activity station (TSE systems, GmbH, Germany). Grey shades indicate the dark cycle.

2) Fasted mice display higher F-ghrelin labeling in the hypothalamic arcuate nucleus (ARC) that remains elevated after 4 days of refeeding



3) Expression of hypothalamic ARC neuropeptides involved in the regulation of food intake: fasted mice show a higher expression of GHSR and AqRP and a lower expression of POMC and GAD1

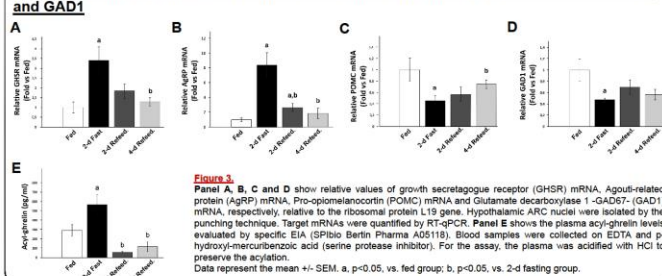
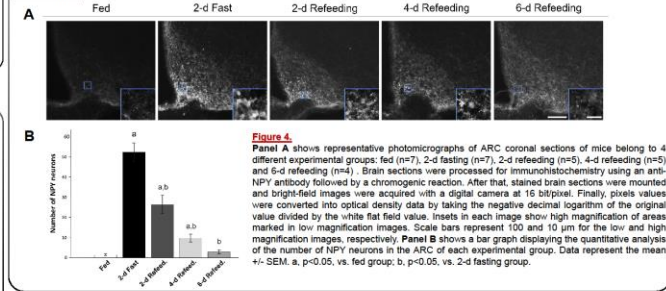
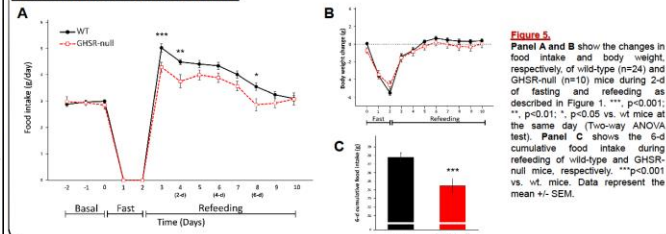


Figure 3. Panel A, B, C and D show relative values of growth secretagogue receptor (GHSR) mRNA, Agouti-related protein (AgRP) mRNA, Pro-opiomelanocortin (POMC) mRNA and Glutamate decarboxylase 1 -GAD67- (GAD1) mRNA, respectively, relative to the ribosomal protein L19 gene. Hypothalamic ARC nuclei were isolated by the punching technique. Target mRNAs were quantified by RT-qPCR. Panel E shows the plasma acyl-ghrelin levels evaluated by specific EIA (SPIBIO Berlin Pharma A05118). Blood samples were collected on EDTA and p-hydroxy-mercuribenzoic acid (serine protease inhibitor). For the assay, the plasma was acidified with HCl to preserve the acylation. Data represent the mean \pm SEM. a, p<0.05, vs. fed group; b, p<0.05, vs. 2-d fasting group.

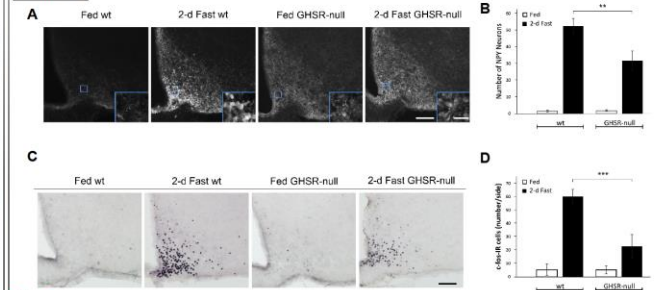
4) Fasted mice show an increase of NPY in the ARC that remains elevated for 4 days of refeeding



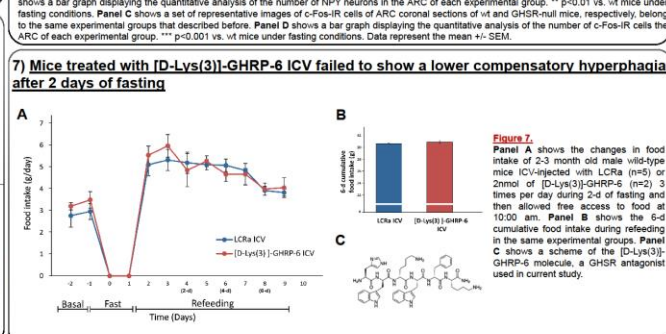
5) Ghrelin receptor deficient mice (GHSR-null) show a lower compensatory hyperphagia when they refed after 2 days of fasting



