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Neurodegenerative diseases

MODULATION OF SYNAPTIC PLASTICITY DEFICITS AS THERAPEUTIC STRATEGY IN HUNTINGTON'S DISEASE

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1. Summary

Selective degeneration of striatal medium spiny neurons (MSN) has been considered the main cause of motor disturbances in Huntington's disease (HD). However, growing evidence demonstrates that corticostriatal synaptic and neuronal dysfunction occurs long before neuronal loss. Hence research to design new therapeutical interventions for HD has focused on the study of the molecular mechanisms underlying neuronal plasticity dysfunction. In this scenario, the current project is aimed to identify mutant huntingtin targeted pathways involved in neuronal plasticity dysfunction to design new therapeutic strategies for preventing or delaying motor and cognitive decline in HD. To achieve this goal, we will analyze whether neuronal plasticity can be restored by modulation of (1) BDNF/TrkB/p75NTR signalling and (2) inhibition-excitation pathways involved in corticostriatal and nigrostriatal circuits.

Methodology: HD mouse models (R6/1, HdhQ7/Q111 and YAC128 mice) and HD human models (MSN neurons derived from HD induced pluripotent stem cells (iPSC) will be used). We will establish a new protocol for differentiation of iPSCs to cortical, nigral and striatal neurons to study cell-cell interaction affected by mutant huntingtin. Changes in plasticity will be evaluated by analyzing dendritic spines using Helios Gene Gun System in iPSC and organotypic cultures. Neuronal sprouting will also be measured by immunohistochemistry and western blot using specific synaptic and cytoskeletal markers. Electrophysiology will be used to analyze NMDA and AMPA receptors activity. Optogenetic stimulus will be used in in vivo and in vitro models. Neuronal network connectivity will be analyzed using high-speed calcium imaging with in silico modelling.

Expected results: Our proposal will provide the proof of principle for BDNF-like new molecules as potential therapeutics for HD, and understanding of the molecular mechanisms involved in the imbalance between inhibitory and excitatory striatal inputs in HD to identify new HD targets. The use of human HD models can help to bring the application of the results closer to clinical trials.

2. Results

WP 1: To study mechanisms of dendritic spine formation and synaptic activity that are affected in HD models in steady-state and after neuronal stimulation.

Pyk2 modulates hippocampal excitatory synapses and contributes to cognitive deficits in a Huntington's disease model

We study the role of Pyk2, a non-receptor calcium-dependent protein-tyrosine kinase highly expressed in the hippocampus. Hippocampal-related learning and CA1 long-term potentiation are severely impaired in Pyk2- deficient mice and are associated with alterations in NMDA receptors, PSD-95 and dendritic spines. In cultured hippocampal neurons, Pyk2 has autophosphorylation-dependent and -independent roles in determining PSD-95 enrichment and spines density. Pyk2 levels are decreased in the hippocampus of individuals with Huntington and in the R6/1 mouse model of the disease. Normalizing Pyk2 levels in the hippocampus of R6/1 mouse rescue memory deficit, spines pathology and PSD-95 localization. Our results reveal a role for Pyk2 in spine structure and synaptic function, and suggest that its deficit contributes to Huntington's disease cognitive impairments (**Nature Communications**, 2017 May 30;8:1559). Continuing with these data we also observed a possible role for Pyk2 in the response to stress and in synaptic markers expression and spine density regulation in the amygdala, suggesting that Pyk2 contributes to stress-induced responses through micro-structural changes and that its deficit may contribute to the resilience to chronic stress (**Translational Psychiatry**, 2019 Jan 15;9(1):3).

