Scientific Workshop

~ New technologies in Neurosciences ~

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Report by
Bérangère BALLION

Foreword by
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The Workshop was introduced by the NEURON Coordinator Dr. Marlies Dorlöchter in order to explain the ERA-NET NEURON scheme and the scope of the Workshop.

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Thematic input for programmes of the NEURON project,
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Foreword

This workshop is the second of a series devoted to new technologies in Neurosciences. The objective was to identify new technologies and frontiers which will allow breakthroughs in this field. The challenges for understanding how our brain develops and functions at multiple levels of integration as well as those related to elucidating the mechanisms and treating disorders of the nervous system, particularly neurodegenerative disorders, remain numerous. After a series of technological advances illustrated in July 2008 in Geneva, six new areas were explored at the Warsaw workshop which should help understanding the normal functioning of the brain and better treat its disorders.

A. Konnerth showed spectacular examples of \textit{in vivo} high resolution imaging using calcium dyes which provides information on the activity of single neurons within complex networks, in physiological and pathological conditions (murine model of Alzheimer’s disease).

J. Rothwell illustrated the use of transcranial magnetic stimulation (TMS), alone or coupled with imaging, for studying functional connectivity in cognitive neuropsychology. Moreover, repetitive TMS which is associated with long term changes of synaptic effectiveness has many potential applications for improving neurological and psychiatric conditions.

J. Livet provided details on a remarkable tool, brainbow, based on the variable combination of fluorescent proteins expressed in neurons for detailed analysis of the structural organisation of individual neurons. This tool should help deciphering the "connectomics" of brain cells and regions.

M. Jaber discussed the issue of cell therapies in the nervous system demonstrating the potential for both anatomical and functional recovery using embryonic cells in murine models of damaged cortical areas and of Parkinson’s disease. The potential of differentiated neurons derived from neural stem cells (e.g. dopaminergic neurons) was also discussed.

J. Mallet gave a comprehensive overview of the promises and challenges of gene therapy for disorders of the nervous system. Using several examples of preclinical and clinical studies, he illustrated the key question of the choice of vectors (vectorology).

B. Müller-Myhsok illustrated the application of convergent "omics" for elucidating the basis of disorder of the nervous system. An exciting example was given with converging evidence for the implication of the SLC6A15 gene in major depression. However, combining data coming from various sources and various types implying non-linear relationships raises difficult methodological and statistical questions which are still unanswered.

This workshop, combined with the previous one in Geneva, gave a large overview of the major frontiers and technological progresses in the field of neurosciences. They should provide solid scientific ground for new calls for proposals coordinated by funding organizations, partners in the Era-Net NEURON.

Alexis Brice
Inserm
New Technologies in Neurosciences:

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TITLE: High-resolution optical imaging in the living brain

Recent technical developments open new avenues for a better understanding of the functional mechanisms in the brain. One of these techniques involves the use two-photon fluorescence excitation (Denk et al., 1990), which allows for the first time to image the processing and the function of neural circuits on the level of individual neurons in the living brain. An important prerequisite for such analyses is the staining of the neurons with appropriate fluorescent markers and dyes. An effective staining procedure is the multi-cell bolus loading method (MCBL) (Stosiek et al., 2003) with calcium indicator dyes that are applied, for example, directly to the visual cortex of the mouse. These calcium dyes are highly effective reporters of the cell’s electrical activity. This technique has been successfully used in a large variety of animal models, including mouse, zebrafish, rat, cat and more recently monkey. Importantly, recent work showed that two-photon calcium imaging can be combined with other optical tools like genetically-encoded fluorescent markers, for example green fluorescent protein (GFP), or with electrophysiological patch clamp recordings. A high potential for future work lies in the development of genetically-encoded calcium indicator proteins that involve the use of troponin C (Heim et al., 2007).

At present, this imaging technique is restricted to a depth of less than a millimetre under the brain surface. Therefore, new tools, like optical fibre-based microendoscopy, are under development (Adelsberger et al., 2005). When placed into regions of the brain that were stained with a calcium indicator dye, the optical fibre detects the activity in the cluster of neurons near the tip of the fibre. This technique allows the analysis of non-anesthetized animals and is, therefore, useful for the investigation of brain region-specific neuronal activities during defined behaviours. It can be used in any region of the brain and at any stage of development. Recent work in primates suggests that this technique works also in humans, where it could find applications for the analysis of neural activity in neurosurgical patients.

