CONICET UBA

FACULTAD DE MEDICINA

IFIBIO HOUSSAY

Workshop Virtual IFIBIO

Libro de abstracts

Facultad de Medicina
Universidad de Buenos Aires

18 de diciembre 2020

Programa

Actividades Sincrónicas

10.00 - 10.10 hs: **Palabras del Director del IFIBIO**

Gustavo Murer

10.10 - 10.40 hs: **Presentación de protocolos institucionales para el retorno a las actividades de laboratorio no esenciales**

Roxana Toriano y Juan José Casal

10.40 - 11.10 hs: **Resultados de las encuestas sobre la situación vivida por los miembros del IFIBIO durante el ASPO.**

Camila Coll, Rodrigo Paz, Azul Silva, Florencia Santos, Karina Fischer Sigel, Agostina Stahl, Flavia Sacerdoti y Nicolás Garimano.

11.10 - 11.30 hs: **Intervalo**

11.30 - 12.00 hs: **Mesa redonda: Mecanismos de evaluación para ingresos y promociones en CONICET**

Juan Belforte y Claudia Capurro

12.00 – 13.00 hs: **Inmunidad y vacunas frente a la infección por SARS-CoV-2**

Jorge Geffner

13.00 – 14.00 hs: **Almuerzo**

Actividades Asincrónicas

14.00 – 16.00 hs: **Sesión de Posters**

Abstracts

(1) RELIABILITY AND VALIDITY OF A NEW MOUSE TIC SEVERITY SCALE (MTSS)

Beccaria, Juan Pablo; Coll, Camila; Murer, Gustavo; Belforte, Juan.

Instituto de Fisiología y Biofísica "Bernando Houssay" (IFIBIO-Houssay). Facultad de Medicina. UBA-CONICET.

Sudden, rapid, recurrent, non-rhythmic motor movements or vocalizations, are the main medical sign of Tourette Syndrome (TS). Since their intensity and manifestation is exceedingly variable, tics are challenging to quantify. The Yale Global Tics Severity Scale (YGTSS) is perceived as the gold-standard clinical scale for tic quantification not only for TS but also for any other disorder that may present tics. YGTSS measures tic complexity, frequency, intensity and interference with normal behavior. In addition, since YGTSS is used world-wide, it provides comparable and reproducible data. However, there is no similar method for scoring tics in animal models. The present work aims to validate a mouse tic severity scale (MTSS) based on YGTSS standards. For that, we used a new murine model of TS developed in our lab, in which selective ablation of striatal Nkx2.1+ derived interneurons lead to exacerbated spontaneous repetitive behaviors including tic-like movements (See Coll et al. poster). High temporal resolution videos of lesioned and control mice were thoroughly watched and scored by two treatment-blinded observers. Lesioned mice showed a higher score than their control littermates both globally and in every scale section. Moreover, total punctuation and presence of particular patterns of movements correlated with lesion extent. These results suggest that MTSS might be a valid and reproducible scale to measure tics in mice.

(2) EFECTOS DEL DESEQUILIBRIO EN LA SEÑALIZACIÓN TIROIDEA SOBRE EL PROCESO DE REGENERACIÓN CELULAR EN LA RETINA DE ZEBRAFISH ADULTO

Claudio Alejandro Bejarano y Maria Paula Faillace.

INTRODUCCION: El zebrafish (ZF, Danio rerio) es un modelo establecido internacionalmente para la investigación biomédica y es capaz de regenerar diversos órganos y tejidos. La retina de ZF se regenera de manera completa tanto morfológica como funcionalmente. Las hormonas tiroideas (HT) regulan la retinogénesis y la formación de fotorreceptores en los vertebrados. El ZF posee un eje hipotálamo-hipófiso-tiroideo y produce HT a partir de folículos tiroideos en la zona branquial. Las HT ejercen sus principales efectos biológicos a través de los receptores nucleares a HT (TRs). OBJETIVO: examinar el efecto de desequilibrios en la señalización tiroidea sobre la reparación del circuito neuro-glio-vascular durante la regeneración de la retina en el ZF. METODOS: se lesionaron las retinas de ZF con una inyección intravítrea de ouabaína y grupos controles con solución salina. A su vez, se separaron los animales en cuatro grupos, se trataron con HT (300 µg/L) o vehículo en el agua de la pecera durante 25 días postlesión (dpl). Se detectaron por inmunofluorescencia tipos neuronales y gliales y vasos sanguíneos intrarretinianos. Se determinó apoptosis por caspasa 3 activada. Se analizaron los niveles relativos de ARNm de los TRs, receptores para VEGF (VEGFRs); HIF1α y diversos marcadores de fenotipos celulares, mediante RT-PCR cuantitativa. RESULTADOS: el tratamiento con una elevada concentración de TH (25 dpl) indujo neovascularización retiniana. El análisis por inmunomarcación de poblaciones celulares y capas demostró cambios significativos en el número y tipo de neuronas, en las capas y conexiones sinápticas. La glia de Müller presentó un prolongado y alto grado de gliosis y se observó apoptosis tardía no debida a la lesión. Se describieron variaciones significativas en la expresión génica relativa de TRs, VEGFRs y marcadores celulares retinianos que correlacionan con los cambios morfológicos observados. CONCLUSION: un desbalance de la señalización tiroidea a largo plazo provoca cambios y una disminución significativa en el número y cambios en el tipo de células retinianas, así como neovascularización aberrante y un grado alto de gliosis, inhibiendo la diferenciación neuronal, lo que a su vez impide la regeneración tisular y recuperación de la función visual que normalmente ocurre en la retina de ZF.

Abstracts

(3) SHIGA TOXIN 2 (STX2) AND LPS FROM ENTEROHEMORRHAGIC ESCHERICHIA COLI (EHEC) PRODUCE A TLR4-INDEPENDENT MICROGLIAL REACTIVITY AND PRO-INFLAMMATORY CYTOKINES

Ana B. Celi1, Alejandro Villarreal2, Lucas Elizagaray1, Adriana Cangelosi3, Patricia A. Geoghegan3, Alipio Pinto1, A. Javier Ramos2, Jorge Goldstein1.

(1) Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Fisiología y Biofísica "Houssay" (IFIBIO), Laboratorio de Neurofisiopatología, Facultad de Medicina, Argentina.
(2) Laboratorio de Neuropatología. Molecular, Instituto de Biología Celular y Neurociencia "Prof. De Robertis", UBA/CONICET.
(3) Centro Nacional De Control De Calidad De Biológicos (CNCCB), ANLIS "Dr. Carlos G. Malbrán", Ciudad Autónoma de Buenos Aires, Argentina.

Stx2 from EHEC is the main cause of hemolytic uremic syndrome that is defined clinically by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Stx2 can also lead to encephalopathy with motor, cognitive and emotional impairments in 30% of cases. We have studied that Stx2 can bind to neurons through its receptor Gb3. Other authors have reported that Stx2 may bind to TLR4 in leukocytes, which leads to its activation and cytokine release. Given that microglial cells (MC) are CNS resident macrophages, we hypothesize that MC would respond to Stx2 through a TLR4 receptor. Therefore, our aim was to determine by in vivo and in vitro studies whether Stx2 produces MC activation through TLR4 and cytokine release. Mice were subjected to the following sublethal treatments: vehicle (control) or Stx2 (3ng)+LPS (800ng), to determine MC reactivity by immunofluorescence and cytokine release by flow cytometry. In vitro assays using cglial mixed cultures containing MC were obtained from TLR4 KO rat brains which were treated with either control, LPS (50ng/ml), Stx2 (50 or 200ng/ml) or Stx2+LPS. One way ANOVA analysis, and Bonferroni post-hoc analysis were performed for in vivo and in vitro studies. After 24h of treatment, Stx2+LPS treated mice showed an increase in the expression of IBA1 as well as in the number of MC ($p<0.001$). Further, after 6 hours of toxin treatments a significant increase of IL6, TNF alfa and INF gamma was observed ($p<0.001$). In vitro studies showed that 200ng/ml of Stx2 and Stx2 (200 ng/ml)+LPS produced an increase in the area occupied by MC respect other treatments ($p<0.001$). Further, the expression levels of IBA1 and the number of MC were increased in Stx2 (50 and 200ng/ml) and Stx2 (50 and 200ng/ml)+LPS with respect to LPS alone and the control ($p<0.05$). We concluded that co-treatment of Stx2+LPS produced early anti-inflammatory cytokines in the brain which could be related with MC reactivity through a TLR4 independent pathway.

(4) MODULATORY ACTIVITY OF DOPAMINE NEURONS IN A SELF-PACED BEHAVIORAL TASK

Marcos Coletti, Mariano Belluscio, Gustavo Murer

Midbrain dopamine neurons (DAn) signalize the occurrence of a reward as a result of an action taken. Several studies analyze the DAn activity during a behavioral task mediated by a cue that predicts the value of the action to be taken. However, little is known about DAn activity during the performance of a behavioral task in which the action to be taken should be self-initiated. This work aims to characterize the activity of DAn that innervate the striatum in a self-paced instrumental task. The animal has to estimate a minimum waiting time to enter into a port and initiate a licking sequence to obtain a reward. If the waiting time is not reached the probability of obtaining a reward is zero, even if the licking sequence is done. Neuronal activity in the ventral tegmental area (VTA) and the substantia nigra (SN) was recorded using tetrodes during the performance of the self-initiated behavioral task. Preliminary results show that there is a modulation of the neuronal activity in VTA that marks the boundaries of the action sequence, while the SN neuronal activity is modulated around the time for the reward. Interestingly though, some DAn responses differentiate a licking sequence initiated on time from sequences initiated prematurely, both before port entry and at the time of reward. These results suggest that DAn could be marking an expectation signal to achieve a reward.