6) GHSR-null mice show an impaired fasting-induced increase of both c-fos and NPY levels in the ARC



7) Mice treated with [D-Lys(3)]-GHRP-6 ICV failed to show a lower compensatory hyperphagia after 2 days of fasting



CONCLUSIONS

GHRS signaling is required in order to develop a full compensatory hyperphagia in response to a fasting period

Acknowledgements

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SIP238. Impact of the ghrelin signaling in the response of the hypothalamic – pituitary–adrenal axis to fasting

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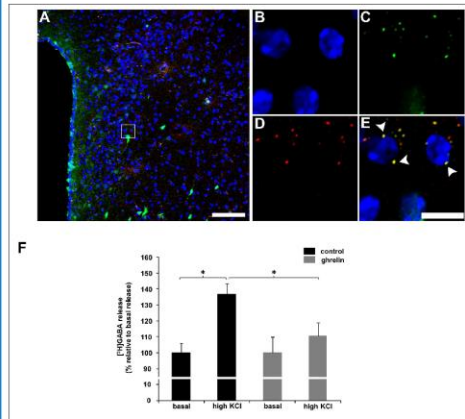
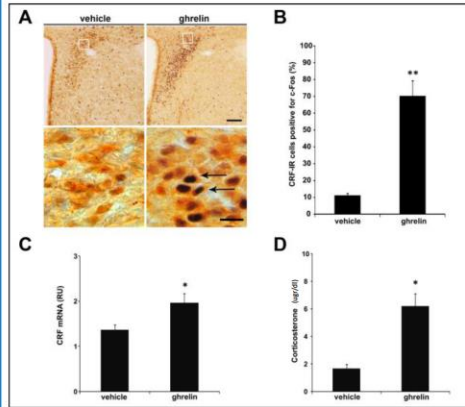
Ghrelin is a stomach-derived hormone that regulates food intake and several neuroendocrine functions. We had shown that the administration of ghrelin activates the hypothalamic–pituitary–adrenal (HPA) axis and corticotropin-releasing factor (CRF) neurons of the hypothalamic paraventricular nucleus (PVN) via inhibition of the local γ -aminobutyric acid (GABA)ergic tone and independently of the arcuate nucleus (ARC). However, the impact of physiological increments of plasma ghrelin on CRF neurons and HPA axis is currently unknown. Here, we studied the response of the HPA axis, at hypothalamic and peripheral level, of 48-hour fasted wild-type or ghrelin receptor (GHSR)-null mice. In addition, we tested the GABA release from PVN explants of fed and fasted mice. We found that the lack of GHSR influences the peripheral response of the HPA axis as well as induction of the marker of cellular activation c-fos in the PVN CRF neurons. In contrast, GHSR expression is not necessary for fasting-induced increase of the neuropeptide Y-fiber density in the PVN. We also found a reduction of basal and high K-stimulated GABA release in PVN explants from fasted mice as compared to the release detected in explants from ad libitum fed mice. Thus, these data support the notion that ghrelin signaling is relevant for the normal response of the HPA axis to fasting and these suggest that de NPY/GABA neurons engaged in this response. Supported by PICTO 2013-0065.

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INTRODUCTION

Ghrelin, the only known orexigenic hormone, is a stomach-derived hormone that regulates food intake and several neuroendocrine functions. Ghrelin acts via its specific receptor named growth hormone secretagogue receptor (GHSR), which is highly expressed in numerous brain nuclei regulating appetite as the nucleus arcuate (ARC). As shown Figure 1 and Figure 2, we had shown that the administration of ghrelin activates the hypothalamic-pituitary-adrenal (HPA) axis and corticotropin-releasing factor (CRF) neurons of the hypothalamic paraventricular nucleus (PVN) via inhibition of the local γ -aminobutyric acid (GABA)ergic tone and independently of the ARC.

However, the impact of endogenous increments of plasma ghrelin on CRF neurons and HPA axis is currently unknown.



In this study we studied the response of the HPA axis, at hypothalamic and peripheral level, of 48-hour fasted wild-type or GHSR-null mice. In addition we studied the Neuropeptide Y (NPY) and GABA in the PVN and ARC of 48-hour fasted wild-type mice.