Two-photon imaging has many applications for the analysis of the healthy and, importantly, the diseased brain. For example, the approach has been recently used for the analysis of the brain function in a mouse model of Alzheimer's disease (Busche et al, 2008). By using a triple staining procedure involving the staining with a calcium indicator, a glial cell marker and staining of beta amyloid plaques, it was found that neurons in the immediate vicinity of plaques are particularly active. Such hyperactive neurons fire more frequently and in a correlated manner, thus increasing the risk for seizure-like activity. Thus, hyperactive neurons are likely candidates to trigger epileptic activity in Alzheimer disease patients, known to have an increased incidence of epileptic seizures.

In conclusion, these new in vivo optical techniques are relevant for (1) the analysis of the molecular mechanism in the intact brain, (2) a comprehensive investigation of neural networks with single cell resolution and (3) the investigation of dendrites and even single synapses in vivo. Moreover, these approaches can be easily combined with other techniques, ranging from optogenetic tools to MRI. These imaging approaches are powerful and indispensable tools for the analysis of neurodegenerative and other diseases in animal models, like for example mutant mice (Rochefort et al., 2008).
References


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**TITLE:** Advances in Transcranial Brain Stimulation.  

Using an electrical stimulus to activate the brain through the scalp is still a problem because the scalp and the skull have a high resistance barrier to electricity. So, brain stimulation by putting electrodes on the scalp remains difficult and painful. Transcranial Magnetic Stimulation (TMS) mitigates this drawback by using a magnetic field to carry the electrical stimulating current across the barrier of the skull and directly into the brain. This approach is based on Faraday’s principle of electromagnetic induction with a large magnetic field that induces an electrical signal in the brain. Even if this method has some limitations (a low focal precision (1cm x 1cm) and a restricted activation of the superficial cortex), advantages are numerous:

- Non-invasive,
- No pain,
- Induction of a single pulse being similar to a pulse coming from peripheral nerve stimulation
- Activation of neuronal axons,

Moreover, classic experiments with stimulation and recordings from intact animal brains provide the basic knowledge of a functional system. Combined with imaging methods (fMRI, EEG, MEG etc) they can provide the same information in human.

This experimental method can be (1) conducted in conscious subjects who can report experience and perform complex tasks with little practice and (2) can be used to test the effect of disease and therapy on brain function.

In addition, the TMS is already used in the diagnosis of some neurological diseases and in the motor cortex and visual area stimulations. Moreover, repetitive TMS induces beneficial effects in some neurological conditions such as in the cortical control of muscles in patients with dysphagia. Nevertheless, the use of TMS can induce some reported negative consequences such as transient scotoma produced by visual cortex stimulation (Amassian et al, 1989). In the same way, a study showed an activation of occipital cortex when blind readers read Braille (Sadato et al., 2004) but the TMS of the occipital cortex disrupted the Braille reading (Cohen et al).

The new technologies develop (1) the new protocols of magnetic stimulation (multiple pulses at different rates, intensities and durations) to recruit mechanisms of synaptic plasticity, (2) new coil design to improve the focality and the depth of stimulation and (3) the combination between TMS and the imaging techniques to illustrate the functional connectivity of networks. Functional connectivity studies are important in terms of providing (1) the interactions between the various cortical areas implicated in cognitive psychology, and (2) neurological disease and response to therapy (examination in functional connectivity changes associated with recovery or the understanding in treatments effects on network activity).

Using repetitive TMS (rTMS) can affect the cortical activity. Indeed, animal studies show that repetitive stimulation of neural pathways can lead to long lasting changes in synaptic effectiveness. rTMS may have similar effects on pathways in human cortex. Some of these are abolished by drugs that interfere with NMDA receptor function. So, they may be involved in LTP/LTD-like effects at synaptic levels. Different patterns of rTMS (frequency, intensity, sequences) can increase or decrease excitability. In healthy brain, the effects of rTMS only seem to last minutes or few hours.

