Spinal cord circuit reorganization induced by stem cell transplantation and activity-dependent plasticity in an in vitro model of spinal cord injury

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Regeneration in the central nervous system is very limited following a lesion compared to peripheral nerves. In the context of spinal cord injury (SCI), only a restricted spontaneous recovery can be observed and at present it exists no established cure. Promising therapeutic strategies however have been tested over the last decades. The use of embryonic stem cells as transplant grafts has shown interesting results, but the exact cellular mechanisms involved are still unclear. In a previous project our group developed a new ***in vitro*** model for the study of functional regeneration after SCI consisting of organotypic slice co-cultures of E14 embryonic rat spinal cord on multi-electrode arrays. Using this model and optogenetic tools we aim to better characterize the specific plasticity of endogenous neuronal circuits as well as the effect of the transplantation of pre-differentiated embryonic stem cells into spinal cord lesions.

Transplantation of embryonic stem cells pre-differentiated into embryoid bodies (EBs) induced a re-establishment of neuronal connections between the two transected slices, allowing propagation of activity over the lesion site and thus, improving functional recovery. A greater impact on recovery occurred when the transplantation was performed at the time of the lesion compared to the transplantation of the EBs with a delay of one week after the lesion. Furthermore, modulation of the culture activity through sodium channel or synaptic transmission blockers before or after EBs transplantation pointed out that activity-dependent circuit reorganization influences functional recovery.

Together these findings suggest that pre-differentiated embryonic grafts are able to form new functional connections between two transected spinal slices ***in vitro*** to induce functional regeneration.

Evaluation of autonomic nerve fiber density in small fiber neuropathy patients by skin biopsy analysis.

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Background. Small fiber neuropathy (SFN) is a peripheral disease due to damage of unmyelinated axons, responsible for sensory and autonomic function. SFN is currently diagnosed by skin biopsy analysis showing intraepidermal nerve fiber density (IENFD) reduction. No systematic quantitative assessment of autonomic nerve fiber density (ANFD) of dermal structures is routinely performed.

Aim. To study if intraepidermal denervation in SFN is associated also to dermal autonomic structures denervation.

Methods: A quantitative technique to determine ANFD of sweat glands (SG), sebaceous glands (SbG) and muscle arrector pilori (MAP), was assessed in SFN patients, compared to control (CNT) subjects with normal IENFD. Skin sections from ankle and thigh were stained with panaxonal neuronal marker (PGP9.5) and sympathetic nerve marker (TH). Using confocal microscope, serial pictures through z-axis of selected structures were taken. Five representative images were chosen and for all of them a standardized grid was overlapped and the number of grid intersections crossed by a fiber was counted. The numbers obtained from five images were summed and normalized to area of each structure, to obtain ANFD value.

Results: Nerve fibers loss affected also dermal autonomic structures in SFN group. In particular, MAPs showed an important denervation mainly in non-adrenergic fibers while SbG and SG were not involved. Interestingly SbG showed an exclusively adrenergic innervation instead MAP had also a non-adrenergic innervation. Finally, a trend toward a possible proximal ANFD denervation was observed in SFN, although no significant ANFD difference between SFN and CNT was found.

Conclusions: Skin biopsy is a versatile tool allowing analysis of autonomic structures innervation of which to date very little is known. A deeper analysis with a higher number of subjects and groups of different disease is warranted to better characterize autonomic involvement in SFN.

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ROLE OF SLEEP IN CERVICAL DYSTONIA: A RTMS STUDY

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Introduction: Cervical dystonia (CD) is the most frequent type of dystonia. Although still debated, a growing body of literature suggests that an alteration of brain plasticity may play a key role in the physiopathology of CD. Since sleep is strictly implicated in the regulation of cortical plasticity, the general hypothesis of the project is that sleep disturbance and changes in cortical plasticity in CD may both reflect some common brainstem basic neural circuit deficits.

Aims: To investigate sleep effect on cortical plasticity in CD.

Methods: CD patients and age-matched healthy subjects (HS) underwent two repetitive transcranial magnetic stimulation (rTMS) sessions (potentiation and a depotentiation protocol), within a 12 hours interval (evening and morning session). The amplitude of the motor evoked potentials (MEPs) recorded at baseline has been compared to the amplitude recorded after each protocol.

