

REVUE

Integrative biology in the discovery of relevant biomarkers monitoring cognitive disorders pathogenesis and progression

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Abstract: Cognitive disorders are highly heterogeneous in terms of symptoms, clinical aetiologies, disease progression and therapeutic responses. Furthermore, their potential biological causes remain largely unknown. Progress at these different levels is currently mired in a vicious circle. The identification of coherent biomarkers, essential for clinical and therapeutic progress, requires an understanding of either the relevant pathogenic processes or, at the very least, of the parameters that need to be monitored. But, syndrome-dominated conceptual thinking has become a barrier to understanding the biological processes linked to diseases characterized by clinical and therapeutic heterogeneity. As a result, current biomarkers of cognitive disorders are much too numerous, too heterogeneous and too variable to serve useful purposes. This leads to an untenable situation that precludes coherent therapeutic developments since it effectively prevents defining what could constitute valid biological, clinical and therapeutic approaches. How to escape from this situation? The problem could be partly resolved by adopting the much wider views allowed by “system-wide” approaches: indeed, by constructing predictive theoretical models of what could constitute pathological cognitive processes. This, naturally, shall require the integration of massive amounts of highly heterogeneous and often conflicting information. The following article aims to provide a necessarily brief overview of the concepts, the breadth of data and the variety of network dynamics that will have to be considered while proposing a functional model-building approach, experimentally validated in vitro and in vivo, that could be fruitfully utilised.

Keywords: Biomarkers – Pathologies – CNS (central nervous system) – Therapies – Integrative biology – Systems biology

La biologie intégrative dans la découverte de biomarqueurs spécifiques au suivi de la pathogénèse de désordres cognitifs et leur évolution

Résumé : Les désordres cognitifs sont caractérisés par une extrême hétérogénéité en termes de symptômes, de présenta-

tion clinique, de progression pathologique et de réponses thérapeutiques. De plus, leurs causes restent très largement inconnues. Aujourd'hui, les progrès dans ces différents domaines sont embourbés dans un cercle vicieux. L'identification de biomarqueurs cohérents, essentiels à tout progrès clinique et thérapeutique, requiert, sinon une compréhension des processus pathogéniques, à tout le moins une identification des paramètres qui devront faire l'objet d'un suivi dans un cadre défini. Mais les approches conceptuelles, aujourd'hui largement dominées par la « syndromologie » sont de fait devenues des barrières à la compréhension des processus biologiques liés aux maladies caractérisées par une hétérogénéité clinique et thérapeutique. Il en résulte que les biomarqueurs disponibles dans le domaine des maladies cognitives sont bien trop nombreux, trop hétérogènes et trop variables pour être utiles. Ceci entraîne une situation intenable où tout développement thérapeutique cohérent est très largement compromis par le fait qu'il devient impossible de définir ce qui pourrait constituer des approches biologiques, cliniques et thérapeutiques valides. Comment échapper à cette situation ? Le problème pourrait être en partie résolu par l'adoption de la vision très large que permettent les approches systémiques. En d'autres termes, par la construction de modèles théoriques prédictifs représentant des mécanismes pouvant constituer des processus cognitifs pathologiques. Pour ce faire, il sera bien entendu nécessaire d'intégrer des masses énormes d'informations souvent contradictoires. Le présent article a pour but de dresser un inventaire nécessairement succinct des concepts, de la diversité de données et des dynamiques de réseaux qui devront être prises en compte tout en proposant une méthode fonctionnelle de modélisation, validée expérimentalement in vitro et in vivo, qui pourrait être ici mise à profit.

Mots clés : Biomarqueurs – Pathologies – SNC (système nerveux central) – Thérapies – Biologie intégrative – Biologie des systèmes

I - INTRODUCTION

Biomarkers are at the roots of evidence-based medicine (who should be treated, how and with what) and without valid and trustworthy biomarkers, advances in better-targeted therapies will be extremely limited and treatments shall remain largely empirical.

It must be said however, that in many clinical contexts, and particularly in the domain of cognitive and psychiatric disorders, the current definitions of biomarkers leave much to be desired. There are three broadly accepted definitions addressing the issue of what should a biomarker be.