Nevertheless, rTMS are used in therapeutic approaches with a beneficial effect in, for example, the treatment of depression. Other studies show an improvement on stroke (Khedr et al, 2005), tinnitus, pain after rTMS. Other types of TMS are available like the Transcranial Direct Current Stimulation (TDCS) using DC electrical stimulation through scalp electrodes (1mA typically for several minutes). This technique polarises neurones and affects ongoing levels of activity and induces LTP/LTD-like effects similar to rTMS.
Jean Livet  
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TITLE: Current approaches to visualize neuronal circuits: Engineering tools for “connectomics”

Data sought in studies of neural circuits include: (1) structural data (how are circuits organized), (2) functional data (which neurons are activated and when), and (3) temporal data (how do circuits change over time). This presentation focused on techniques to access the first type of data, concerning the structure of neuronal circuit. The development of our conceptual framework on the organization of brain circuit has strongly benefited from single-neuron labeling techniques. Initially, the Golgi staining technique has been used to create a sparse labeling of neurons and their anatomy. This technique has allowed Cajal and others to describe the different neuronal types of the nervous system and their pattern of interconnection. More recently, the usage of fluorescence microscopy and specifically the green fluorescent protein (GFP) has opened new avenues for accessing cellular neuroanatomy. Transgenic mice expressing GFP, showing in situ labeling of neurons, can be generated by genetic engineering. For instance Thy1-GFP transgenic mice generated by Feng et al 2000 using a Thy1 promoter (Caroni 1997) show very high level of GFP expression in several neuronal types; sparse Golgi-like labeling is seen in several lines as an effect of transgene variegation. Those two features have made these mice extremely useful for in vivo neuronal imaging.

While the techniques mentioned above provide access to single-neuron anatomy, adjacent cells remain difficult or impossible to visualize simultaneously and distinguish. Techniques that would generate a more complete and quantitative rendering of neuronal circuitry would be useful. Ideally, one would like to visualize all neurons of a circuit individually and in this way obtain full wiring diagram of circuits at the cellular level. Approaches are currently emerging to obtain such ‘connectomic’ data, using either electron microscopy (EM) or fluorescence imaging. The EM-based approach is exemplified by the work of White and collaborators (1986), who obtained the first connectome in C.elegans using serial electron microscopy pictures from which they built wiring diagrams of neuronal connectivity. New automated serial EM data collection techniques have been introduced by Briggs and Denk (2006) to reconstruct circuitry in larger animals. However these serial EM approaches have drawbacks: reconstruction of large samples is difficult, and no marker allows for verifying the identity of the neurons that are reconstructed. An alternative to EM is the use of fluorescence microscopy. The transgenic Thy1-GFP mouse lines presented above make an ideal tool for this approach, allowing for instance one to visualize peripheral motor axons and the synapses they form on muscle fibers (neuromuscular junctions). Taking advantage of these animals, J. Lu and J.W. Lichtman (MCB department, Harvard University) have recently established 3D reconstructions of the complete set (~15-20) of peripheral motor axons innervating small muscles in Thy1-YFP mice (J. Lu and J.W. Lichtman, unpublished results). These 3D reconstructions were performed using high resolution confocal imaging followed by iterated tracing of axonal contours in serial optical sections. This technique was successful in reconstructing the large axons of peripheral motor circuits, however smaller axons typically encountered in the CNS are much more difficult to distinguish with a single color of label, due to the intrinsic resolution limit of optical microscopy. The tracing would be strongly facilitated if axons could be labeled with distinct colors (realizing in essence a multicolor Golgi stain). A large number of distinct hues can be generated by mixing 3 primary colors (red, green and blue) at different levels. A restricted set of spectrally distinct fluorescent proteins such as RFP, YFP and CFP would thus be sufficient to generate a multicolor neuronal labeling if one had a way to express random combinations of these proteins in a cellular population. Recently, Brainbow-1 and Brainbow-2 transgenes were introduced which use the Cre/lox recombination system to create a random choice of expression among 2-4 fluorescent proteins. This random choice is created as follows: Brainbow-1 uses Cre-mediated excision between pairs of incompatible lox sites (such as loxP and lox2272) to create distinct recombination possibilities which each trigger expression of a distinct “XFP” gene. Brainbow-2 uses Cre-mediated inversion between head-to-head oriented loxP sites. Invertible DNA cassettes are positioned in tandem to create up to 4 recombination possibilities, each triggering the expression of a specific XFP. Brainbow-1 and -2 transgenes have first been tested in HEK 293 cells and then introduced in transgenic mice under the control of the Thy1 promoter, where they drive random XFP expression upon Cre recombination. When multiple copies of the Thy1-Brainbow transgenes are present and recombine independently, combinatorial XFP expression is observed, resulting in a multitude of distinct hues (~100).