Results: As expected, HS showed changes of MEP amplitude in accordance to the potentiation/depotentiation protocols. Moreover, in the evening session, HS had a more evident potentiation whereas in the morning there was a prominent depotentiation. We observed a similar response after the potentiation protocol in CD patients. On the contrary, after the depotentiation protocol CD patients had a paradoxical hyperpotentiation that was even stronger in the morning session.

Conclusion: Our preliminary results seem to point in the direction of a hypersynaptic plasticity in CD patients and are thus consistent with the hypothesized alteration of cortical activity in the physiopathology of CD. Furthermore, sleep seems to play a role in the rTMS-mediated response in CD patients that will needs to be further investigated.

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Neurophysiological Differentiation in Processing of Facial Aesthetics by the Fusiform Gyrus

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Introduction

The Systems Neuroscience of Psychosis (SyNoPsis) project uses a dimensional approach to disentangle the underlying neurobiology of psychosis. Previous clinical research suggests that psychotic symptoms can be grouped according to their respective underlying dysfunction of anatomically and functionally distinct brain circuitries: the language, affect and motor function domain. To further understand the pathological system dynamics of the affect domain, we investigate the association of normal behavioral function and neurophysiological correlates of emotional perception in healthy controls.
This should provide new insight into the validity of the continuum hypothesis of psychopathology for psychotic disorders, and their specific functional neuroanatomy.

Methods

Neural activation patterns using functional magnetic resonance imaging (fMRI) and behavioral data from healthy controls (n = 99) were investigated. During fMRI examination, a specifically developed face perception task was used. The presented stimuli were short animations of faces that varied in four different characteristics: gender (male, female), aesthetics (aesthetic, unaesthetic), head movement (animation up, down) and gaze direction (direct, averted). All faces were rated on several rating scales outside the scanner.

Results

Participants rated unaesthetic faces significantly less attractive than unaesthetic faces. Furthermore, there was a significantly increased activity in the fusiform gyrus during the perception of animated faces for unaesthetic faces in contrast to aesthetic faces (Family-Wise-Error corrected, $t(98)=4.69$, $p < .01$).

Conclusion

The fusiform gyrus has been shown to be higher activated during the perception of unaesthetic faces. Further analysis of the interaction between face processing areas, the limbic systems and a person's emotional abilities must be done to further comprehend the dimension of affect.

Spatial Distribution of Perseveration in Neglect Patients Depends on the Integrity of the Right Putamen

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Deficient inhibitory control leading to perseverative behaviour is often observed in neglect patients. Previous studies investigating the relationship between response inhibition and visual attention have reported contradictory results: some studies found a linear relationship between neglect severity and perseverative behaviour
whereas others could not replicate this result. The aim of the present study was to shed further light on the interplay between visual attention and response inhibition in neglect, and to investigate the neural underpinnings of this interplay. We propose the use of the Five-Point Test, a test commonly used to asses nonverbal
fluency, as a novel approach in the context of neglect. In the Five-Point Test, participants are required to generate as many different designs as possible, by connecting dots within forty rectangles. We hypothesised that, because of its clear definition of perseverative errors, the Five-Point Test would accurately assess both visual attention as well as perseverative behaviour. We assessed 46 neglect patients with right-hemispheric stroke, and performed voxel-based lesion-symptom mapping (VLSM) to identify neural substrates of perseverative behaviour as well as the spatial distribution of perseverations. Our results showed that the Five-Point Test can reliably measure neglect and perseverative behaviour. We did not find any significant relationship between neglect severity and the frequency of perseverations. However, within the subgroup of neglect patients who displayed perseverative behaviour, the spatial distribution of perseverations significantly depended on the integrity of the right putamen. We discuss the putative role of the putamen as a potential subcortical hub to modulate the complex integration between visual attention and response inhibition processes.

Creativity after focal brain lesions – a matter of paradoxical facilitation?

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Creativity is a complex neuro-psycho-philosophical phenomenon that may be unique in humans. It is a multifaceted cognitive process rather than a specific cognition that requires different skills (cognitive and social) and can lead to new and useful ideas in various fields (e.g. arts, crafts, science).

However, it is unclear how creativity evolves after focal brain lesions. Brain injury is usually associated with a deterioration of cognitive functions. Nevertheless, it has been pointed out that positive changes such as phenomena associated with plasticity could be overlooked. The paradoxical facilitation approach, for example, states that a lesion in one brain region can lead to a reversal of inhibition to linked areas or to a compensatory functional enhancement of the ipsilesional site. This could result in a contra-intuitive improvement of the functions of the affected area.