Abstracts

(5) ABLATION OF NKX2.1 DERIVED STRIATAL INTERNEURONS RESULTS IN TOURETTE – LIKE PHENOTYPES

Camila Coll, Juan Pablo Beccaria, Bárbara Braz, Analía López Díaz, Juan Belforte, Gustavo Murer

Tourette Syndrome (TS) is a neurodevelopmental disorder that usually starts during childhood. Although it is characterized by motor and vocal tics, most patients also present comorbid conditions including OCD and ADHD. Pathophysiology of TS is unknown, however, there are studies showing a reduce number of PV+, nNOS+ and ChAT+ striatal interneurons (SIs) in the brain of TS patients. Previous studies have tried to reproduce TS phenotype in mice by generating an ablation of a specific type of SI, but none have shown spontaneous tics. In order to reproduce more closely the striatal changes reported, we performed a combined ablation of SIs using a Cre/loxP transgenic system to express human diphtheria toxin receptor in Nkx2.1+ cell lineage, combined with intrastriatal diphtheria toxin administration. Immunohistochemistry assays showed that lesion exclusively affected SIs. Lesioned mice not only developed abnormal involuntary movements resembling motor tics (See Beccaria et al. poster) but also behaviors reminiscent of common comorbid conditions, including an increase in stereotypies (grooming), locomotion, and spontaneous repetitive behaviors (rearing and head poking), as well as a reduction in immobility time, compared to their control littermates. These phenotypes suggest perseverative-like behaviors and hyperactivity, compatible with OCD and ADHD comorbidities. In summary, animals with ablated Nkx2.1 derived SIs develop TS-like phenotypes.

(6) ROLE OF AQUAPORIN-2 AND TRPV4 IN RENAL CELL MIGRATION

Cutrera N, Rivera MF, Beltramone N, Rivarola V, Ford P, Capurro C, Di Giusto G.

Instituto de Fisiología y Biofísica “Bernardo Houssay” (IFIBIO) UBA-CONICET. Facultad de Medicina, Universidad de Buenos Aires. Argentina

We have previously shown that Aquaporin-2 (AQP2) promotes renal cell migration. This promigratory effect is due, at least in part, to the modulation of Na+/H+ exchanger (NHE1) activity, responsible for lamellipodia alkalinization, which would generate the appropriate microenvironment for actin and focal adhesion dynamics. Taking into account that we have demonstrated a physical and functional interaction between AQP2 and Ca2+ channel TRPV4, and that NHE1 activity is modulated by Ca2+, we propose to investigate the contribution of TRPV4 in the AQP2-dependent renal cell migration. We used two renal cell models: one WT not expressing AQPs and another one expressing AQP2. First, we determined TRPV4 expression in lamellipodia of migrating cells by immunofluorescence assays. Then, we evaluated TRPV4 participation in collective cell migration through wound healing assays in presence of a specific activator (GSK1016790A, 3nM). Finally, we characterize focal adhesion complexes by revealing the mechanosensor Vinculin. Our results showed that TRPV4 is present in lamellipodia of both cell types, but AQP2-expressing cells have a higher intensity ratio per area analyzed (WT: 794±84, n=32; AQP2: 1371±79, n=74; ***p<0,001). In AQP2-expressing cells, the activation of TRPV4 produces a decrease in migration indicating that, probably, TRPV4 is already in an activated state and overactivation results in a harmful excess of Ca2+ (Control: 29.65±1.25%, n=10; GSK: 19.14±1.18%, n=6; ***p<0,001). Moreover, lamellipodia of AQP2-expressing cells have focal adhesions of small size evidencing the rapid turnover of active migrating cells (WT: 6.68±0.78µm2, n=29; AQP2: 2.50±0.23µm2, n=10; *p<0,05). These results let us to propose that during lamellipodia protrusion the presence of AQP2 activates it's partner TRPV4, leading to a regulated Ca2+ entry. Furthermore, we propose that Ca2+ entering in the vicinity of focal adhesions would favor the assembly/disassembly cycles of these adhesive sites.

Abstracts

(7) EFFECTS OF SHIGA TOXIN TYPE 2 IN PREGNANT AND NON-PREGNANT FEMALE RATS

Fischer Sigel LK, Sacerdoti F, Ibarra C, Zotta E, Silberstein C.

Shiga toxin-producing Escherichia coli causes acute renal failure and Hemolytic Uremic Syndrome. It was reported that inhibition of nitric oxide (NO) by Shiga toxin type 2 (Stx2) enhanced renal damage in mice and baboon models of Stx-mediated HUS. The aim of the work was to study the evolution of the damages caused by Stx2 in P compare with NP rats. Pregnant Sprague-Dawley rats, at day 8 of gestation, and NP rats were ip inoculated with 0.5 ng Stx2/g body weight (PS, NPS) or diluent (PC, NPC). Some PS and PC rats were treated with 1mg/ml L-NAME, NO inhibitor, in drinking water (PLS, PLC) from 24h before ip injection to 4 days post-injection (dpi). Rats were individually housed, checked for water and food intake, and weighted every 24h until 30 dpi. At 4 dpi, blood and 24h-urine samples were collected to determine urinary flow and free water clearance (CH20). Then, rats were euthanized and kidneys were removed for histopathological observations. NPS and PS rats showed a decrease in food intake and weight with respect to controls ($p<0.05$). PS rats increased food intake and recovered weight at 5 dpi, while NPS rats showed an improvement at 14 dpi. The water intake increased in NPS and PS rats compared to controls until 7 dpi ($p<0.05$). In NPS at 4 dpi, the rise in water intake coincided with an increase in urinary flow and CH20 respect to NPC ($p<0.05$), different from what was observed in PS. The renal cortex of NPS presented significantly more necrosis tubules than PS ($p<0.05$). Preliminary results in PLS rats showed that L-NAME significantly increased renal necrosis compared with PLS and PLC rats ($p<0.05$). In conclusion, PS rats suffered less renal damage and recovered from the Stx2 effect faster than NPS rats. L-NAME increased Stx2 effect in PLS suggesting that physiology changes caused by pregnancy, like increasing in NO production, may contribute to protect maternal kidney from Stx2 effects.

(8) PRESERVATION OF PROTECTIVE EFFECTS OF HYPERIMMUNE ANTI STX2 BOVINE COLOSTRUM AGAINST EHEC O157:H7 PATHOGENICITY AFTER PASTEURIZATION AND SPRAY-DRYING PROCEDURES

N. Garimano1, L.I. Diaz Vergara2, A.D. Kim1 E.E Badin2, S. Sodero2, A. M. Bernal3, D. D. Gonzalez4, M. M. Amaral1, A. R. Lespinard2, C. Porporatto1 M. A. Montenegro2, M. S. Palermo3, M. Larzabal5 A. A. Cataldi5, C. Ibarra1, F. Sacerdoti1.

1 Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay-CONICET), Facultad de Medicina, Universidad de Buenos Aires.
2 Centro de Investigaciones y Transferencia de Villa María (CIT-VM-CONICET), Universidad Nacional de Villa María (UNVM), Villa María, Argentina.
3 Laboratorio de Patogénesis e Inmunología de Procesos Infecciosos, Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, Buenos Aires, Argentina.
4 Instituto de Virología/INVIT-CICVyA, INTA-Castelar, Buenos Aires, Argentina.
5 Instituto de Agrobiotecnología y Biología Molecular (IABIMO)-CICVyA, INTA-CONICET, Buenos Aires, Argentina.

Enterohaemorrhagic Escherichia coli (EHEC) O157:H7 is a major etiologic agent responsible for bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). Shiga toxin (Stx) is the main virulence factor of EHEC and the main responsible for the onset of HUS. Although many efforts have been made to develop an effective treatment for Stx-mediated HUS, a specific therapy has not been found yet. It is well known that human consumption of bovine colostrum has therapeutic effects against several gastrointestinal infections, as it contains a range of peptides and proteins (including antibodies) with antimicrobial and endotoxin-neutralizing effects. Consistent with these findings, we have previously demonstrated that colostrum from Stx type 2 (Stx2)-immunized pregnant cows effectively prevents Stx2 cytotoxicity and EHEC O157:H7 pathogenicity. This study evaluated different pasteurization (72°C for 15 s vs 60°C for 60 min) and spray drying paramenters (Inlet temperature 110°C vs 120°C and outlet temperature lower or higher than 55°C) in order to optimize the preservation of the protective properties of hyperimmune colostrum (HIC-Stx2) against Stx2 after processing.