In order to test the potential of Brainbow mice for circuit tracing, T. Weissman used the multicolor neuronal labeling to reconstruct mossy fiber axons in one confocal stack of images from the cerebellum. A large
number of axons (~300) were reconstructed despite their proximity. In addition, several synaptic contacts between granule cells and mossy fiber axons could be visualized. The distinct colors identifying neurons also allowed for investigating the neuronal convergence in this circuit, showing that in most cases granule cells appear polynuronally innervated. It is to be noted that in addition to studies of neuronal circuits, the Brainbow approach can also be used to study interactions in other cell types. Indeed a few lines of Thy1-Brainbow mice showed expression in specific glial cell types (astrocytes, Bergmann glia, non-myelinated Schwann cells), allowing for studying cellular interactions in those non-neuronal cell types.

To develop and improve this multicolor labeling technique, imaging could in the future be combined with thin sectioning techniques (serial electron microscopy and array tomography) or emerging super-resolution "nanoscopy" techniques that overtake the diffraction limit. Image analysis, in particular regarding the segmentation of cellular objects, needs to be optimized and automated. In addition, advances in genetic engineering are needed to provide amplified and pan-neuronal expression.
Mohamed Jaber  
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**TITLE:** Current aims and hopes in cell based therapies of neurodegenerative diseases.

In the adult CNS, there is an absence of spontaneous axonal regeneration following trauma attributed to inhibitory factors associated to the CNS white matter and to reactive astrocytes that form a physical and biochemical barrier scar. The strategies in brain repair are numerous: the self repair, the neuroprotection, the embryonic cell transplantation and the transplantation of stem cells-derived neurons. The neural transplantation of embryonic neurons has been widely assessed as a potential approach overcoming the generally limited capacity of the mature CNS to regenerate axons or to generate new neurons in response to cell loss. Concerning the embryonic neuron transplantation, this approach is studied in two different systems: point to point systems as in the cortex or in diffuse systems, as in the nigro-striatal pathway.

Previously, in the case of adult motor cortex damage leading to severe and irreversible deficits in motor function, transplantation of embryonic cortical neurons into the damaged adult motor cortex was practised and can induce a partial recovery, however, no direct demonstration was ever provided of adequate reinervation of target areas following transplantation. This was mainly due to the absence of neuronal markers enabling to distinguish the graft from the host. During his talk, M Jaber presented data obtained following grafts of embryonic cortical tissue from transgenic GFP mice into the damaged motor cortex of adult mice. The time-course of developed axonal projections following grafting was studied at 4, 11, 15 and 60 days after the graft. 4 days following grafting, the graft appeared as small tissue blocks opposed to the walls of the lesion cavity. Few GFP-fibers without any apparent preferential orientation were identified in the host cortex. The transplant filled the lesion cavity from 8 days following grafting. GFP-fibres proceeded toward the underlying corpus callosum and axons were found in the motor cortex and striatum. Transplanted neurons continue to proliferate and express DCX in soma (DCX is a marker of corticogenesis and is found in processes of migrating and differentiating neurons). 11 days following grafting, GFP-fibres progressed further tangentially in the host cortex and the most fibers terminate within the deep cortical layers. They cross the corpus callosum and enter in the caudate putamen. Leading axons were found in the internal capsule. 15 days following grafting, dense bundles of radiating neurites occurred within the ipsilateral cortex and cingulum, oriented parallel to the white matter. The density of GFP axons was considerably increased both ipsi and contralaterally in the cortex and striatum. Fast developing fibers were identified within the thalamic and subthalamic regions, cerebral peduncle and, in some cases, at pontine nuclei level. 2 months later, the transplants can develop and maintain a set of projections towards most of the cortical and subcortical targets normally contacted by motor cortex neurons including distant target areas such as the spinal cord. Nevertheless, transplanted neurons retain their specificity: indeed, not all neurons are capable of successfully integrating any brain region and emitting significant and appropriated axonal projections. For instance, motor cortex embryonic neurons need to be grafted in the lesioned motor cortex to observe correct rewiring of the brain.