RESULTS

1) Plasma Acyl-ghrelin and Body weight of Fed and Fasted mice

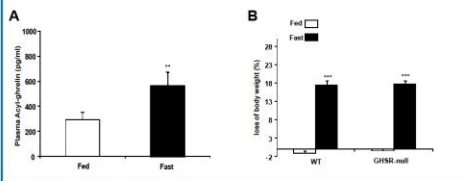
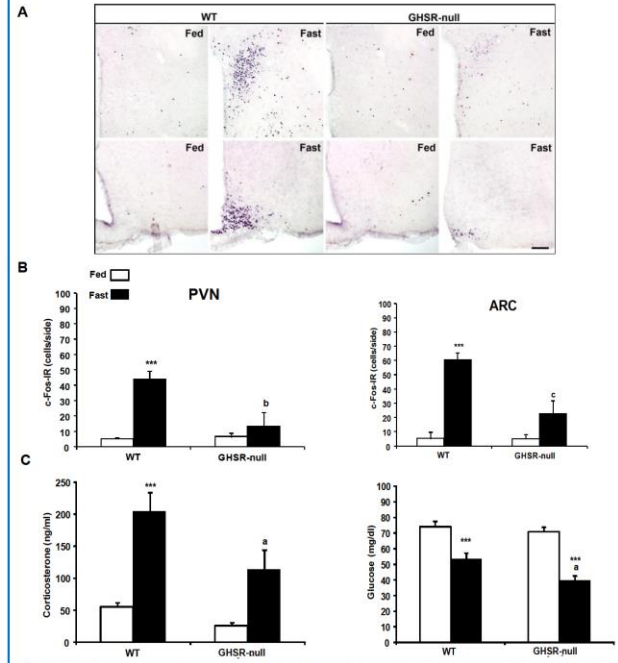
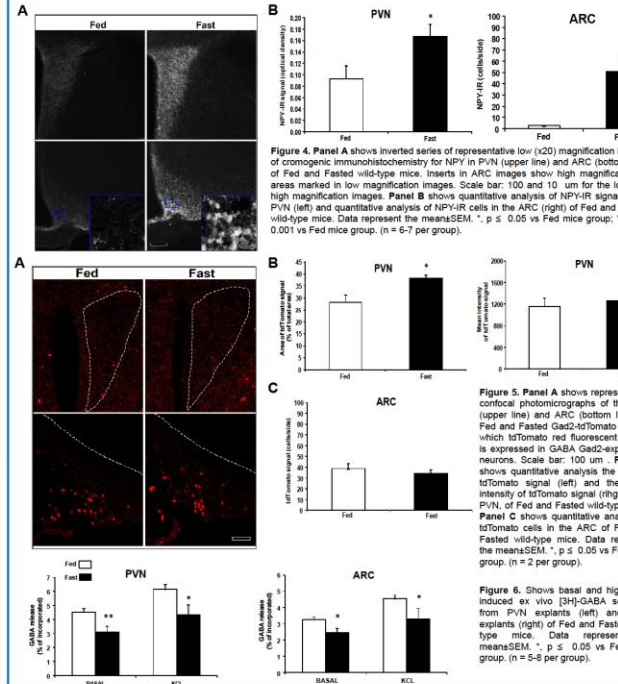


Figure 3. Panel A shows the plasma acyl-ghrelin (EIA kit) in fed and 48-h fasted mice. Data represent the mean \pm SEM. ** p \leq 0.01 vs. Fed mice group (n = 6 per group). Panel B shows the loss of body weight in Fed and 48 h Fasted wild-type or GHSR-null mice. Data represent the mean \pm SEM. ** p \leq 0.001 vs. Fed mice group (n = 6 per group).

2) Response of the hypothalamic-pituitary-adrenal axis to fasting



3) NPY and GABA in PVN and ARC of Fed and Fasted mice



CONCLUSIONS

- The ghrelin signaling is not required for the fasting-induced body weight loss.
- The ghrelin signaling is required for fasting-induced increase of c-Fos levels in the PVN and ARC.
- The ghrelin signaling is required for a full fasting-induced increase of plasma corticosterone levels and the glucose levels.

- Fasting increases the NPY levels in the PVN and the ARC.
- Fasting increases the density of GABAergic fibers in the PVN.
- Fasting decreases the basal and high K⁺ stimulated GABA release from PVN and ARC explants.

Thus, these data support the notion that ghrelin signaling is relevant for the normal response of the HPA axis to fasting and these suggest that NPY/GABA neurons engaged in this response.