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Characterization of neutralizing properties of colostrum were assayed in vitro and in vivo. Our results showed that pasteurization at 60°C for 60 min, combined with spray-drying at inlet and outlet temperatures of 120°C and >55°C, respectively, showed the highest yield and lowest humidity on final samples ($p<0.05$). Reconstituted HIC-Stx2 colostrum after processing under optimized conditions preserved specific IgG quantity and effectively neutralized Stx2 cytotoxicity on Vero cells. Furthermore, this pasteurized/dehydrated and reconstituted HIC-Stx2 preserved the protective properties against EHEC infection in a weaned mice model. In this regard we propose that hyperimmune bovine colostrum has the potential to be administered to patients with the aim of protecting children against EHEC infection.

(9) ELIGLUSTAT AS A POSSIBLE STRATEGY TO PREVENT THE DETRIMENTAL EFFECTS OF SHIGA TOXIN TYPE 2: PRELIMINARY RESULTS IN VIVO

Gómez F, Sacerdoti F, Toytoyndjian E, Ibarra C, Amaral MM.

Typical Hemolytic Uremic Syndrome (HUS) is a complication of Shiga toxin (Stx)-producing Escherichia coli (STEC) infection and the most frequent cause of acute renal failure in children in Argentina. Stx2 binds to globotriaosylceramide (Gb3) receptor and causes direct damage on human renal microvascular endothelial cells (HGEC). Previously, we found that Eliglustat (EG), a Gb3 synthesis inhibitor, prevents the cytotoxic effects of Stx2 on HGEC. In this work, we evaluated the action of EG against the effects of Stx2 in vivo. Male BALB/c mice at weaning (17-21 days) received 3 doses of EG (0.6 mg/g body weight (bwt) administered intraperitoneally (i.p.) every 24 h. After a rest period of 5 days, mice were i.p. injected with a lethal (1ng/g bwt) or sublethal (0.1 ng/g bwt) dose of Stx2 (EG+Stx2) or PBS (EG). Two additional groups of mice without EG pre-treatment were injected one with PBS (Ctrl) and another with Stx2 (Stx2). Survival, body weight (Δ weight= body weight after Stx2 or PBS injection-body weight at a day before injection) and food intake were registered daily. EG did not affect body weight gain (Δ weight: EG: 0.61 ± 0.07 g vs. Ctrl: 0.76 ± 0.09 g; n=3, ns). showed a body weight decrease and a survival time (48-72 h) similar to Stx2 mice. On the contrary, after 3 days of Stx2 sublethal dose injection, while Stx2 mice exhibited piloerection and inactivity and body weight loss, EG+Stx2 mice did not show signs of illness and gained weight (Δ weight: EG+Stx2: 0.81 ± 0.09 vs. Stx2: -0.65 ± 0.05 g; n=3, $p<0.05$). Body weight loss in Stx2 mice was associated with a significant decrease (70%) in food intake, unlike EG pre-treated mice that reduced intake by only 15% (n=3, $p<0.05$). These results suggest that EG may reduce the disease symptoms caused by Stx2, such as poor appetite and the resulting body weight loss. Future studies will analyze if EG prevents the renal damage and will improve EG treatment to avoid mortality.

(10) NOCICEPTIVE RESPONSES OF CORTICO-STRIATAL NEURONS OF THE ANTERIOR CINGULATE CORTEX IN AN ANIMAL MODEL OF CHRONIC PAIN

Constanza Ilarraz1, Mario Acuña2, Thomas Nevian2 and Fernando Kasanetz1.

1. Grupo de Neurociencias de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Houssay, CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina.
2. Department of Physiology, University of Bern, Switzerland.

Pain is a sensory and emotional experience mediated by distributed brain networks. Maladaptive functional changes in the so called “pain matrix” may participate in the chronification of pain. In particular, during chronic pain (CP), the representation of noxious stimuli shifts from the typical nociceptive circuits to the limbic system, involved in the affective and motivational assessment of pain. Cortico-striatal (CS) neurons of the Anterior Cingulate Cortex (ACC) may play a key role in linking nociceptive and limbic systems during CP. The ACC is a main area of the pain matrix and is essential for the affective connotation of pain. Its projections to the medial striatum (MS) converge with other limbic inputs and the midbrain dopamine system. However, it is unknown how this neurons process nociceptive signals and how this is affected during CP. To address this issue we used a mice model of neuropathic pain and recorded specifically CS-ACC neuronal activity in response to noxious stimuli in freely behaving animals. For that, we imaged neuronal calcium signals through a GRIN lens implanted over the ACC and a miniature

microscope mounted on the head. Our preliminary results show that a set of CS-ACC neurons were activated by nociceptive stimuli and that injured animals exhibited a differential pattern of affective behavioral responses to those stimuli. Further experiments will permit us to better understand the role of CS-ACC neurons on the affective expression of CP.

(11) EFFECTS OF ESTROGENS ON RENAL PROXIMAL TUBULE EPITHELIAL CELLS

Jove P1, Vlachovsky SG2, Sánchez DS1, Azurmendi PJ2, Oddo EM2, Ibarra FR1; 2, Silberstein C1

1. Departamento de Ciencias Fisiológicas, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO-Houssay) UBA-CONICET, Facultad de Medicina, Universidad de Buenos Aires
2. Laboratorio de Nefrología Experimental y Bioquímica Molecular, Instituto de Investigaciones Médicas Alfredo Lanari, Facultad de Medicina, Universidad de Buenos Aires

We have previously demonstrated that 17β-Estradiol (17βE) stimulates cell proliferation through classic estrogen receptors (ER) and the G protein-coupled estrogen receptor 1 (GPER-1), in primary cultures of human renal cortical tubular epithelial cells (HRTEC). We also observed that 17βE decreases the expression of Na+ K+ ATPase (NKA) in primary cultures. The aim of the present work is to study the effects of 17βE on cell proliferation and the expression of NKA in a human renal proximal tubular epithelial cell line (HK-2) and its comparison with previous results in HRTEC and in studies in vivo with ovariectomized (oVx) Wistar rats. HK-2 were treated with 17βE (10 nM, 24 h) with or without an agonist (G-1) or an antagonist (G-15) of GPER-1. Cell proliferation was measured by bromodeoxyuridine (BrdU) uptake. The expression of NKA was assayed by western blot. In HK-2, 17βE stimulated the BrdU uptake (26%) compared with control cells ($p<0.05$). The treatment of HK-2 with G-15 (100 nM) inhibited 17βE effect on cell proliferation. The treatment with G-1 (1000 nM) inhibited the BrdU uptake as observed in HRTEC ($p<0.05$). The treatment of HK-2 with 17βE and with G-1 (10 nM, 24 hs) decreased NKA expression compared with control cells ($p<0.05$), demonstrating that estradiol exerts these effects through GPER-1. These results agree with previous studies in HRTEC, where an increase of D1DR (dopamine receptor) expression was associated with the decrease of NKA. These results also match with previous studies in female adult Wistar rats, in which the oVx produced an increase of NKA expression in renal medulla while there was a decrease of D1DR, both in cortex and medulla. Likewise, hormonal replacement on oVx animals with 17βE diminished the expression of NKA in renal medulla. In conclusion, our present results show that HK-2 cell line can be a valid in vitro model for better understanding of molecular and cellular renal mechanisms regulated by female sex hormones like estrogen.

(12) CORTICOSTRIATAL CONNECTIVITY BALANCE IN L-DOPA-INDUCED DYSKINESIA UNDER AND AFTER THE ACUTE EFFECT OF L-DOPA

Ettel Keifman1, Mariela Verónica Escande1, Jesica Nahir Unger1, Juan Emilio Belforte1, Mario Gustavo Murer1.

Striatal medium spiny neurons (MSNs) are key in action selection. A balanced activity of direct pathway MSNs (dMSNs) and indirect pathway MSNs (iMSN) is needed for an appropriate motor performance. Midbrain dopaminergic neurons provide this balance by modulating MSNs' response to cortical inputs. In Parkinson's disease (PD) nigrostriatal dopaminergic neurons degenerate and an imbalance in favor of iMSNs over dMSNs appears. Chronic treatment with L-DOPA, a DA precursor widely used to treat PD symptoms, causes abnormal movements known as L-DOPA-induced dyskinesia (LID) in up to 30% of patients and is thought to emerge from an imbalance in MSN activity. Under the hypothesis that chronic L-DOPA treatment may produce aberrant plasticity phenomena that affect corticostriatal connectivity that underlie LID, we studied functional and structural changes that emerge from PD and from an acute and chronic L-DOPA treatment. We used in vivo juxtacellular recordings on transgenic mice showing MSN type-specific expression of fluorescent proteins, and we assessed MSN responsiveness to motor cortex stimulation before (off) and following (on) an acute L-DOPA challenge. Off L-DOPA, we did not find differences from PD corticostriatal connectivity. However, on L-DOPA dMSN reverted from their previous disconnection from cortical inputs. Therefore, during LID dMSN prevailed over iMSN. Interestingly, iMSN seemed to contribute less to this imbalance after chronic treatment with L-DOPA.