To date, there is only a limited number of single-case studies, which have found that patients can become more creative after a focal brain lesion. However, it is virtually impossible to measure creativity as a non-confounded factor. Individual differences in brain damage and the associated symptoms have to be taken into account.

With the planned long-term study, patients are repeatedly measured over a one year period post stroke. Insights could be gained into how acute brain injuries influence creativity. A creativity test battery will be used to investigate the development of patients' creative output over time. With extensive testing neurological and neuropsychological deficits will be identified.

The following research questions are to be clarified: 1) The role of the lesion side, whereby noticeable differences in creativity is expected, especially when the right hemisphere is affected; 2) Development of creativity over time, which will indicate the mechanisms behind it; 3) Whether creativity is a question of paradoxical facilitation or of plasticity and repair processes; 4) Furthermore, lesion symptom mapping will be carried out in order to correlate places of focal lesions with creative performance.

This research is relevant and useful, as creativity contributes greatly to patients' life satisfaction and their management of everyday life.

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Snap-shooting the "Eureka! Brainwave": How Does Instruction Affect Motor Task Understanding? A Pilot Study.

Authors: *Joaquín Peñalver-Andrés, Karin A. Buetler, René M. Müri, Laura Marchal-Crespo*

Understanding the underlying mechanisms of motor learning is crucial to improve neurorehabilitation. Skillful motor performance is the result of a sequence of cognitive and motor achievements: from understanding the task to correct recruitment and activation of involved muscles. Thus, tracking the subject's cognitive and neuromotor footprint during motor training may help to optimize motor learning. However, to date, less is known about how to optimally adapt motor training paradigms to support this hierarchical processing in order to optimize motor learning. The goal of this pilot study was to assess the feasibility of identifying and characterizing neurocognitive markers for *task understanding* during motor learning using EEG (Electroencephalography). The impact of different modes of instruction (implicit versus explicit) on attentional-related EEG components (e.g. P300) were studied during motor task performance. The results of this study present a first step to improve motor learning and neurorehabilitation by understanding the cognitive correlates of learning during the acquisition of novel motor skills.

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New approaches to influence 5-ALA-induced Protoporphyrin IX (PpIX) fluorescence and accumulation in GBM cell lines with different epidermal growth factor (EGFR) expression

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Background

Glioblastoma (GBM) is the most frequent and devastating brain tumor. Approximately 50% of GBM expresses epidermal growth factor receptor (EGFR) and from these another 50% expresses the mutated form EGFRvIII. In GBM cell lines the EGFR expression status influences the 5-aminolevulinic acid (5-ALA)-induced fluorescence by inducing the rate limiting enzyme Heme Oxygenase-1 (HO-1). We hypothesized that by co-treatment with different drugs such as Deferoxamine (DFO), Tin-Protoporphyrin (SnPP) and Genistein, which are respectively an iron (Fe^{2+}) chelator, an inhibitor of HO-1 and an inhibitor of ABCG2 PpIX efflux transporter, 5-ALA-induced, Protoporphyrin IX fluorescence can be augmented.

Methods

U87MG (low EGFR expression), U87wtEGFR (EGFR overexpression), U87vIII (EGFR expression/EGFRvIII+) cells were incubated with exogenous 5-ALA 1mM, alone or in combination with DFO, SnPP and Genistein. After 8 hours of combined treatments, PpIX fluorescence was analyzed by PpIX extraction, by flow cytometry and/or confocal microscopy.

Results

SnPP treatment was able to inhibit HO-1 activity and restore the PpIX fluorescence in the three GBM cell lines, independently of EGFR quantitative and qualitative expression. Co-treatment with 5-ALA and DFO and co-treatment with 5-ALA and Genistein of U87 cells lead to a significant increase in PpIX fluorescence. Furthermore, we demonstrated that the treatment that led to a greater PpIX accumulation was the combination of the three drugs for all the three U87 cell lines.

Conclusion

Modification of PpIX fluorescence may be helpful during intraoperative imaging of GBM and surgical resection of the tumor. Moreover, it allows to act on PpIX accumulation with different combinations of drugs, regardless of the EGFR expression status. These findings will be further investigated in animal models.