The World Health Organisation (WHO) provided the first definition in 1993. The WHO defined a biomarker as “any parameter that can be used to measure an interaction between a biological system and an environmental agent, which may be chemical, physical or biological”. The broadness of this definition raised so many problems that in 1999 the FDA proposed a new definition. According to the FDA, a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention”. This definition in effect presupposes a knowledge of what functionally distinguishes a “normal” biological processes from “pathogenic” processes and it further presupposes relative homogeneity in terms of both mechanisms of pathogenesis and responses to a given drug treatment.

While certainly valid for many infective and metabolic diseases, this definition turned out to be inapplicable in a plethora of medical domains dominated by heterogeneous and multifactorial aetiologies, and in particular in neuro-psychiatry. In 2001, in an attempt to accommodate the evident functional complexities attached to the distinctions between “normal” and “pathogenic” biological processes, the Biomarkers Definitions Working Group (BDWG) at the NIH proposed that a biomarker should be viewed as “a molecular indicator of a specific biological property, a biochemical feature or facet that can be used to measure the progress of disease or the responses to a therapeutic intervention” [1]. Taking into account the state of our current understanding of pathological processes, this definition literally opens a Pandora box. In the context of functionally very heterogeneous disorders, there might be as many biomarkers as there are affected individuals. Furthermore, this definition does not distinguish between biological markers and clinical markers. Yet the goals of experimental therapies in CNS disorders are twofold: 1) better symptomatic therapies, and 2) treatments that slow disease progression or delay disease onset [2]. In the latter case, clinical endpoints are used as biomarkers (clinical measures such as blood pressure have been used in the past), and they are not measured for the purpose of detecting clinical benefit but for their reflection of the underlying pathological process.

Thus, since a “biomarker” is typically defined as a laboratory measurement that reflects the activity of a disease process, there are many such markers identified for many diseases of the nervous system, such as, for example, various magnetic resonance imaging (MRI) measures in multiple sclerosis and Alzheimer's

disease treatments, positron emission tomographic (PET) scanning of dopamine transporters in Parkinson's disease, etc. [3–6]. In essentially all cases, these markers quantitatively correlate, either directly or inversely, with disease progression. A “surrogate marker” however, can be defined as “a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy.” [7]. Hence, the major difference between a biomarker and a surrogate marker is that a biomarker is a “candidate” surrogate marker, whereas a surrogate marker is a test used and taken as a measure of the effects of a specific treatment.

Mental disorders are highly prevalent, heterogeneous, and of multifactorial aetiologies. Collectively, they are associated with significant morbidity, mortality, and economic cost. However, major difficulties are encountered in translating and quantifying critical end-points into therapeutic activity that benefit patient outcome. In the absence of a validated biomarker for psychiatric illness activity, symptomatic remission and functional restoration are the only available markers of wellness in psychiatry [8]. Furthermore, it is increasingly accepted that the imprecision of categorical psychiatric diagnoses can be a limiting factor in understanding both the genetic and functional basis of human behavioural abnormalities [9]. Each of the current clinical forms of mental disorders, be it depressive disorder, bipolar disorder, schizophrenia, anxiety disorders, attention-deficit/hyperactivity disorder (ADHD) or neurodegenerative diseases, is defined by a number of symptoms that differ considerably between affected individuals with respect to their presence, frequency, severity, and topography. This heterogeneity in symptoms has complicated the search for the aetiology of the diseases and the mechanisms for their treatment. For more than a century, it has been uncertain whether or not the major diagnostic categories of psychosis, namely schizophrenia and bipolar disorder, are distinct disease entities with specific genetic causes and neuro-anatomical substrates [10, 11]. Indeed, the results of studies directed toward aetiologies and the interpretation of the complex relationships between genes and behaviour have shown very limited levels of reproducibility [12]. So much so that the reputation of the field of psychiatric genetics has become tarnished in the view of many human geneticists [13, 14]. Too many linked loci have been claimed and withdrawn, too many association studies published and not confirmed and too many new and different chromosomal regions have been implicated for the same disorder.

It is becoming generally accepted that the current diagnostic system often guarantees, rather than diminishes, disease heterogeneity. In fact, syndrome-dominated conceptual thinking has become a barrier to understanding the biological causes of diseases characterised by clinical and therapeutic heterogeneity such as cognitive and psychiatric disorders.