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(13) EFFECT OF ALKALINE GRADIENT ON CLEAR RENAL CELL CARCINOMA PROLIFERATION: ROLE OF ISOFORM 1 OF NA+/H+ EXCHANGER FUNCTION

Mechali A, Cabral B, Di Giusto G, Beltramone N, Ford P, Capurro C, Rivarola V

The association between proliferation and intracellular pH elicits the possibility that extracellular pH (pHe) may modify cell survival. Moreover, as tumor extracellular acidity is a hallmark of cancer, is probable that pHe affects differently cancer or normal cells. Our previous studies showed that cells derived from renal cell carcinoma (RCC) were more sensible to cell death after 72h exposition to 9.6 mM NaOH (mild alkalosis) than normal cells. The aim of this study was to investigate if this alkaline condition also affects cell proliferation. We use three renal cell models: HK2, derived from normal human proximal epithelial cells, 786-O and Caki-1, both derived from human RCC. We exposed cells to media with 9.6 mM NaOH for 72h. Then, we estimated cell proliferation by BrdU experiments. Our results show at pH 7.4 both RCC derived cell lines proliferate more than normal HK2 cells (% BrdU+ cells: HK2= 31±2; 786-O= 46±3 and Caki-1= 58±3, $p<0.05$ vs HK2 n=10). Normal HK2 cells were not affected by exposure to 9.6mM NaOH for 72hs. However, malignant Caki-1 significantly reduced their proliferation (% BrdU+ cells, pH 7.4= 58±3 vs pH 7.5= 18±3, $p<0.001$ n=8). Previous studies showed that NHE1 isoform of Na+/H+ exchanger (NHE1) can favor or inhibit proliferation in some experimental models. Then, we inhibited NHE1 during the 72h exposition to 9.6 mM NaOH. In normal cells, NHE1 inhibition significantly reduced proliferation in alkaline condition (% BrdU+ cells, +NHE1= 37±1 vs -NHE1= 31±1, $p<0.05$ n=8). On the other hand, inhibition of NHE1 in RCC derived 786-O cells rises proliferation in media at pH 7.4. This effect is partially reverted in the presence of alkalosis (Difference in % BrdU+ cells without NHE1 pH 7.4: 14±3 vs pH 7.5: 3±1, $p<0.05$ n=8). In summary, the combination of alkali plus NHE1 activity reduces tumor proliferation with little effects in normal tissue. Then, this combination of treatment could be an interesting new approach to control RCC cancer.

(14) AQP9 MEDIATES LACTATE TRANSPORT IN HUMAN PLACENTA AS AN ALTERNATIVE ENERGY SUBSTRATE

Medina Yollyseth1, Damiano Alicia E.1,2

1Laboratorio de Biología de la Reproducción. Facultad de Medicina. IFIBIO-CONICET, UBA. Argentina.
2Cátedra de Biología Celular y Molecular, Dpto. de Ciencias Biológicas, Facultad de Farmacia y Bioquímica. UBA. Argentina.

BACKGROUND: Emerging evidence shows that placental aquaporin-9 (AQP9) is not involved in the transfer of water between the mother and the fetus. However, its role in human placenta is still unknown. AQP9 is an aquaglyceroporin that also permeates other solutes such as lactate. In brain, AQP9 may transport lactate as an alternative energy substrate. OBJECTIVE: Our aim was to evaluate the participation of AQP9 in the lactate transfer across the human placenta. METHODS: This study was approved by the ethics committee of the Hospital Nacional Dr. Prof. A. Posadas. Explants from normal term placentas were cultured in low glucose with or without L-lactate, and in presence and absence of AQP9 inhibitors (0.3 mM HgCl2, a general blocker of AQPs, or 0.5mM Phloretin, to block AQP9). Normal glucose medium was used as control. Cell viability was assessed by MTT assay and LDH release. Apoptosis indexes were analyzed by Bax/Bcl-2 protein expression ratio and TUNEL assay. RESULTS: In low glucose medium, MTT decreased while LDH release did not change compared to controls, suggesting that cell death is not due to necrosis. Moreover, Bax/Bcl-2 ratio and apoptotic nuclei increased (n=5, $p<0.02$) and the blocking of AQP9 did not abrogate apoptosis. However, when explants were cultured in low glucose medium supplemented with L-lactate, explant viability and apoptotic indexes were similar to controls indicating that L-lactate could be replacing glucose as an energy substrate. In this case, the blocking of AQP9 resulted in an increase in cell death (n=4, $p<0.05$), proposing that this protein has a role in lactate transport. CONCLUSION: Our results show that placental AQP9 may have a key role in lactate transport as an alternative energy substrate. Thus, the blocking of lactate transport mediated by AQP9 negatively affects the survival of trophoblast cells.

Abstracts

(15) HOMOLOGY MODELING AND MOLECULAR DYNAMICS SIMULATIONS TO STUDY HUMAN AQP4 ISOFORMS

Netti V, Casal JJ, Molina Ponce T, Cocca A, Capurro C.

Laboratorio de Biomembranas, Instituto de Fisiología y Biofísica "Bernardo Houssay" (IFIBIO-HOUSSAY), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Facultad de Medicina, Universidad de Buenos Aires.

Aquaporin-4 (AQP4) is expressed at the plasma membrane as 2 isoforms, AQP4-M1 of 323 amino acids (aa) and AQP4-M23 of 301 aa. Recently, a new AQP4 isoform with a 29 aa C-terminal (Ct) extension (AQP4-Ex) generated by translational readthrough was described. Crystalized AQP4-M1 (32-254 aa) lacks N-terminal (Nt) and Ct ends. Our aim was to model AQP4-M1 and AQP4-Ex by homology modeling to study and compare their properties by molecular dynamics (MD) simulations. Human AQP4 sequences were obtained from UniProt (entry P55087). CBS-DTU tools were used for post-translational modification analysis. AQP4 crystalized structure was obtained from the Protein Data Bank (entry 3GD8) and each Nt and Ct ends were modeled with PEP-FOLD 3 (RPBS Web Portal). Peptides were linked to the 3GD8 AQP4 model to build AQP4-M1 and AQP4-Ex homotetramers by UCSF Chimera software. A 10 ns MD simulation was run in GROMACS 2019 for both isoforms embedded in a bilayer of lipid POPC molecules and solvated with TIP3P as a solvent model. AQP4-Ex (352 aa) had a 100% identity with AQP4-M1. Ct of AQP4-Ex had only two Serine residues (331 and 335) with high score for phosphorylation motif prediction. Homology modeling of AQP4-Ex showed that the extended Ct is a random coil. MD simulations evidenced that AQP4-Ex has a larger mean square displacement and radius of gyration as compared to AQP4-M1, indicating that AQP4-Ex would be less compact and stable. The distance from His201 to Arg216, representative of the selectivity filter (AQP4-M1: 4.32 ± 0.03 nm vs. AQP4-Ex: 4.27 ± 0.03 nm, n=4, ns), showed that water permeability of these isoforms should be similar. Bioinformatics tools allowed us to model both full-length AQP4 isoforms for the first time. MD simulations of AQP4-Ex provide valuable insights into its water permeation mechanism, which agree with recent experimental observations. This is a promising starting point for performing MD simulations to elucidate the function of this novel extended isoform.

(16) L-DOPA CAUSES OSCILLATORY ACTIVITY IN STRIATAL CHOLINERGIC INTERNEURONS FROM PARKINSONIAN MICE VIA DOPAMINE D1/D5 RECEPTORS

Rodrigo Manuel Paz, Cecilia Tubert, Agostina Stahl, Yimy Amarillo, Lorena Rela and Gustavo Murer

Striatal cholinergic interneurons (SCIN) are key modulators of the striatal circuitry controlling voluntary movement and goal-directed behavior. Aberrant striatal cholinergic signalling contributes to the symptoms of Parkinson's disease (PD) and L-dopa induced dyskinesia (LID), a major complication of antiparkinsonian L-dopa therapy. However, the mechanisms causing SCIN dysfunction in PD and LID remain uncertain. Here we used slice electrophysiological approaches to show that SCIN exhibit enhanced Kir and reduced leak currents in a mouse model of LID. These changes cause exacerbated hyperpolarizing responses that coexist with an enhanced excitability, resulting in a burst-pause firing pattern that persists after the dyskinetic effect of an L-dopa dose has worn off. Additionally, we show that a negative slope region of the Kir conductance curve is responsible for the oscillatory behaviour. Stimulation of dopamine D1/D5 receptors mimics the physiological changes induced by L-dopa administration, but D1/D5 receptor blockade does not modify the persistent hyperexcitability and oscillatory activity observed in dyskinetic mice. However, blunting intracellular cAMP signaling restores normal hyperpolarizing responses and dampens oscillatory activity in dyskinetic mice. Our data unravel a mechanism causing aberrant SCIN activity in LID and point at D1/D5 receptor regulation of Kir2 and leak channels as potential targets to restore normal striatal cholinergic function in PD and LID.