GluN3A promotes NMDA spiking by enhancing synaptic transmission in Huntington's disease models

GluN3A subunits have been linked to synapse loss and death of spiny projection neurons of the striatum in Huntington's disease. Our results show that suppressing GluN3A expression prevents a multivariate synaptic transmission phenotype that precedes morphological signs at early prodromal stages. Using a highly sensitive electrophysiological method in corticostriatal slices, we showed an enhancement in synaptic drive as a mechanism that triggers a type of NMDAR-dependent electrical excitability in dendrites. Our results also rule out a role for glutamate spillover activating extrasynaptic NMDA receptors. Therefore, these data implicate GluN3A reactivation in a broad spectrum of early-stage synaptic transmission deficits in YAC128 mice, question the current concept that NMDAR mislocalization is the

pathological trigger in HD, and also introduce NMDA spikes as a new candidate mechanism for coupling NMDARs to neurodegeneration (**Neurobiology of Disease**, 2016 Sep;93:47-56).

Increased levels of rictor prevent mutant huntingtin-induced neuronal degeneration

Rictor associates with mTOR to form the mTORC2 complex, which activity regulates neuronal function and survival. Here, we analyzed whether mTORC2 activity could be altered by the presence of mutant huntingtin. We observed that rictor levels are specifically increased in the striatum of HD mouse models and in the putamen of HD patients. Rictor-mTOR interaction and the phosphorylation levels of Akt, one of the targets of the mTORC2 complex, were increased in the striatum of the R6/1 mouse model of HD suggesting increased mTORC2 signalling. Interestingly, acute downregulation of Rictor in striatal cells in vitro reduced mTORC2 activity, as shown by reduced levels of phospho-Akt, and increased mutant huntingtin-induced cell death. Accordingly, overexpression of rictor increased mTORC2 activity counteracting cell death. Furthermore, normalization of endogenous rictor levels in the striatum of R6/1 mouse worsened motor symptoms, suggesting an induction of neuronal dysfunction. In conclusion, our results suggest that increased rictor striatal levels could counteract neuronal dysfunction induced by mutant huntingtin (**Molecular Neurobiology**, 2018 Oct;55(10):7728-7742)

<u>WP2: To study the balance between BDNF and TrkB/p75NTR in HD-iPSC-derived MSNs.</u>

HPSC-derived neurons are functionally matured in vitro and integrated in in vivo mouse striatum

We develop a novel and fast feeder-free protocol for the differentiation of hPSCs to mature and physiologically active telencephalic neurons in 37 days, which is useful for human disease modelling, drug screening and cell therapy applications. An induced hPSC line (33Q#1) and an embryonic hPSC line (Genea 19) were used in the present study. Our data show that 33Qn1 hiPSC-derived neuronal cultures have spontaneous activity with differentially neuronal features DIV 37 (**Comella et al. manuscript in preparation**).

WP3: To generate new molecules that restore neural plasticity and TrkB/p75NTR equilibrium in HD models.

7,8-dihydroxyflavone ameliorates cognitive and motor deficits in a Huntington's disease mouse model through specific activation of the PLCγ1 pathway

Chronic administration of 7,8-DHF delayed motor deficits in R6/1 mice and reversed deficits on the Novel Object Recognition Test (NORT) at 17 weeks. Morphological and biochemical analyses revealed improved striatal levels of enkephalin, and prevention of striatal volume loss. We found a TrkBY816 but not TrkBY515 phosphorylation recovery in striatum concordant with in vitro results. Additionally, 7,8-DHF treatment ameliorated the imbalance of p75/TrkB. Our results provide new insights into the mechanism of action of 7,8-DHF suggesting that its effect through the TrkB receptor in striatum is via selective phosphorylation of its Y816 residue and activation of PLCγ1 pathway, but pleiotropic effects of the drug also contribute to its therapeutic potential (**Human Molecular Genetics**, 2017 Aug 15;26(16):3144-3160).