The question is how to have specific connections? The cortical lesion induces an axonal degeneration and destruction of myelin. Transplant-derived axons preferentially follow demyelinated pathways: patches of GFP-fibres were found within striatal white matter where host myelin was reduced while fewer GFP-fibres occurred where host myelin was still present. Electronic microscopy approach confirmed that transplanted cells integrate correctly within the host. 2 months following grafting, axons of the transplanted are myelinated. Nevertheless, previous studies showed that transplanted cells could fuse with cell hosts and induce false positive signals of pathway reconstruction. To examine this possibility, DNA analysis of transplanted cells was assessed by fluorescence-activated cell sorting technique. Results show that within the transplant a single cellular population with a single nucleus was present. In addition, GFP-neurons contained a single To-Pro3-labeled nuclei, and female embryonic cells (XX chromosomes) transplanted within a male host (XY chromosomes) do not show any labelling for the Y chromosome. In conclusion, the projections do not result from the fusion of the embryonic cells with the host neurones. What happens if embryonic cells are replaced by stem cell derived neurons? Use of neural stem cells should solve logistic and ethical problems associated with embryonic cells. Embryonic stem cells were forced to generate cortical progenitors expressing markers of the presumptive cerebral cortex. These cortical excitatory neurons exhibit a pyramidal or unipolar morphology, characterized by the presence of one dendrite that is wider than the others. Neural progenitors expressed vesicular glutamate transporters and markers of pyramidal neurons of the cerebral cortex. In a
recent study of transplantation in vivo of the newly generated neurons in vitro, results show that the projections of the grafted neurons markedly resemble the pattern of cortical efferents, providing further evidence that most neurons generated have a cortical identity.

One example of embryonic cell grafts in an animal model of Parkinson’s disease: In the early 80’s, the transplants of embryonic dopaminergic neurones consisted to graft dopaminergic neurons in the striatal target. But, results were not satisfactory. Then, intra-nigral, instead of intra-striatal, transplantation was performed and the optical imaging shows that GFP embryonic transplant from the mesencephalon integrates well within the lesioned substantia nigra. 5 days following transplantation, pioneer axons express dopaminergic markers and GFP axons reach the striatum within 9 days. 2 months following transplantation, GFP/DAT axons are present in the striatum and GFP cells co-express dopaminergic markers. This approach allows a reconstruction of the lesioned pathway and the functional restoration of DA levels (DA levels are increased by 35% following transplantation) and behaviour (unilaterally lesioned animals no longer rotate after transplantation). What is guiding these axons to target areas? It’s well-known that EphrinA5 is widely expressed in all the brain in development while its expression is absent in adult excepted in areas with a high plasticity (ex, hippocampus). This factor is notably involved in the development of thalamocortical and retinotectal projections and prevents the nonspecific connections. The next step is now to understand the involvement of ephrinA5 in the guiding of nigrostriatal system to increase the graft efficiency and the cellular therapy.

Another transplantation alternative is studied using dopaminergic neurons generated from neural stem cells. Stem Cells are derived from the SVZ of transgenic mice over-expressing GFP under the control of the TH promoter. In a first time, neuroD1, a dopaminergic differentiation factor in neural stem is identified. This factor is then included by electroporation in the TH-GFP mice. Then the GFP expressed-DA neurons, which are derived from stem cells, are isolated by FACS and transplanted.

And finally, what about promoting self-repair or neuroprotection (by injection of molecules) in the brain repair? In the SVZ cells, Hepatocyte Growth Factor promotes proliferation of SVZ cells in vivo. In addition NPY stimulates the proliferation and migration of endogenous stem cells and promotes the neuroprotection against parkinsonian toxins.
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TITLE: Gene Therapy for Diseases of the Nervous System.

The use of gene therapy today requires a perfect knowledge of several parameters: the disease, the therapeutic gene, the vector and finally the ways of administration. The knowledge of disease will determine the organ to target, the therapeutic gene, and the validity of a gene transfer approach. In addition it’s necessary to know the therapeutic gene properties in terms of recessive or dominant heredity and the aetiology. The choice of vector depends on the organ to target. Actually, adenoassociated virus-derived expression vectors (AAV) and lentiviruses are the most promising today but adenoviruses need to be developed. Finally, the way of administration is variable: ex vivo, hematopoietic cells, embryonic or adult stem cells are chosen by opposition to in vivo in the muscle or CNS for which retrograde transport is used and combined with physical, biological or chemical methods of diffusion.