Analysis of Tau-KO cells reveals a new role of Tau protein in modulating cell death

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Support by the Synapsis Foundation and EOC

Age-dependent neurodegenerative tauopathies are characterized by the neuronal accumulation of pathogenic forms of Tau leading to neuronal dysfunction and cell death (i.e., gain-of-cytotoxic function). The role of Tau in causing disease is demonstrated by the existence of dominant mutations in its *MAPT* gene in frontotemporal dementia with Parkinsonism (FTDP-17). In the absence of mutations, the reasons provoking the switch from normal to pathogenic Tau remain poorly understood. The best proven risk factor for neurodegenerative disorders is aging, for which a progressive accumulation of DNA damage is described. Here we addressed a possible role of Tau in the cellular DNA damage response (DDR), in particular with regards to the induction of cell death. The objective is to test the hypothesis that progressive tau pathology and accumulation of DNA damage may act jointly in these devastating progressive conditions.

Strategy: analyse the DDR in the absence or presence of Tau based on cellular and biochemical assays.

A new Tau-KO cellular model was generated from human SH-SY5Y neuroblastoma cell line by targeting exon 1 of the *MAPT* gene with the CRISPR/Cas9 technology. With Tau-expressing lines and by comparing them to our validated Tau-KO lines, we analysed the DDR following a short treatment with Etoposide, a topoisomerase II inhibitor. Parental SH-SY5Y cells respond to DNA damage consistently with what is described in the literature, in particular with regards to the activation of all phases of the DDR, including induction of apoptosis when cells fail to repair the DNA. Whilst we were not able to assign a role of Tau in the DNA damage and in the early phase of the DDR, the use of different cytotoxic assays (LDH, MTS and pro-apoptotic cleaved caspase-3) demonstrated that the absence of Tau significantly reduced DNA damage-induced cell death by an apoptotic mechanism. Interestingly, this was compensated by the induction of cellular senescence. From the point of view of the mechanism, we found that Tau-KO cells present a perturbation of p53 expression and function. Finally, restoring p53 expression with Nutlin-3, which inhibits MDM2-mediated degradation of p53, partly restored DNA damage-induced apoptosis in Tau-KO cells.

Overall, our data indicate that Tau is able to modulate a p53-driven response to cellular stress in balancing cell fate between cell death and senescence. A loss-of-function role of Tau in inducing cellular senescence, possibly also occurring during disease-associated Tau deposition, represents an intriguing and new finding that may further improve the understanding of tauopathies. A modulatory role of Tau in p53 biology also suggests a possible involvement of Tau in cancer.

BENEFRI 2019 Workshop

Name:Praveen Bathini

Abstract

Title

Progressive signaling changes in the olfactory nerve of patients with Alzheimer's disease

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Background

The olfactory system has been extensively studied in rodents and has received much interest in the clinical setting based on the incidence of olfactory impairment in a variety of neurodegeneration. Olfactory dysfunction is present in up to 90% of Alzheimer's disease (AD) patients. Although deposition of hyperphosphorylated tau and β-amyloid substrates are present in olfactory areas, the structural and signaling changes associated with this sensory area are not completely understood. We addresses here progressive changes in known hallmarks of AD, signaling molecules, and cytoskeletal markers occurring in the human olfactory nerve.

Methods

A cross-sectional histoanatomical study was conducted on the postmortem olfactory nerves of patients with different stages of dementia which includes Mild cognitive impairment (n=9), Moderate Alzheimers (n=8), Severe Alzheimers (n=11) and Healthy controls (n=10).

Results

The olfactory bulbs and tracts show a prominent and progressive tauopathy in contrast to a weaker amyloid pathology localized to the glomerular region. Topological analysis of Notch signaling components reveals a transient increase in Jagged1 expression in mitral cells of the olfactory bulb of patients with MCI and a gradual decline onwards. Analysis of the olfactory tract reveals an abundance of corpora amylacea, which declines starting from the MCI stage. With the increasing severity of dementia, corpora amylacea are characterized by a gradual shift in cytoskeletal proteins, tau, MAP2 and glial fibrillary acid protein, as well as by a decrease in their Reelin and Jagged1 content.

Conclusions

Our research indicates that the olfactory nerve undergoes early and sequential morphological and signaling alterations that correlate with the development of dementia suggesting that this structure may capture and propagate neuronal network imbalances to connected higher brain centers of the entorhinal cortex and hippocampus.

Results

Bathini, P., Mottas, A., Jaquet, M., Brai, E. and Alberi, L., 2018. Progressive signaling changes in the olfactory nerve of Alzheimer's disease patients. *Neurobiology of Aging*.