Pituitary adenylate cyclase-activating polypeptide (PACAP) enhances hippocampal synaptic plasticity and improves memory performance in Huntington's disease

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that exerts neuroprotective actions, mainly through the PAC1 receptor. We investigated the ability of PACAP receptor stimulation to enhance memory development in HD. We observed a hippocampal decline of all three PACAP receptor expressions in different HD mouse models from the onset of cognitive dysfunction. In hippocampal post- mortem human samples we found a specific decrease of PAC1 without changes in VPAC1 and VPAC2 receptors. We also found that PACAP treatment rescued PAC1 level in R6/1 mice promoted expression of the hippocampal brain-derived neurotrophic factor and reduced the formation of mutant huntingtin aggregates. Furthermore, PACAP administration counteracted R6/1 mice memory deficits as analyzed by the novel object recognition test and the T-maze spontaneous alternation task. Importantly, the effect of PACAP on cognitive performance was associated with an increase of VGlut-1 and PSD95 immunolabelling in hippocampus of R6/1 mice. Taken together, these results suggest that PACAP, acting through stimulation of PAC1 receptor, may have a therapeutic potential to counteract cognitive deficits induced in HD (**Molecular Neurobiology**, 2018 Nov;55(11):8263-8277).

WP4: To develop new strategies to control the inhibition-excitation equilibrium in corticostriatal and nigro striatal circuits in vitro and in vivo.

NETCAL: An interactive platform for large-scale, NETwork and population dynamics analysis of CALcium imaging recordings

We developed NETCAL, a complete software platform, developed within MATLAB, to record, manage and analyze high-speed high-resolution calcium-imaging experiments. Its ease of use, interactive graphical interface and exhaustive documentation are tailored to wet lab researchers, but also to experienced data scientists through its plug-in and scripting system. We have developed a large set of tools and incorporated state of the art algorithms and toolboxes for the large-scale analysis of network and population dynamics, including: automated cell detection (both static and dynamic); trace and population sorting through machine learning, clustering and pattern recognition; bursting dynamics; spike detection; network inference (from functional networks to causal relations); and many more. Several of these tools are also available in real time, cells and spikes can be monitored while the experiment is being recorded, giving the researcher extensive feedback of how an experiment is developing.
We have tested and used the software in several different experimental preparations and laboratory equipment. NETCAL has already been used to test the viability and performance of differentiation protocols from human induced pluripotent stem cells (hISPCs); to characterize the individual and collective behaviour of dissociated cortical and striatal cultures from Huntington's disease mouse models; to reveal the communication between neurons and astrocytes in rat hippocampal cultures; and to detect propagating activity patterns in cortical cultures. Although it has been developed for calcium recordings in cultures, we have successfully tested and used it for in-vivo recordings and to analyze data from multi-electrode arrays.

NETCAL has been developed by scientists for scientists to promote and foster the development of tools for the replication and validation of experimental results. The software is highly modular, and its implementation provides easy extendability to adapt it to the specific requirements of any research group. **Orlandi et al. Zenodo. http://doi.org/10.5281/zenodo.1119026 www.itsnetcal.com**

JG. Orlandi; S Fernández-García; A Comella-Bolla; M Masana; G García-Díaz Barriga; M Yaghoubi; A Kipp; JM. Canals; MA. Colicos; J Davidsen; J Alberch; J Soriano. (2017). NETCAL: An interactive platform for large-scale, NETwork and population dynamics

analysis of CALcium imaging recordings (Version 7.0.0 Open Beta). Zenodo. http://doi.org/10.5281/zenodo.1119026 www.itsnetcal.com

Neuronal network connectivity and dynamics dysfunction in Huntington's Disease striatal cultures

Using high-resolution calcium imaging, we showed that R6/1 striatal cultures showed less active neurons than WT. Moreover, both striatal cultures are able to self-organize and produce collective events of nearly- synchronous activity, indicating the presence of a functional network. Network bursts present a duration and amplitude similar between genotypes whilst the interval between bursts is significantly decreased in R6/1 HD striatal cultures respect to WT. Blockade of GABAA receptors by bicuculline (BIC) increased number of active neurons and boosted coherent activity throughout the culture reaching similar levels in both WT and R6/1 HD, suggesting an impairment in striatal GABAergic inhibition in HD. Additionally, functional connectivity analyses revealed decreased input degree in R6/1 striatal network as well as fewer connected modules of neurons, pinpointing a defective communication in R6/1 striatal neuronal network. Nevertheless, striatal network dysfunction in HD may arise not only from local inhibition but probably also from aberrant afferent activity. Modulation of striatal activity with NMDA partially restored the network dynamics. Global efficiency was increased and community statistic decreased specifically in R6/1 striatal cultures, both parameters reaching similar levels to WT basal striatal network. Moreover, the number of connector hubs slightly increased after addition of NMDA in both WT and R6/1 striatal networks. These results suggest that increasing the excitatory drive of the network can partially restore the information flow across the striatal R6/1 network **(Fernández-García et al., manuscript in preparation)**