Abstracts

(17) SYNAPTIC OUTPUT OF DOPAMINERGIC NEURONS ONLY MODULATES POSITIVELY CONTEXTUAL MEMORY

Melina Petrissans, Carolina D`Odorico, Micaela Vicente Biscardi, Mario R. Pagani

IFIBIO-Houssay, Grupo de Neurociencia de Sistemas, Facultad de Medicina, Universidad de Buenos Aires, CONICET, 2155 Paraguay Street, Buenos Aires, Argentina

An emerging model in associative learning and memory in Drosophila, suggests that dopaminergic neurons (DANs) modulate the synaptic output of mushroom body neurons driving the appropriate behavior (approaching or avoidance). However, whether DANs are involved in contextual memory is unclear. Here, we examined the role of dopaminergic neurons in contextual memory in freely behaving flies. We blockade neuronal activity of specifics subset of DANs by using the thermosensitive allele Shits1. We found that contextual memory is promoted by two kinds of DANs. The first kind corresponds to those DANs that prevented habituation during training in the contextual learning (PPM2). The second kind of DANs corresponds to neurons that were not involved in the contextual learning (PAL). Therefore, suggesting that the second kind of DANs are not involved in sensory or motor activity during the assay, but specifically participate in memory. Interestingly, there are no DANs preventing contextual memory, as it was found for learning. Contextual memory required synaptic output from a smaller number of dopaminergic neurons than that required for contextual learning. Of note, DANs involved in contextual memory are not the same neurons implicated in learning as it is reported in associative learning.

(18) PARADOXICAL ANTINEOPLASTIC EFFECT OF SHIGA TOXIN 2 FROM ENTEROHEMORRHAGIC Escherichia coli IN TRIPLE-NEGATIVE BREAST CANCER CELLS

Pinto A1, Miret N2, Zárate L2, Randi 2, Goldstein J1

(1) Universidad de Buenos Aires, Facultad de Medicina, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Fisiología y Biofísica ''Houssay'' (IFIBIO), Laboratorio de Neurofisiopatología, Buenos Aires, Argentina.
(2) Universidad de Buenos Aires, Facultad de Medicina, Departamento de Bioquímica Humana, Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina.

Shiga toxin 2 (Stx2) is a virulence factor responsible for hemolytic uremic syndrome. Classically, the Stx2´s cytotoxic effect is mediated by its receptor globotriaosylceramide (Gb3). Furthermore, it has been observed that malignant cells represent a great source of Gb3. One of these Gb3-producing malignant tumors is breast cancer, which is the most common cancer type in women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive and difficult to treat from all breast tumors. Our goal is to determine the antineoplastic effect of Stx2 in the TNBC cell line MDA-MB-231. The non-tumorigenic mammary epithelial cell line NMuMG, which lacks Gb3 (negative control), and VERO cells (positive control) were used. Gb3 expression was immunolocalized in MDA-MB-231 and VERO cells, and Stx2 uptake was also observed in both cell lines, with higher levels in VERO cells ($p<0.0001$). Besides, Gb3 levels were increased after Stx2 treatment in MDA-MB-231 cells ($p<0.007$). MTT results showed that 1 and 10 ng/ml Stx2 reduced cell viability after 12, 18, 24 and 48h ($p<0.05$). A Stx2 dose-dependent cytotoxic effect was found in MDA-MB-231 cells after 48h ($p<0.05$). This action was accompanied by an increase of karyorrhexis ($p<0.0001$) and a reduction of mitosis only in MDA-MB-231 and in VERO cells ($p<0.0004$), analyzed by immunofluorescence. With the purpose of evaluating whether an anti-Gb3 antibody would be able to trigger a cellular response, 10 ng/ml of anti-Gb3 or combined with Stx2 was assayed. A cytotoxic effect of anti-Gb3 was observed by MTT in MDA-MB-231, but with less potency than that produced by Stx2 itself ($p<0.05$) and no synergism was found between them. These results suggest that MDA-MB-231 cells are susceptible-dose dependent to Stx2 and sensitive to anti-Gb3 antibodies. Further studies are

necessary to evaluate the safety and effectiveness of Stx2 or anti-Gb3 as a potential treatment in TNBC.

(19) A MULTIVARIATE RELATIONSHIP BETWEEN LABORATORY DATA DURING THE EVOLUTION OF TYPICAL HEMOLYTIC UREMIC

Porporato M(1), Casal JJ(1), Toriano R(1), Dorr R(1), Ibarra C(1), Zotta E(1;2)

(1) UBA, Facultad de Medicina, Departamento de Ciencias Fisiológicas, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO-Houssay)
(2)UBA Facultad de Farmacia y Bioquímica Departamento de Ciencias Biológicas, Catedra de Fisiopatología.

Hemolytic uremic syndrome (HUS) is a systemic disease characterized by variable degrees of acute nephropathy, thrombocytopenia and microangiopathic hemolytic anemia. Laboratory and clinical parameters contribute very closely to progression of HUS. To better understand HUS evolution, the association between a set of laboratory data and a set of clinical parameters of a HUS population is investigated in this study. We conducted a retrospective study of patients (n = 20) attended with diagnosis of typical HUS in the Pediatric Service of the Hospital Posadas from January 2012 to July 2020. 70% were women, with a mean age of 2.19 year. All laboratory data including those from the emergency department (admission), hospitalization, up to the first post-discharge check-up by external clinics were standardized in innovative report formats. We perform the graphical representation of the evolution over time of several of the important clinical parameters (creatinine, hematocrit, hemoglobin, among others). We find the creatinine curve relevant with well-defined moments in its evolution: rise, plateau and decline. We emphasize that 50% of the patients present a similar descent slope (- 0.353 +/- 0.022 mg/dL/day) regardless of the maximum value reached by creatinine. Also, analytic platform KNIME was used to evaluate the multivariate relationship between laboratory data and the evolution plasma creatinine values. We observed a strong correlation between the plasma values of creatinine-urea (positive, r = 0,818), platelets-uric acid (negative, r = 0,610) and direct bilirubin-uric acid (positive r = 0,735). The study should be complemented with the comparison of qualitative variables, as well as with new parameters such as albuminuria, podocyturia, etc.), in order to generate a model of prediction of patient evolution during the acute period of HUS the disease and after it.

(20) SPARSE LABELLING WITH AAV-PHP.EB, A NONINVASIVE GENE DELIVERY METHOD: OPTIMIZATION OF A PROTOCOL FOR MORPHOLOGICAL AND ANATOMICAL CONNECTIVITY ANALYSES

Carlos A. Pretell Annan1, L. Teresita Tribbia2, Germán La Iacona3, Irene R.E. Taravini2, Diego E. Pafundo1, Juan E. Belforte1

1 Grupo Neurociencia de Sistemas, IFIBIO-Houssay, UBA-CONICET, Ciudad de Buenos Aires, Argentina.
2 Laboratorio de Neurobiología Experimental. LNE-FBRO-UNER, Gualeguaychú, Entre Ríos, Argentina.
3 Servicio de Microscopía del IFIBIO-Houssay, UBA-CONICET, Ciudad de Buenos Aires, Argentina.

Since its inception, neuroscience has been captivated by the morphological understanding of neural elements and their intertwining into complex circuits. Studies concerning structure-function relationships at the single cell level and those aimed at delineating anatomical connectivity at neural network level are fundamental for the elucidation of both physiological and pathophysiological processes of the central nervous system (CNS). Although various methods exist for studying neuronal morphology, drawbacks can be encountered in certain settings: e.g., heavy metal staining can hamper simultaneous neurochemical analyses, and genetically-directed labelling can result in insufficient sparseness for adequate reconstruction of complex arborizations. If a detailed study of neuroanatomical projections with conventional or viral tracers is to be combined with a morphological analysis of the cellular elements receiving these inputs, a sparse labelling allowing visualization of axonal processes and performance of immunofluorescence (IF) would be favored. Hence, we optimized a protocol for sparse neuronal labelling taking advantage of a commercially available adeno-associated virus (AAV) variant developed for efficient noninvasive CNS gene delivery: AAV-PHP.eB.

We present data supporting its use for 3D neuronal reconstruction, spine density analysis, and the complementation with AAV tracers and IF to analyze local and distal afferents to individual neurons in the prefrontal cortex of mice.

(21) HYPEROSMOLARITY INDUCES CAVEOLAE INTERNALIZATION IMPAIRING EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION

Reppetti J1, Damiano AE1;2, Martínez N1.

1 Laboratorio de Biología de la Reproducción. Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay) UBA-CONICET. Buenos Aires, Argentina.
2 Cátedra de Biología Celular y Molecular. Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, UBA. Buenos Aires, Argentina

Introduction: During placentation, human extravillous trophoblast (EVT) cells need to proliferate, migrate, and differentiate correctly to ensure proper placental development. Previously, we reported that caveolae are required for the proper migration and endovascular differentiation of EVT. Recently, we found that hyperosmolarity alters EVT cell migration and invasion. However, up to now, the molecular mechanism is unknown. We hypothesized that hyperosmolarity increases caveolae endocytosis and caveolin-1 (Cav-1) degradation, altering EVT cell differentiation. Objectives: Our aim was to explore the effect of hyperosmolarity on caveolae microdomains and the impact on the EVT cell differentiation. Methods: The human EVT Swan-71 cell line was cultured in complete DMEM/F-12. 100 mM of sucrose was added to generate the hyperosmolar condition. Cell viability was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The localization of caveolae was analyzed by Transmission Electron Microscopy (TEM). Cav-1 expression was determined by WB in different conditions (isoosmolarity or control and hyperosmolarity, with or without MG-132- a proteasome inhibitor- and NH4Cl- a lysosomal inhibitor). Endovascular differentiation was analyzed by the formation of tube-like structures in plates coated with Matrigel®. Results: Cell viability was not affected by the experimental conditions. TEM showed that hyperosmolarity induced the internalization of caveolae. In addition, hyperosmolarity also increased Cav-1 protein degradation by lysosomal proteolysis ($p<0.05$, $n=3$) and significantly reduced the formation of tube-like structures compared to control ($p<0.05$, $n=4$). Conclusion: Our results show that hyperosmolarity leads to the internalization of caveolae and the degradation of Cav-1, impairing endovascular differentiation of EVT cells.