This leads to an untenable situation that precludes coherent therapeutic developments since it effectively prevents defining what could constitute valid biological, clinical and therapeutic biomarkers.

2 - THE NEEDS FOR BIOLOGICAL MODELS AND THE ROLE OF SYSTEMS BIOLOGY

How to escape from this vicious circle? There seem to be very few choices. We must imperatively obtain a functional understanding of the pathological processes associated with these disorders. But this primarily means that we must first and foremost understand what we call "normal" processes so as to distinguish what could constitute potentially pathological deviations or events, thereby identifying markers for such events.

In other words, we have to develop predictive functional models sufficiently detailed so as to enable the precise identification, in mechanistic terms, of events leading to pathological consequences, hence identifying the markers associated with these events together with the modes of intervention most likely to prevent or alleviate the problems.

All very well, but psychiatric disorders arise from adverse events, both internal and external, affecting cognitive processes. Could we really have the pretension to "model" the cognitive processes in the brain? And assuming that anyone could even dare hold such pretension; could we only start doing this? To have the least chance of success, one would have to integrate massive amounts of information, pertaining to literally every domain of functional biology, if not into a coherent whole, at least into coherent pathways, leading to defined mechanisms, leading in turn to defined networks and interactions and finally to defined processes and functional modes. Could this be seriously contemplated?

Not only it can be seriously contemplated, but it must be undertaken.

Psychiatric conditions result from a complex interaction of genetic susceptibility and environmental effects. None of the many psychiatric conditions investigated to date has been shown to present a purely genetic background. Furthermore, molecular biology studies have indicated that gene expression is influenced by several environmental factors, including early experiences, traumas, learning, and memory processes [15]. However, the heterogeneity of available information together with the entire absence of functional models of pathogenesis and pathological evolution leads to a situation where not only psychiatric illness activity cannot be coherently approached but where therapy also become highly problematic since unidentified or evolving medical conditions may precipitate rapid and unanticipated changes in status [16–21].

Acquisition of the necessary knowledge can be obtained, in parts, using in-silico models produced through analytical approaches and processes collectively known as "Systems Biology".

1 What is "Systems biology"?

Systems biology is the discipline that specifically addresses the fundamental properties of the complexity that living systems represent. Living organisms present systems complexities that span at least five dimensions: 1) molecular complexity; 2) structural complexity; 3) temporal complexity; 4) abstraction and emergence; and 5) algorithmic complexity [22].

In effects, systems biology addresses the need to shift from a component-based reductionist view of biology to a system-wide perspective. It can be described as a global qualitative analysis of how all components in a biological system interact to determine its phe-

notype. Although the definitions may vary, systems biology can usually be characterized as interdisciplinary, iterative, computationally intensive, and information greedy. An explanation of these terms is beneficial to set the stage for the remainder of this discussion:

Interdisciplinary: Although it is sometimes possible to answer a reductionist question by a collaboration of scientists from the same discipline, a global view requires a multidisciplinary team. Scientists from multiple quantitative disciplines (mathematics, computer science, statistics), medical specialties (clinicians, radiologists, epidemiologists), and life sciences disciplines (molecular and cell biologists, geneticists, chemists, physiologists) need to work together for a real system-wide model to be developed.

Iterative: An intriguing aspect of systems approaches is the iterative nature of the process, in which scientific publications provide information required for modelling; based on this information, a model is created and tested by experiments. The results of these experiments are in turn used to refine the model.

Computationally intensive: The integration of multiple levels of information, multiple datasets from high-throughput technologies, the use of advanced mathematical and computational tools, and the iterative nature of the analysis require high-powered and parallel computing.

Information greedy: In contrast to reductionist approaches, where only recognized, directly relevant information is used, in systems biology all information is potentially relevant.

A recent review [23] described three concepts that are also worth introducing in any discussion of systems biology:

Emergence: The most basic principle in systems biology. Biological systems will express emergent characteristics that cannot be predicted from knowledge of their parts only. The colloquial equivalent of this concept is "the whole is bigger than the sum of its parts." For example, the presence of collagen I and III and matrix metalloproteases (MMPs) in the lung does not necessarily result in fibrosis; it is the combination of multiple factors and their deregulation that leads to the abnormal phenotype.