Optogenetic stimulation of corticostriatal pathway ameliorates motor behaviour in Huntington's disease

Using in vivo MRI, optogenetics and microdialysis and ex vivo multielectrode arrays, we characterized corticostriatal dysfunction in HD. Then we applied repeated optogenetic stimulation in symptomatic R6/1 HD mice and evaluated motor learning and coordination. Structural and functional MRI showed loss of corticostriatal function in R6/1 HD mice. Also, we measured a reduction of striatal glutamate levels (GluCEST and MRS) and corticostriatal release (optogenetics coupled to microdialysis). The electrophysiological response of striatal neurons to optogenetically induced

corticostriatal function was also reduced in HD mice (MEA). Finally, repeated corticostriatal optogenetic stimulation in symptomatic HD mice (R6/1-ChR2) improved motor learning (accelerating rotarod), coordination (balance beam test), exploratory activity (rearings) and stereotypic behaviour (grooming), compared to control R6/1-YFP mice, almost reaching WT levels. This improvement was accompanied by an increase in spine density measured from Golgi-staining. Thus, our results demonstrate for the first time an in vivo effective optogenetic-induced recovery of HD motor symptoms. **(Fernández-García et al., manuscript in preparation)**

RNAi-based GluN3A silencing prevents and reverses disease phenotypes induced by mutant huntingtin.

We tested the therapeutic potential of silencing GluN3A expression in YAC128 mice, a well established HD model. Recombinant adeno-associated viruses encoding a short-hairpin RNA against GluN3A (rAAV- shGluN3A) were generated, and the ability of different serotypes to transduce MSNs was compared. A single injection of rAAV9-shGluN3A into the striatum of 1-month-old mice drove potent (>90%), long-lasting reductions of GluN3A expression in MSNs, prevented dendritic spine loss, and improved motor performance in YAC128 mice. Later delivery, when spine pathology is already apparent, was also effective. Our data provide proof of concept for GluN3A silencing as a beneficial strategy to prevent or reverse corticostriatal disconnectivity and motor impairment in HD and support the use of RNAi-based or small-molecule approaches for harnessing this therapeutic potential. (**Molecular Therapy, 2018 Aug 1;26(8):1965-1972.**

3. Relevance and possible clinical applicability of the final results

This project is a preclinical study that provides data to design new treatments for Huntington's disease directed (1) to signalling the BDNF / TrkB / p75 system, Pyk2 and PACAP, and (2) to a new therapeutic target, the GluN3A subunit of NMDA receptors, which was recently found elevated in HD pathologies. These results provide important information for the development of therapies directed not only to Huntington's disease but to other cognitive disorders, such as Alzheimer's disease. The lack of effective treatments for these diseases has a great social and economic impact, as well as psychological problems not only for patients but also for their families. Therefore, there

is the need to develop effective therapies for neurodegenerative disorders and, in this scenario, our results provide information to develop new treatments to improve the quality of life of the community of Huntington's disease.

4. Publications, communications and training of personnel derived from this research

Papers

1. Pyk2 in the amygdala modulates chronic stress sequelae via PSD-95-related micro-structural changes. Montalban E, Al-Massadi O, Sancho-Balsells A, Brito V, de Pins B, Alberch J, Ginés S, Girault JA, Giralt A. **Transl Psychiatry**. 2019 Jan 15;9(1):3.