(22) NEUTRALIZING PROPERTIES OF HUMAN MILK AGAINST SHIGA TOXIN TYPE 2

Flavia Sacerdoti, Edith Romero, Maria Marta Amaral, Cristina Ibarra

Shiga toxin (Stx) producing Escherichia coli (STEC) is a foodborne pathogen responsible for Hemolytic Uremic Sindrome (HUS). Stx is the main virulence factor responsible for this disease and Stx type 2 (Stx2) has been associated with more severe cases affecting mainly children under 5 years of age. Breastfeeding is one of the least cost-effective public health tools to protect the newborn from diarrhea and its effectiveness can be attributed to different bioactive compounds transmitted through milk. We have previously demonstrated that rats immunized against Stx2 can transfer through lactation protection against a lethal dose of Stx2 to their offspring. In this work we aim to evaluate whether human milk from human healthy donors may have protective properties against Stx2. Human milks (n=107) were collected under informed consent at the National Hospital Prof. Alejandro Posadas by the manual method from healthy women (18-45 years). After collection, milks were stored at 20°C until used. Milks were delipidated by centrifugation at 3000 rpm for 45 min. Supernatant was collected from samples and used for the evaluation of: 1) Neutralizing capacity of Stx2 in vitro on Vero cells, 2) Total protein content by the BCA kit 3) Total IgA content by radial immunodiffusion (RID) and 4) Ig Anti STEC titer by ELISA. Collected milk samples showed a mean of 229 mg/dL of total IgA levels (range: 10-675 mg/dL). Total protein content of samples was heterogeneous and ranged from 5.5 to 166.5 mg/ml. Fourteen milk samples (14/107; 13%) showed neutralizing properties against Stx2 in vitro.

When comparing IgA levels and protein content of positive and negative samples for Stx2 neutralization, no significative differences were observed. Neutralizing capacity of Stx2 has no correlation with high Ig titer against STEC. These results indicate that some human milk may have neutralizing properties against Stx2. These data may be important for HUS prevention of newborn by promoting breastfeeding.

(23) ELIGLUSTAT PROTECTS FROM DAMAGE CAUSED BY SHIGA TOXIN TYPE 2 IN HUMAN RENAL TUBULAR EPITHELIAL CELLS

Sánchez DS1, Fischer Sigel LK1, Balestracci A2, Ibarra C1, Amaral MM1, Silberstein C1

1. IFIBIO HOUSSAY, Departamento de Ciencias Fisiológicas, Facultad de Medicina, Universidad de Buenos Aires.
2. Hospital General de Niños Pedro de Elizalde.

Shiga toxin-producing Escherichia coli is responsible for Hemolytic Uremic Syndrome (HUS), a cause of renal failure in children. We have previously shown that C-9 and Eliglustat (EG), inhibitors of glucosylceramide synthase and globotriaosylceramide (Gb3), prevent the cytotoxic effects of Shiga toxin type 2 (Stx2), in human cortical renal tubular epithelial cells (HRTEC) primary cultures and HK2 cell line. The aim of this work was to evaluate the efficacy of EG, elucidating EG treatments necessary to achieve total protection against Stx2 in HRTEC and HK2. Cells were incubated with Stx2 (1 ng/ml, 24 and 72 h) and pre-incubated with or without EG (1-500 nM, 6 and 24 h), followed by co-incubation with same dilutions of EG and Stx2 (24 and 72 h). Total number of cells stained with Hoechst was counted in microphotographs and compared with cell viability measured by neutral red uptake. Early and late apoptosis and necrosis was evaluated by annexin V/propidium iodide staining. Tubulogenesis was evaluated in HRTEC grown on matrigel. Treatment of cells with Stx2 significantly decreased cell confluence and viability and the number of cells attached ($p<0.001$). In HRTEC, Stx2 increased early and late apoptosis, and necrosis compared to non-treated cells ($p<0.01$). Furthermore, Stx2 inhibited cell aggregation and tubulogenesis on matrigel. HRTEC preincubated with EG (50 nM, 24 h or 500 nM, 6 h) totally prevented Stx2 effects on HRTEC measured as cell count, viability, apoptosis, necrosis and tubulogenesis ($p<0.05$). Preincubation of HK2 cells with EG (1 nM, 24 h or 10 nM, 6 h) totally prevented Stx2 effects on cell viability and confluence. EG alone did not produce cytotoxic effects per se. In conclusion, EG protects human renal tubular epithelium against Stx2 cytotoxicity being HRTEC more sensitive than HK2. Treatment with EG could be a novel substrate inhibition therapy to neutralize Stx2 action and prevent renal damage in patients with HUS. Study supported by PUE0041, CONICET.

(24) A PROJECT TO STUDY HOW REWARD AND PUNISHMENT MODULATE VISUALLY EVOKED RESPONSES IN THE PRIMARY VISUAL CORTEX

María Florencia Santos, Camila L. Zold.

Grupo de Neurociencias de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Houssay, CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina.

The primary visual cortex (V1) neuronal activity encodes basic properties of visual stimuli. Experience dependent plasticity has been observed in V1 as a way to improve visual perception. However, recent studies show that V1 neural plasticity is also related to reinforcement learning. When rodents experience an association between a visual stimulus and a contingent future reward, a proportion of V1 neurons develop reward timing activity. Cholinergic projections from the basal forebrain (BF) have been shown to be necessary and sufficient to induce the reward timing activity in V1. However, little is known about whether this activity evolves simultaneously in the BF and V1 during learning. On the other hand, if V1 encodes the behavioral significance of visual stimuli in a general way, we hope that it may also encode the time interval between visual stimuli and contingent punishments. To unveil this, we implanted C57BL/6 adult male mice with a microelectrode array in V1 and performed electrophysiological recordings in head-fixed mice learning a visually cued rewarded task. Mice were trained to perform a lick sequence in order to receive a water reward in 70% of cases. We trained 4 mice that successfully learned the task and we identified neurons responding to visual stimulus. To continue with the project, we plan to carry out simultaneous recordings on V1 and BF and also analyze V1 activity in animals exposed to Pavlovian stimulus-reward and stimulus-punishment associations.

Abstracts

(25) PRESENCE OF SHIGA TOXIN PRODUCING ESCHERICHIA COLI IN ENDOCERVIX OF ASYMPTOMATIC PREGNANT WOMEN: NOVEL PATHOGEN RESPONSIBLE FOR ADVERSE PREGNANCY OUTCOMES?

Scalise ML1; Garimano NE1; Porporato M1; Leonino P2; Pereyra A2; Ferreiros JA2; Casale R2; Amaral MM1; Sacerdoti F1; Ibarra C1.

Grupo de Neurociencias de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Houssay, CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina.

E. coli can colonize the vagina, usually asymptomatically, although epidemiologic studies have showed that the presence of this bacterium in the endocervix microbiota could be a risk factor for pregnancy. We have previously reported that Shiga toxin (Stx) producing E. coli (STEC) infections during pregnancy may cause maternal or fetal damage mediated by Stx2 in rats in early or late stage of gestation. The goal of this study was to detect STEC in the endocervix from asymptomatic pregnant women. Endocervical swabs from 103 asymptomatic pregnant women with gestational age of 12 to 30 weeks from the National Hospital Posadas were enrolled. Swab samples were enriched in Tryptic Soy Broth and then streaked on sorbitol-MacConkey (SMAC) agar. E. coli was confirmed by the presence of uidA gene detected by polymerase chain reaction PCR. The positive samples for E. coli were analyzed for STEC virulence factors genes such as: stx1, stx2, eae, rfbO157, lpfAO113 and hcpA genes. The stx2 positive E. coli samples were grown in Luria-Bertani Broth and the filter-sterilized bacterial supernatants (SN) were used to evaluate Stx2 activity on Vero, Swan and Hela by cell viability assay. Our results showed that 14.6% (15/103) of the endocervical samples were positive for uidA gene. Additionally, we found that 8.7% (9/103) was positive for stx2 and 5.8 % (6/103) for lpfAO113 and hcpA genes. The SN of one of them expressing stx2 gene had a high cytotoxic activity on Vero, Swan 71 and HeLa cells. Stx2 identity was checked using an anti-Stx2 antibody in order to neutralize the cytotoxic effects. In conclusion, we demonstrate that STEC can be asymptomatically present in the endocervix and that can potentially express Stx2. This study may open a new perspective to understand whether STEC can be a novel pathogen involved in adverse pregnancy outcomes.