Robustness: This concept describes how stable the system is in response to environmental stresses and genetic variation. This includes understanding of internal regulatory loops and positive and negative feedback mechanisms. In general, negative feedback loops increase system robustness and positive feedback loops reduce it. It is worth considering that similar organ phenotypes may differ in their robustness [24]. Understanding the system's robustness and the factors that increase its sensitivity may be important in design of therapeutic interventions.

Modularity: complex biological systems are organised by functional modules, where multiple components are co-regulated by a process and, when activated, lead to a similar outcome. A module can be a set of genes located in a similar genomic region that are activated by the same transcription factors, or a set of chemokines that are bound to a similar glycoprotein. An increase in the peptidase that degrades this protein will cause predictable response through all of the associated molecules. The benefit of modular analysis is that it reduces dependence on the relative variability in levels of single molecules. Modularity often helps resolving conflicting pieces of information by allowing for spatial, temporal, and contextual organization of the information.

2 The current state of systems biology

A generic approach to biological systems modelling and analysis, called biochemical systems theory (BST) [25, 26] was originally designed for studying the dynamics and other features of biochemical and gene regulatory systems, but is not restricted to these application areas in terms of its mathematical foundation. BST is almost forty years old, and its development, expansions, and applications have been documented in several books and hundreds of journal articles, proceedings, and book chapters (see for example [27]). However, the real obstacle to fast progress in bio-mathematical modelling is the determination of unknown parameters from biological information. Even within the same modelling framework, this task may be attacked in distinctly different ways, leading to entirely different results [28] because one is never short of possible mathematical manipulations.

Furthermore, despite the identification of a number of novel disease-predisposing genes [29–32], progress in uncovering the mechanisms by which these genes lead to disease has been far slower. Even for cases in which genes validated as causal for disease are known to operate in what are thought to be well-understood pathways, it is often unclear whether the connection to disease regarding such genes involves the known pathways, whether these "known" pathways are more general than is presently known, or whether the disease-associated genes operate in multiple pathways, some of which are yet to be defined [33]. An example is the gene *Tgfb β 2*, a key component of the transforming growth factor- β signalling pathway, that involves only a modest number of proteins, but whose expression in the liver of mice from an F2 inter-cross population was shown to associate with thousands of other genes ostensibly unrelated to this classic signalling pathway. The gene was subsequently identified and validated as causal for obesity in a segregating mouse population [34], but how variations in this gene can lead to obesity is not yet understood.

There currently exists a vast diversity of systems biology programs with a wide variety of goals [22]. However, in spite of numerous technological advances [22, 35–39], systems biology remains in its early infancy and models accurately describing complex pathophysiological processes remain extremely rare, with the notable exception of those published in 2003 and 2005 by one European group [40, 41]. While these models, addressing breast cancer, were experimentally validated a posteriori using cell lines, their latest model, describing the mechanisms leading to neuronal death and vacuolation in Creutzfeldt-Jakob disease, has been experimentally validated in vivo (J-P Deslys, personal communication).

Hence, efficient appropriation and data integration across the "five-dimensional" manifold leads to the production of highly detailed predictive models, that, although entirely theoretical in nature, indicate very precisely what should be experimentally investigated, where, when, how and, most of all, why.

Although the models arising from these integrative approaches cannot, by any means, be regarded as biologically true in the absolute, they do represent a "least biased" and detailed view of the mechanisms potentially associated with a given physiological

state and/or governed by the biological components under consideration, together with precise indications of the means whereby these could be manipulated.

Thus, although such a model only represents an approximation of biological reality, it has the considerable advantage of providing the investigators with precise indications of what should be scrutinized and why. The new data arising from these experimentations can then be re-injected into the model, rapidly leading to a clear and factual understanding of the biological processes under investigation.

3 "Information" is a double-edged tool to be manipulated with caution

Nevertheless, it remains certain that, if predictive models of psychiatric pathogenesis are to ever be constructed, enormous masses of information originating from a multitude of biological investigations and encompassing an enormous functional complexity will have to be integrated.