2. Conditional BDNF Delivery from Astrocytes Rescues Memory Deficits, Spine Density, and Synaptic Properties in the 5xFAD Mouse Model of Alzheimer Disease. de Pins B, Cifuentes-Díaz C, Farah AT, López-Molina L, Montalban E, Sancho-Balsells A, López A, Ginés S, Delgado-García JM, Alberch J, Gruart A, Girault JA, Giralt A. **J Neurosci**. 2019 Mar 27;39(13):2441-2458.

3. Increased Levels of Rictor Prevent Mutant Huntingtin-Induced Neuronal Degeneration. Creus-Muncunill J, Rué L, Alcalá-Vida R, Badillos-Rodríguez R, Romaní-Aumedes J, Marco S, Alberch J, Perez-Otaño I, Malagelada C, Pérez-Navarro E. **Mol Neurobiol.** 2018 Oct;55(10):7728-7742.

4. RNAi-Based GluN3A Silencing Prevents and Reverses Disease Phenotypes Induced by Mutant huntingtin. Marco S, Murillo A, Pérez-Otaño I. **Mol Ther.** 2018 Aug 1;26(8):1965-1972.

5. Human alpha 1-antitrypsin protects neurons and glial cells against oxygen and glucose deprivation through inhibition of interleukins expression. Cabezas-Llobet N, Camprubí S, García B, Alberch J, Xifró X. **Biochim Biophys Acta Gen Subj**. 2018 Sep;1862(9):1852-1861.

6. Huntington's disease: novel therapeutic perspectives hanging in the balance. Saavedra A, García-Díaz Barriga G, Pérez-Navarro E, Alberch J. **Expert Opin Ther Targets**. 2018 May;22(5):385-399.

7. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Enhances Hippocampal Synaptic Plasticity and Improves Memory Performance in Huntington's Disease. Cabezas-Llobet N, Vidal-Sancho L, Masana M, Fournier A, Alberch J, Vaudry D, Xifró X. **Mol Neurobiol.** 2018 Nov;55(11):8263-8277.

8. Pyk2 modulates hippocampal excitatory synapses and contributes to cognitive deficits in a Huntington's disease model. Giralt A, Brito V, Chevy Q, Simonnet C, Otsu Y, Cifuentes-Díaz C, de Pins B, Coura R, Alberch J, Ginés S, Poncer JC, Girault JA. **Nat Commun**. 2017 May 30;8:15592.

9. 7,8-dihydroxyflavone ameliorates cognitive and motor deficits in a Huntington's disease mouse model through specific activation of the PLCγ1 pathway. García-Díaz Barriga G, Giralt A, Anglada-Huguet M, Gaja-Capdevila N, Orlandi JG, Soriano J, Canals JM, Alberch J. **Hum Mol Genet.** 2017 Aug 15;26(16):3144-3160.

10. Helios expression coordinates the development of a subset of striatopallidal medium spiny neurons. Martín-Ibáñez R, Pardo M, Giralt A, Miguez A, Guardia I, Marion-Poll L, Herranz C, Esgleas M, Garcia- Díaz Barriga G, Edel MJ, Vicario-Abejón C, Alberch J, Girault JA, Chan S, Kastner P, Canals JM. **Development**. 2017 Apr 15;144(8):1566-1577.

11. GluN3A promotes NMDA spiking by enhancing synaptic transmission in Huntington's disease models. Mahfooz K, Marco S, Martínez-Turrillas R, Raja MK, Pérez-Otaño I, Wesseling JF. **Neurobiol Dis**. 2016 Sep;93:47-56.

12. Emerging roles of GluN3-containing NMDA receptors in the CNS. Pérez-Otaño I, Larsen RS, Wesseling JF. **Nat Rev Neurosci.** 2016 Oct;17(10):623-35.

Communications: 28 in Spanish and 19 in international meetings

Training of Personnel:

PhD Students:

Alvaro Murillo (2019)

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