One example of gene therapy applied in the SCID-X trial, an incurable disease affecting young children: this monogenic disease is well-known in term of gene (IL2GR (gammaC)). The ex vivo gene therapy uses CD34+ stem cells which are optimized in culture conditions (FCS, IL3, Flt3 ligand, Stem cell factor). The oncoretrovirus MLV is used as vector and can induce a risk of genotoxicity. The route of administration is performed by infusion of modified cells. However, the promoter is so strong that this gene therapy induced the development of Leukaemia-like Syndrome in 4 patients.

Indeed, there are failures of gene therapy and more specifically because of (1) the clinical trials launched prematurely (1989), (2) the immunogenicity of the therapeutic factor (F-IX etc), (3) the vector biology which was not studied until recently, (4) the need for systematic studies at industrial scale (e.g. for testing various serotypes/pseudotypes or promoters….) and (5) the biosecurity negligence. In addition, the needed time to launch a clinical trial is too long (leading to the use of a not updated vector version). Thus all these points incite us to propose a more « basic » research (virology, molecular biology, chemistry etc…) and a stronger implication of industry.

Gene therapy is a multidisciplinary domain and the success relies necessarily on the optimization of a multitude of parameters, including the therapeutic strategy (choice of therapeutic gene depending on the physiopathology), the choice of vector, the optimization of vector (in terms of efficacy and biosecurity), the optimization of the vector dose, the optimization of cell culture conditions for ex vivo approach, the optimization of the expression cassette (choice of promoter …), the optimization of the delivery method etc… And to be successful, the multidisciplinary nature of gene therapy must be taken into account. Actors in various domains must be involved: medicine, virology, vectorology, biotechnology etc…

Vectorology is the heart of gene therapy. The choice and design of vectors used in gene therapy are the first parameters to be considered once the therapeutic strategy is established. This choice will depend on: the desired duration of expression of the transgene, the desired level of expression of the transgene, the need for regulation or not of the transgene expression, the type of transgene (ADNc or siRNA), the target cell type (quiescent cell or not), the target organ, the desired diffusion, the immunogenic status of the patient (neutralizing antibodies ….) etc…Today, four types of vectors are optimized and efficient: “gutless” adenovirus, adeno-associated virus (AAV), oncoretrovirus and lentivirus.

There are a lot of limits and challenges of vectorology: the scale-up of GMP production, the transduction efficacy, the biosafety (replication competent viruses during production, genotoxicity), the immune reaction (vs vector and vs transgene), the specific targeting (pseudotyping, transcriptional targeting) and finally the regulation of transgene expression (humanized regulation systems, non-protein-based systems). But for all these points, technical answers exist.

One efficient and secured vector is the non-integrating lentiviral vector which is used for gene transfer into the CNS. This vector is not integrated and forms a mini-chromosome. Six months later, the transgene is still expressed.

In term of vectorology, new technologies of interest are developed to improve the synthetic DNA-binding domains, the site-specific recombinases, the transcriptional control by endogenous miRNA, the silencing in antigen presenting cells (prevent immune reaction against transgene) and the negative transcriptional targeting (targeting, cell lineage). One of emerging project is INTEGRA which is a European project (NEST-Adventure project) aimed at improving the “research in emerging areas of knowledge and on future
technologies...which is highly innovative and involves correspondingly high (technical) risk”. It’s a consortium of 7 European laboratories including CNRS (J. Mallet, for vectorology), CRG (M. Isalan, for ZFPs), ULP (A. Griffiths, for directed evolution), UPS (M-C Serre, for recombinases), INSERM (S. Hacein-Bey, for SCID), ROSLIN (B. Whitelaw, for transgenesis) and CeBRA (P. Sandoe, for ethic laws).