However, while we might be suffering from too much data complexity, we are certainly not suffering from lack of available information nor from lack of redundancies in this information. Thus, although daunting in amplitude, if approached coherently, the flood of highly heterogeneous and often conflicting information, that currently hampers most biological fields, may become an invaluable tool.

But this tool must be approached and manipulated with extreme caution.

The information shockwave is of such amplitude that the scientific literature has become complex almost beyond measure. But to make matter worse, most of that information arose from reductive approaches that, in attempts to compensate for the enormous and often insurmountable experimental difficulties presented by in vivo systems, utilised in-vitro experimentation on material far removed from functional physiological reality. Hence, the enormous diversity of information obtained in association with most physiological networks poses a particular challenge in that, per se, it represents a highly distorted compounded view of the various modulations that can potentially affect each such network, albeit without any distinctions of means nor of actual effectors. Thus, due to the enormous diversity of biological systems that gave rise to this information, and the often complete lack of physiological compatibilities between experimental systems, the information thereby generated is characterized by three very burdensome properties that cannot be controlled by neither the producers of this information nor its potential users. All information is always 1) incomplete, to an unknown extent; 2) biased, in unknown manners and to an unknown extent; and 3) erroneous, to an unknown extent. "As a consequence, without analytical approaches that specifically incorporate the facts that 1) all that is called "information" is neither necessarily useful nor utilisable, and 2) all information should be considered as a priori suspect, modelling attempts will fail because of the much too numerous conflicting and, although correct in molecular terms, physiologically invalid reports". This is particularly true for psychiatric disorders since these are characterised by functionally fuzzy concepts such as "behavioural" and "cognitive" "dysfunctions".

A rapid survey of the types of data and concepts that will need to be integrated to generate models applicable to these disorders will demonstrate the fact.

3 - THE CONCEPT OF ENDOPHENOTYPES AND THE FUNCTIONAL GENETIC REALITY

Since 1972, a new conceptual approach has slowly emerged around the idea of “endophenotypes”. Reducing complex behaviors into components, whether they are of neurophysiological, biochemical, endocrine, neuroanatomical, cognitive or neuropsychological nature, is described as an “endophenotype strategy” [42]. Symptoms and clinical sub-typing (i.e. depression with or without psychosis) generally are not considered endophenotypes. Sub-typing in this manner amounts to little more than altering the defining observations of a complex behaviour. Decades of applying this approach have resulted in only slightly greater reproducibility than with the broad definition disorders themselves, whether in schizophrenia [43–46], bipolar disorder [47–49], depression [50, 51], attention-deficit hyperactivity disorder [52–55], obsessive compulsive disorder [56, 57]. It is increasingly obvious that there exist an overwhelming number of potential biological markers associated with psychiatric diseases. However, these often solitary findings frequently have limited reproducibility, both among and within patients, and may only represent state-dependent results. Even with a better definition of what might constitute a credible endophenotype, namely that it must 1) be heritable, 2) be associated with illness in the population, 3) be manifest in an individual whether or not illness is active (state independent) but age-normed, 4) co-segregate with illness within families, and 5) be found in unaffected relatives of probands at a higher rate than in the general population [58], the results remain far below expectations [59–61]. This may not be surprising since the biology of psychiatric disorders is not only complex but further complicated by epigenetic and stochastic contributing factors as well as by a plethora of gene/gene and gene/environment interactions and co-actions. Many gene products interact at many levels, leading to activation of multiple neuronal circuits, which results in behavioral variations. This is further complicated by the fact that there can be more than one pathway to a given behaviour.

1 Biological functions results from interactions between integrative and discontinuous mechanisms

In effects, the idea that endophenotypes could represent defined and quantifiable measures may be somewhat over simplistic. The concept is clearly dependent upon the argument that endophenotypes involve few genes, and therefore fewer interacting levels, ultimately affecting only a few, if not a single, set of neuronal circuits.

This is reminiscent of the views that were put forward in other medical fields, including gerontology, with respect to the benefits to be derived from knowledge of the human genome sequence [62–67].

It simply does not take into account the fact that biological functions result from integrative and non-linear processes subject to discontinuities.