Acknowledgements. This work was supported by grants of the National Agency of Scientific and Technological Promotion of Argentina PICT2013-0005 to MP



"Ciencia, Tecnología e Innovación para una Provincia en Crecimiento"

LA COMISIÓN DE INVESTIGACIONES CIENTÍFICAS DE LA PROVINCIA DE BUENOS AIRES

certifica que:

REYNALDO, MIRTA BEATRIZ

ha participado del Tercer Congreso Internacional Científico y Tecnológico realizado el 1º de septiembre de 2016 en el Teatro Argentino de la ciudad de La Plata.

Se extiende el presente certificado en la ciudad de La Plata, a los 6 días del mes de septiembre de 2016.

Ing. Luis P. TRAVERSA
Vicepresidente
Comisión de Investigaciones Científicas

Ing. Armando E. DE GIUSTI
Presidente
Comisión de Investigaciones Científicas

1.) Centro Científico Tecnológico CONICET La Plata

Consejo Nacional de Investigaciones Científicas y Técnicas
30/05/2017 | CIENCIAS BIOLÓGICAS Y DE LA SALUD

Confirman que la hormona del hambre también ayuda a vaciar el estómago

Investigadores del CONICET en nuestra ciudad mostraron cómo actúa en el cerebro además de hacerlo en el tracto gastrointestinal

2.) Científicos platenses confirman que la hormona del hambre acelera la digestión

eldia.com/nota/2017-5-31-3-26-42-cientificos-platenses-confirman-que-la-hormona-del-hambre-acelera-la-digestion-informacion-general/

El hallazgo contribuye a echar luz sobre un mecanismo que podría encerrar la clave contra la obesidad

3.) LA NACION Miércoles 31 de Mayo de 2017. 16.08 Ciencia

Científicos de La Plata realizaron un hallazgo que podría servir para luchar contra la obesidad

También podría servir para luchar contra la bulimia, anorexia, y atracones compulsivos

4.) **Entrevistas** del 2 de Mayo de 2017 realizadas para Telefe Noticias y Buenos Días América de A24



Los autores del trabajo integran el Laboratorio de Neurofisiología del IMBICE. FOTO: gentileza investigadores.

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Maia Uriarte. Becaria doctoral, IMBICE.

Jeffrey M. Zigman. Centro Médico del Sudoeste de la Universidad de Texas, EEUU.

Enrique Portiansky. Investigador principal, FCV, UNLP.

Mirta Reynaldo. Profesional principal (CICPBA), IMBICE.

Mario Perelló. Investigador independiente, IMBICE.

ESTUDIO DEL ROL MODULADOR DEL RECEPTOR DE GHRELINA SOBRE LAS CONDUCTAS
HIPERFAGICAS AGUDAS

CARÁTULA DE GRUPO

Convocatoria

Tipo	Proyectos de Investigación Científica y Tecnológica Orientados (2013)
Categoría	GlaxoSmithKline
Tipo de Proyecto	Equipo de Trabajo

Datos generales del proyecto

Áreas Temáticas	Principal : Ciencias Médicas Secundarias : Ciencias Biológicas de Organismos y Sistemas
Prioridad Regional	No Aplica
Duración	3 años
Ubicación	Argentina, Buenos Aires
Investigador Responsable	Perello, Mario
Fecha de alta	25-09-2013

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Conformación de Grupo Colaborador

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Agustina Soledad	Cabral	Becario	
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Lucila Anabela	Patrone	Becario	

Becas

Tipo	Descripción
Tipo de la Beca	

PROYECTO ENVIADO

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Página 5 de 7

ESTUDIO DEL CIRCUITO NEURONAL POR EL CUAL LA GHRELINA COORDINA ACCIONES OREXIGENICAS Y SEÑALES DE ESTRES

CARÁTULA DE GRUPO

Convocatoria

Tipo	Proyectos de Investigación Científica y Tecnológica (2016)
Categoría	Temas Abiertos
Tipo de Proyecto	Jóvenes

Datos generales del proyecto

Áreas Temáticas	Principal : Ciencias Médicas II: Fisiología y Fisiopatología de tejidos. Órganos y Sistemas de Órganos Secundarias : Fisiología y Biología Experimental
Prioridad Regional	No Aplica
Duración	2 años
Ubicación	Argentina, Buenos Aires
Investigador Responsable	CABRAL, AGUSTINA SOLEDAD
Fecha de alta	20-07-2016

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Guadalupe	García Romero	Personal Técnico	
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Becas

Tipo	Descripción
Tipo de la Beca	



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P - UE 2017**

CONVOCATORIA: P - UE 2017

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TIPO Y NRO DE DOCUMENTO: DNI 24644088

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Lugar y Fecha

.....
Firma

Para pegar en la tapa de cada carpeta



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