(26) REMAPPING OF HIPPOCAMPAL PLACE CELLS IN AN UNREWARDED CONTEXTUAL MEMORY TASK

Azul Silva 1, Pedro Bekinschtein 2 y Mariano Belluscio 1

1. Instituto de Fisiología y Biofísica Bernardo Houssay, IFIBIO-UBA CONICET
2. INCYT, Universidad de Favaloro-INECO-CONICET

In order to retrieve an episodic memory our brain needs to integrate the contextual information available. But usually, the information is incomplete, so which memory is retrieved in a particular situation? Pattern completion allows us to generalize and retrieve memories although information is partial. On the other side, when we need to encode a new memory without interfering with a pre-existing one, pattern separation is involved. The hippocampus has a key role in these memory processes. Some hippocampal neurons, place cells (PC), are tuned to spatial location and generally change their tuning when sensory inputs change (remapping). But sometimes, although the context changes, PC doesn't remap. Accumulating evidence has suggested that the hippocampal ability of storing and distinguishing between different situations and contexts, can be related with place cell's remapping. The aim of this project is to understand how the remapping observed in CA1 and CA3, two hippocampal regions, correlates with the evocation of contextual memories. To tackle this question, we use a behavioral task that allows us to discriminate if an animal recognizes a context as new (pattern separation), or as one it already knows (pattern completion). We carried out electrophysiological recordings in CA3 and CA1 while the animal was performing the task. Preliminary results show that there is a correlation between the amount of remapping and the memory that the animal is recalling.

Abstracts

(27) LOCAL COUPLING BETWEEN SLEEP SPINDLES AND SLOW OSCILLATIONS SUPPORTS THE CONSOLIDATION OF MOTOR MEMORIES.

Agustín Solano[1], L. A. Riquelme[1], D. Perez Chada[2], V. Della-maggiore[1]

[1]IFIBIO-Houssay, Univ. de Buenos Aires, Buenos Aires, Argentina; [2]Dept. de Med., Austral Univ. Hosp., Pilar, Argentina

The precise coupling between slow oscillations (SO) and spindles is critical for sleep-dependent consolidation of declarative memories. Here, we examined whether this mechanism also operates in the stabilization of human motor memories during NREM sleep. We hypothesized that if the coupling of these oscillations is instrumental to motor memory consolidation then only SO-coupled spindles would predict long-term memory. We found that motor learning increased the density of fast (≥12 Hz) spindles during NREM3. This modulation was manifested locally over the hemisphere contralateral to the trained hand. Contrarily, spindles density was no altered during NREM2 sleep. Although motor learning did not affect the density of SOs, it substantially modulated the coupling between fast spindles and SOs in an inter-hemispheric manner, suggesting it may rather increase the ability of slow oscillations to promote thalamic spindles. The fact that only the modulation of coupled fast spindles predicted long-term memory points to the association of these oscillations as a fundamental signature of motor memory consolidation. Our work provides evidence in favor of a common mechanism at the basis of the stabilization of declarative and non-declarative memories.

(28) ROLE OF STRIATAL SOMATOSTATINERGIC INTERNEURONS IN PARKINSON'S DISEASE MOTOR SYMPTOMS AND L-DOPA-INDUCED DYSKINESIAS

Agostina Stahl, Verónica Risso, Juan Belforte, Lorena Rela and Gustavo Murer

Action selection relies on the coordinated activity of striatal direct and indirect pathways, strongly modulated by dopamine (DA) and cholinergic neurons. Loss of mesencephalic DA neurons in Parkinson's disease (PD) is thought to disrupt the balance between these modulators resulting in an alteration of basal ganglia circuits and motor disabilities. Striatal non cholinergic interneurons also play key roles in modulating striatal projection neurons, but their potential contribution to motor symptoms of PD is poorly understood. The goal of this project is to identify the role of striatal somatostatinergic interneurons (iSOM+) in the expression of motor deficits and the development of L-dopa-induced dyskinesias (LID). To this aim, we use Som-Cre mice unilaterally lesioned with 6-OHDA as a model of PD and evaluate behavioral performance while modulating iSOM+ activity using chemogenetic tools. As a first approach, we delivered a viral vector that directs the expression of an inhibitory DREADD in iSOM+, unilaterally into the striatum, and evaluated motor deficits by using three behavioral assays in the presence and absence of its synthetic ligand. Subsequently, mice were treated with increasing doses of L-dopa and we evaluated whether LID expression is altered by iSOM+ inhibition. Preliminary results showed that after L-dopa treatment (6 mg/kg), inhibition of iSOM+ increased LID expression, while no effects were found on basal locomotion nor on the development of motor deficits.

Abstracts

(29) AN APPROACH TO ADDRESS THE EFFECTS OF MICROGLIA DEPLETION ON DEGENERATION AND REGENERATION AFTER OLFACTORY NERVE DAMAGE

Diego M. Topsakalian, Luis E. Acosta, Ana P. Piantanida, Jésica N. Unger, Juan E. Belforte, Lorena Rela

Olfactory ensheathing glia is recognized for its ability to promote axonal growth, a property that is normally used to explain the regenerative capacity of the olfactory nerve. However, little attention has been paid to the possibility that immune cells (microglia/macrophages) present in the afferent olfactory pathway participate in its repair, although they show signs of activation after damage. To determine whether microglial cells present in the afferent olfactory pathway mediate damage severity and/or recovery, we propose to analyze the effects of microglia depletion by PLX5622 in a mouse model of damage to the olfactory nerve by the olfactotoxin methimazole. The functional status of the olfactory nerve will be evaluated by a habituation/dishabituation olfactory test at four or fourteen days after damage. In addition, the same day of the behavioral test, we will collect tissue to obtain an anatomical correlate from histological preparations of the bulb and olfactory mucosa, to verify the microglial depletion and to evaluate the sensory neuron integrity. If microglial cells play a role in damage severity and/or resolution, we expect that the animals show partially conserved olfactory function or slower recovery, respectively, with PLX5622 treatment. This approach sets the basis to analyze whether inflammation modulates the neurotrophic properties of olfactory ensheathing cells.

(30) ROLE OF EspF FROM ENTEROHAEMORRAGIC Escherichia Coli O157:H7 ON THE MECHANISMS RELATED TO PRODUCTION OF SHIGA TOXIN TYPE 2

Toytoyndjian EV, Garimano N, Amaral MM, Ibarra CA.

Laboratorio de Fisiopatogenia, Departamento de Fisiología, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay-CONICET), Facultad de Medicina.

Enterohaemorrhagic Escherichia coli O157:H7 (EHEC) strains are responsible for multiple clinical syndromes including bloody diarrhea, hemolytic uremic syndrome (HUS). HUS is a systemic disease caused mainly by Shiga toxin type 2 (Stx2). EHEC employs a type III secretion system to colonize the bowel and to inoculate effector proteins such as EspF. This protein is responsible to disrupt tight junctions, inhibit phagocytosis and induce effacement of microvilli and apoptosis. However, the role of EspF on the mechanisms related to Stx2 production across human intestine are not well known. The EHECΔespF mutant was constructed by Chengsong Wan and co-workers (Zhao et al. PlosOne 2013). We have evaluated the equivalent amount of Stx2 on Vero cells (ATCC CCL-81) cultured with the supernatants of EHEC wild type (SNwt) and EHEC ΔespF (SNΔespF).

Both strains, EHEC O157:H7 wt and EHEC O157:H7 ΔespF were grown in LB medium for 18 h at 37 °C in LB with shaking at 150 rpm and then diluted 1:10 in DMEM/F12 medium with the addition of 10 mM of HEPES and grown to exponential phase (optical density at 630 nm of 0.3-0.4). Bacterial supernatants were collected after centrifugation at 10,000 g for 5 min and sterilized by filtration through a 0.22-µm-pore-size filter. The titers of Stx2 of filter-sterilized supernatants were determined on Vero cells.

A significant cytotoxic effect was observed when monolayers of Vero cells were exposed to different concentrations of purified commercial Stx2 under growth-arrested conditions. The CD50 was maximal after 72 h of incubation. Both, SN wt and SN ΔespF showed similar degrees of cytotoxicity on Vero cells corresponding to equivalent amount of Stx2 calculated by a non-parametric Mann-Whitney test.

This initial trial shows that the absence of the gene EspF might not significantly affect secretion of Stx2 in the bacterial culture. More studies are needed to evaluate the effect of EspF on Stx2 production of EHEC.

Abstracts

(31) HYPOTHALAMIC PROOPIOMELANOCORTIN EXPRESSION RESTRICTED TO GABAERGIC NEURONS PREVENTS OVERFEEDING AND NEUROPEPTIDE Y OVEREXPRESSION

Milagros Trotta, Estefania Pilar Bello, Ramiro Alsina, José Luis Ferrán, Marcelo Rubinstein, Viviana Florencia Bumaschny.