The human genome contains much fewer open reading frames (ORFs; about 30 000 ORFs), encoding functional proteins, than was generally predicted. Like all other completed genomes, it contains many “novel” genes with no ascribed functions. Moreover, because of processes such as alternative mRNA splicing, RNA editing, and post-translational protein modifications, one gene can encode several functionally different proteins. Therefore, the functional complexity of an organism far exceeds that indicated by its genome alone. There is often a poor correlation between mRNA abundance and the quantity of the corresponding functional protein present within a cell [68, 69]. Additionally, co-translational and post-translational modification (PTM) events result in a diversity of protein products from a single ORF. These modifications include phosphorylation, sulfation, glycosylation, hydroxylation, *N*-methylation, carboxymethylation, acetylation, prenylation, and *N*-myristoylation. These dynamic processes, together with protein maturation and degradation, control the amount of functionally active protein within a cell [70]. However, most proteins carry out their physiological functions by interacting with other proteins. And this is more particularly the case in the CNS where, for many receptor sub-units, the variety of potential interaction partners can be bewildering with very significant functional consequences [71–76]. Hence, it is not merely a matter of what genes are expressed and to what level. It is rather a matter of what other potential interaction partners are present and in which state. Thus qualitative aspects are important here, and not quantitative considerations. A protein deemed “physiologically important” may be entirely absent or constitutively non-functional without producing deleterious phenotypic effects. This is amply demonstrated by the numerous examples observed in knockout mice [77–84]. Furthermore, the deleterious effects of inactivating mutations affecting a given protein can often be compensated by inactivating mutations simultaneously affecting another protein [85–88] or by the corrective mechanisms, involving many genes, that its functional absence induces [89–92]. Ultimately, what appears most likely to be at play in the realm of psychiatric disorders could be the effects of complex and extensive haplotypes, involving numerous genetic loci, with positive as well as negative complementation effects. While providing a coherent explanation for the numerous apparent conflicts associated with psychiatric genetics [93–97], this view is also in agreement with numerous puzzling and so far unexplained observations, including discordance amongst monozygotic twins [98–102] and non-mendelian segregation within proband families [103–106].

2 The effects of time, gene expression switching and complex haplotypes

With the exception of clear congenital malformations or maternal drug abuse, there are few reported cases of “constitutive” mental disorders (onset in early infancy) [107] and most of these are various forms of autism [108–110]. While this certainly could be due to diagnostic difficulties, it could just as equally be due to the mechanisms associated with cognitive development. The CNS, much more so than many other organs, is eminently affected by time (the act of being actively alive), in terms of both structural anatomy as well as networks interactions [111–113]. >>

▷▷ Functional evolution over time or, to use the term under which this is better known, ageing, implies numerous switches in gene expression patterns [114–116]. This is evident in many organs including the CNS [117, 118]. Interpreted in terms of functional genetics, this leads to the inescapable conclusion that, depending upon complex haplotypes, the functional situation implemented when time-point “B” will be reached may be radically different from what it was at time-point “A”. The numerous reported cases of spontaneous remission in a variety of severe psychiatric conditions [119–123] may well be striking examples of this.

4. THE PHYSIOPATHOLOGICAL ASPECTS, USING ANXIETY AS AN EXAMPLE

According to studies carried out on a random sample of non-institutionalised inhabitants from Belgium, France, Germany, Italy, the Netherlands and Spain aged 18 or older ($n = 21\ 425$), 13.6% reported a lifetime history affected by an anxiety disorder and over 6% reported suffering from an anxiety disorder during the past year [124]. Panic disorder, with a mean lifetime prevalence ranging from 1.4 to 3.5%, is among the most frequently occurring psychiatric disorders [125, 126]. It is also one of the most common and important conditions in primary care: 8% to 13% of patients seen in general practice fulfil the criteria for panic disorder [127]. The term “anxiety disorders” encompasses several clinical conditions:

- panic disorder, in which feelings of extreme fear and dread strike unexpectedly and repeatedly for no apparent reason, accompanied by intense physical symptoms,
- obsessive-compulsive disorder (OCD), characterized by intrusive, unwanted, repetitive thoughts and rituals performed out of a feeling of urgent need,
- post-traumatic stress disorder (PTSD), a reaction to a terrifying event that keeps returning in the form of frightening, intrusive memories and brings on hypervigilance and deadening of normal emotions,
- phobias, including specific phobia, a fear of an object or situation and social phobia, a fear of extreme embarrassment, and
- generalized anxiety disorder (GAD), exaggerated worry and tension over everyday events and decisions.