Finally, to close this article, an example of the therapeutic gene transfer in the disease of CNS. In some CNS’s diseases, the therapeutic factors used (neurotransmitter synthesis enzyme, neurotrophic factors, free radical scavenging enzymes, RNAi) are difficult to administrated with systemic way. Indeed, most of these factors are degraded systemically and induce unwanted side effects. The interest of gene transfer is the prolonged production of therapeutic factor and the local production then a strong limitation of side effects. Then the strategy of the gene transfer (cellular vectors -ex vivo gene transfer- ; viral vectors -in vivo gene transfer-) is used in lot of CNS’s diseases (Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, spinal cord trauma, Alzheimer’s disease, ear and eye diseases) to improve the restoration and the neuroprotection. In a rat model of Parkinson’s disease, intrastriatal injection of an adenoviral vector expressing GDNF prevents dopaminergic neuron degeneration and behavioural impairment. The use of pseudotyping and specific cell-targeting (vect...
Historically, the understanding of disease was helped by the genomic convergence approaches to identify candidate gene by combining serial analysis of gene expression and genetic linkage. One interesting example is the Genome-Wide case-control Association Study (GWAS) in Major Depression Disease (MDD). The MDD has high lifetime prevalence (16.2%) with a strong heritability (37%). There are several candidate genes (BDNF, ACE, P2RX7, TPH2, PDE9A, PDE11A, DISC1, and GRIK3) and the genetic linkage is well-known (1p, 2q, 12q, and 15q). What about the GWAS in MDD? In a large study performed on suffering MDD-patients, genotyping was performed on an Illumina BeadChip platform with a 100k and 300k SNP-chip. The result shows that Rs1545843 (SNP II single-nucleotide polymorphism) reached genome-wide significant association with MDD. 5 others SNPs were nominally associated with MDD. All SNPs map to a „gene desert” on chromosome 12q21.31. These 6 SNPs were all associated with MDD in an independent larger replication sample.

To establish genotype-specific functional correlates, functional evidence in the genes adjacent to the associated region has to be determined. For that, human mRNA expression data are used and then studied for mouse models. The GENEVAR (GENe Expression VARiation) data is exploited to perform correlation testing between SNP genotypes and gene expression levels on a genome-wide level. The gene expression data availability is checked in a range of 1 Mb on both sides of the associated region. Expression data were available for the TMTC2, SLC6A15 and ALX1 gene and six associated SNPs in this extracted dataset are tested. In summary, the results show that we cannot exclude effects of the associated SNPs on gene expression of TSPAN19 (no probe), LRRIQ1 (no probe), unknown genes and RNAs on 12q21.31 and smaller effects on TMTC2 and ALX1. In addition, there is strongest evidence for MDD risk genotype dependent SLC6A15 mRNA regulation. In addition, the SNP – MR (magnetic resonance) spectroscopy correlations show that N-acetyl-aspartate (NAA) is reduced in the depressive state and reduced NAA reflects reduced white matter and axonal integrity. MDD risk-allele carrying controls (AA+AG) showed lower NAA levels than non-risk allele controls (GG): It might mirror their susceptibility to MDD. The SNP-MR regional volumetry correlations show that risk allele carriers might be more vulnerable to hippocampal reduction under high-risk conditions for MDD (e.g. stress, trauma, previous depressive episodes). There is now evidence from mouse models. Indeed, microarray results show that stress-susceptible mice present a lower SLC6A15 mRNA expression in the hippocampal CA1 region. In addition, TMTC2 and ALX1 were much less or rather not expressed in the CA1 region. These data support evidence for implication for SLC6A15, but not for other genes. SLC6A15 mRNA in-situ hybridisation shows a 2.1 fold reduction of SLC6A15 mRNA in CA1 hippocampal region. In addition, it has been demonstrated a normal anxiety-related behaviour under basal conditions in SLC6A15 knock-out mice but a trend for stress-induced anxiety (Drgonova et al. (2007)). In summary, there are multiple converging evidence for SLC6A15 as a novel candidate gene for Major Depression: genome-wide significance in the GWAS (case-control), positive independent replication of associated SNPs, risk-allele in controls (lower SLC6A15 mRNA expression in lymphoblastoid cell lines), risk-allele in controls (lower NAA levels in hippocampus), risk-allele in patients (lower CA1 volumes), stress-susceptible mice (lower SLC6A15 mRNA expression in the CA1 region of the hippocampus) and a trend for stress-induced anxiety in SLC6A15 knock-out mice.

Nevertheless, there are shortcomings: so far no clear mathematical / statistical basis; simply collecting evidence; work by plausibility and „Bayesian”gut-driven analysis.

What are the possible modes of action? Before envisaging the possible modes of action, it’s necessary to be conscious that there are some problems concerning the studies and specially the data coming from various sources and various types implying non-linear relationships likely. Therefore, it’s necessary to use new methods of analysis as for example the Kernel methods to perform analogies to many statistical approaches, automatic scaling of data. This method is usable with all kinds of data (e.g. experimental data -real values-; e.g. handwriting; e.g. sequences) and support vector machines. The support vector machines are great for classification, but comparatively poor for feature selection („finding genes”) and not really built for testing. These points may be overcome by using algorithmic developments and brute force Monte Carlo methods.
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