The arcuate nucleus is a key regulator of energy homeostasis in which different neuronal populations integrate peripheral signals of energy status. In particular, arcuate proopiomelanocortin (POMC) neurons inhibit food intake and promote energy expenditure. Due to the existence of different subpopulations of POMC neurons secreting antagonistic neurotransmitters such as glutamate or GABA, it is proposed that Arc-POMC neurons could have different physiological roles and targets. In the present study, we aimed to elucidate the contribution of the subpopulation of Arc-POMC GABAergic neurons in the control of energy balance by expressing Pomc exclusively in GABAergic-POMC neurons. We found that Pomc rescue restricted to GABAergic neurons leads to food intake normalization and body weight enhancement. Surprisingly, these physiological improvements were achieved with the recovery of Pomc expression in only 25% of total hypothalamic POMC neurons. Immunohistochemical analysis showed that GABAergic POMC neurons preferentially project to the dorsomedial hypothalamus (DMH), a nucleus that induces food intake by releasing NPY. In addition, we found that DMH-NPY expression is negatively correlated with Pomc expression in GABAergic-POMC neurons, suggesting that food intake may be regulated by an Arc-GABAergic-POMC→ DMH-NPY pathway.

(32) SYNAPTIC AND CELLULAR PLASTICITY OF CORTICO-STRIATAL NEURONS OF THE ANTERIOR CINGULATE CORTEX ASSOCIATED TO NEUROPHATIC PAIN

María Jesús Trujillo, Constanza Ilarraz & Fernando Kasanetz

Grupo de Neurociencias de Sistemas, Instituto de Fisiología y Biofísica (IFIBIO) Houssay, CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina.

Chronic neuropathic pain (NP) is a highly prevalent and debilitating neurological condition. On the cellular level, the elevated pain sensitivity is induced by aberrant neuronal plasticity at all stages of the nociceptive pathway. Whereas a lot is known about the mechanisms mediating NP in the peripheral nervous system and in the spinal cord, less is known about these processes in brain areas where pain is eventually perceived. The Anterior Cingulate Cortex (ACC) is a key area of the nociceptive system, is essential for encoding pain affect and is hyperactive in patients suffering from chronic pain. In addition, synaptic and cellular modifications in ACC neurons are necessary for the expression of nociceptive sensitization in animal models of NP. Recent data suggest that abnormal recruitment of basal ganglia (BG) structures may facilitate the persistence of pain. In this context, we speculate that abnormal nociceptive processing during NP could spread to the BG through aberrant neuronal plasticity in cortico-striatal (CO-ST) neurons of the ACC. However, little is known on how NP affects these neurons. To gain insight on this, we evaluated the synaptic and cellular modification in CO-ST ACC neurons associated with NP. To do this we combined neuronal identification with fluorescent retrograde tracers and ex-vivo electrophysiological recording (brain slices) in a rodent model of NP. Our preliminary data shows that NP impairs the integration of synaptic inputs into CO-ST ACC neurons.

Abstracts

(33) VALIDATING A BEHAVIORAL TEST FOR STUDYING SOCIABILITY USING FAST-SCAN CYCLIC VOLTAMMETRY

Juan M Uehara1, Juan E Belforte1, Estefania P Bello1

1 Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Universidad de Buenos Aires, Facultad de Medicina - CONICET

The three-chamber test and the reciprocal social interactions test are the most widely used paradigms to study sociability in rodents. However, they were not initially developed for simultaneous electrochemical recordings. In the former, the apparatus is not well suited for handling moving wires and it only allows the simultaneous presentation of two stimuli as a maximum, while in the latter the aggressive behaviors initiated by the conspecific are not restricted. This work aims to validate a social interaction test compatible with fast-scan cyclic voltammetry and that allows the free circulation of implanted mice and the presentation of multiple stimuli of different novelty, salience and valence. In the interaction phase, subject mice spent more time exploring an age and sex-matched conspecific than an object. In addition, they preferred to investigate a novel demonstrator over a familiar one in the recognition phase. The number of entries to the exploration zones was also significantly different in both phases. Discrimination indexes remained stable in a retest conducted one week later, although differences within each phase were less significant. Other variables such as latency to first visit and length of the first visit were particularly relevant when the affective state of the demonstrators was modified. Validating this test is the first step towards studying the dopaminergic contribution to social interaction and the social phenotype of a murine model of schizophrenia.

(34) SYNAPTIC OUTPUT OF DOPAMINERGIC NEURONS CONTROLS CONTEXTUAL LEARNING BY PROMOTING ANTAGONISTIC BEHAVIORS IN DROSOPHILA

Micaela Vicente, Carolina D`Odorico, Melina Petrissan, Mario R. Pagani

IFIBIO Houssay, Grupo de Neurociencia de Sistemas, Facultad de Medicina, Universidad de Buenos Aires - CONICET, Buenos Aires, Argentina.

Accumulated evidence supports an emerging model for the neuronal control of behavior in associative learning in Drosophila. In such model, dopaminergic neurons (DANs), which encode the unconditional stimuli, modulate the synaptic output of mushroom body neurons driving the appropriate behavior (approaching or avoidance). However, studies in vertebrates have shown that self-motivated contextual learning and other forms of learning depend on distinct molecular and cellular mechanisms. Here, we examined the role of dopaminergic neurons in contextual learning in freely behaving flies.

We blockade or enhanced neuronal activity of subset of DANs by using the several GAL4 lines to drive the expression of the thermosensitive allele Shits1 or TrpA1. We found that in contextual learning, flies showed habituation of the exploratory activity, which is controlled by DANs. Preliminary studies indicate that, habituation was promoted by two different clusters of DANs. Three neurons from the PAL cluster or one to four PPL2 neurons, or possibly both subsets contributed to promote habituation. Interestingly, habituation also was prevented by two different clusters of DANs. Neurons from the PAM cluster and one to two neurons from the PPM2 cluster were individually sufficient to prevent habituation. Taken together, habituation of a motivated behavior is under positive and negative control of DANs.

Abstracts

(35) THE PRESENCE OF CONSPECIFICS DURING NICOTINE EXPOSURE ALTERS DRUG PREFERENCE IN A DOSE DEPENDENT MANNER IN ZEBRAFISH (DANIO RERIO)

Rocco, Leandro. Bernabeu, Ramón.

Group dynamics in gregarious animals are a complex behavioural aspect with multiple variables influencing the way individuals relate to one another. Drugs of abuse such as nicotine are important reinforcing stimuli which have been proven to alter the way group behaviour takes place. Interestingly, there have not been studies that show whether group dynamics alter the way in which individuals react to a drug. These dynamics may be highly influential on the possibility of an individual becoming addicted to a certain substance. Nicotine rewarding properties have been assessed in zebrafish using a biased conditioned place preference (CPP) protocol, which has proven to be a remarkable tool in evaluating response to drugs of abuse with this animal model. Despite the fact that several studies have been able to describe the way in which group behaviour is altered with the addition of several substances, and how co specifics have altered the response to drugs of abuse by making the animal choose between an addictive substance, food, or the possibility to be a part of a group of its own species, there have not been studies that consider how drug exposure within a group can alter the way an individual responds to a drug long term. In the present study, we aimed to evaluate whether individuals exposed to nicotine as a group developed different responses to those of individuals exposed to the substance in isolation ("classic" CPP) and whether these responses varied in accordance to the concentration of nicotine to which they were exposed. By exposing fish to either a "Group" or an "Alone" CPP Protocol our preliminary results seem to show that Nicotine elicits a stronger, more robust CPP when being exposed to the drug as a group. When Nicotine concentration is risen to 50mg/L, however, the animals exposed as a group show negative CPP scores in comparison to their "Alone" exposure counterparts, that exhibit higher CPP Scores when compared to the ones exposed to a concentration of 15mg/L of Nicotine. These results may indicate that being exposed as a group enhanced the effects of nicotine to a point that higher concentrations resulted in an exacerbation of its negative, anxiogenic effects, outweighing its rewarding, anxiolytic properties. To corroborate our findings we conducted protocols of nicotine exposure coupled with Phenylbutirate, an HDAC inhibitor that has been proven to arrest the development of CPP in isolated animals. We theorized that by blocking the unfolding of CPP in a group-enhanced CPP protocol we should be able to observe a positive CPP score at higher concentrations (50mg/L) whereas the isolated CPP protocol should still be showing negative results regardless of concentration.

(36) ANALYSIS OF BETA BURSTS ACTIVITY ALONG THE PRIMARY MOTOR CORTEX DEPTH IN A RODENT MODEL OF PARKINSON'S DISEASE

Piña Novo, Daniela

The nigrostriatal degeneration developed during Parkinson's disease leads to changes in the oscillatory activity both of the basal ganglia and the motor cortex (MC). In particular, exaggerated beta bursts (15–35 Hz) have been shown to emerge after dopamine depletion. This pathological beta band exacerbation is also correlated with motor symptoms of the disease, such as bradykinesia and rigidity. Thus, along with the symptomatic relief resulting from the administration of L-DOPA, which is the gold standard medication, there is a decrease in the prevalence of beta activity. Our previous characterization of beta bursts in MC of hemiparkinsonian mice disclosed a similar pattern, with an increase in their amplitude, duration and occurrence. Interestingly this pattern reversed during the acute effect of L-DOPA, but reappeared when L-DOPA effects have worn off. Here we evaluated the relationship of beta bursts occurrence and their amplitude with the depth within MC. Additionally, we performed the same analysis but separating in rest and movement periods. We found that in lesioned animals, whether they had not received L-DOPA or were out of its acute effect, the number of beta bursts differed significantly along cortical depth. Particularly, there was an increase around layer 5. Beta burst amplitude profile exhibited a small increment around this depth but it didn't differ significantly.

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