The two main treatments for generalized anxiety disorder are medications and psychotherapy, either alone or in combination. The most commonly used anti-anxiety medications are sedatives, such as benzodiazepines, alprazolam (Xanax), chlordiazepoxide (Librium), clonazepam (Klonopin), diazepam (Valium) and lorazepam (Ativan). Buspirone hydrochloride (BuSpar) is the only non-sedative medication often prescribed for generalized anxiety disorder.

While benzodiazepines ease anxiety symptoms within 30 to 90 minutes, they are often habit-forming if taken for more than a few weeks. For this reason long term treatment relies upon more conventional sedatives. But these cause unsteadiness, drowsiness, reduced muscle coordination, balance problems and long-term use can cause memory dysfunctions. Buspirone hydrochloride typically takes several weeks to improve symptoms but it does not lead to dependence nor to the above side effects. However, in opposition to the above sedatives, its mechanism of action remains

unknown. Buspirone has high affinity for serotonin (5-HT_{1A}) receptors, and moderate affinity for brain D₂ receptors and some pre-clinical studies indicate that it is a 5-HT_{1A} partial agonist [128].

1 Aetiological overview of anxiety

Anxiety is present in most psychopathological conditions [129] and overlaps with major depressive disorder (MDD; [130, 131]). Anxiety is often experienced as an automatic and uncontrollable response with deep roots in psychological processes such as rumination. These processes, central to human anxiety, imply high-order cognitive capacities, such as self-consciousness. Yet, progress in the understanding of the neurobiological substrates of anxiety and in the discovery of new pharmacological treatments often involves rodent models [132, 133]. It is therefore essential to be aware of the processes that are absent in the animal species used [134], in order to be aware of the limits of such models.

Fear, an important component of anxiety [135], develops when an individual is confronted with an identified threat while panic is regarded as a paroxysmic fear, that is, a full-blown fear expressed and experienced at the maximum of its possible intensity that can maladaptively occur when the threat alarm is erroneously triggered. However, fear is physiologically distinct from panic because of the lack of hypothalamic-pituitary-adrenal (HPA) activation in panic [136]. Thus, fear and anxiety are complex phenomena that articulate different mechanisms. When confronted with a danger, a subject may display specific responses that include behavioural components (flight), physiological changes (increase in heart rate), and expressive aspects (specific vocalization or facial expression).

In fear, the threatening factor is perceived but the reaction is independent of cognitive processes. In anxiety, the stressor is not always clearly identified, but in contrast to what happens in fear, cognitive processes are involved. Hence, in panic disorder, anxiety is considered as the fear of spontaneous panic attacks [137]. Such definition implies that anxiety requires more cognitive capacities than fear. Anxiety necessitates the capability to hold a representation of an emotional state and to react to it. This representation might be rudimentary, for instance, the reactivation of the emotional somatic state, but it constitutes a necessary condition to anxiety [138]. This implies that anxiety should appear in higher species when compared to fear.

When a relevant stimulus is identified, physiological, motor, and expressive response systems are activated. This leads to the tendency of performing certain actions or achieving certain relational changes with the environment: in other words, the activation of a behavioural plan aiming at changing the individual-environment relationships. In this respect, avoidance behaviour and flight have been observed in protozoan such as paramecia [139, 140], suggesting that a central nervous system is not necessary for the expression of such behaviours.

In humans and many other species, these stimulus-induced tendencies are not necessarily immediately enacted. The actual reaction requires a sufficient activation of the response systems. However, when occurring in a distressing context, enactment may become automatic [141]. Thus, there appears to be a buffer between the activation of a response mode and its actual enact-

ment and distressing signals may lead to functional disappearance of this buffer with potentially pathological consequences.

2 The neuroanatomy of anxiety

In humans, anxiety is accompanied by specific cognitive response. Threat and anxiety have been shown to powerfully affect attention allocation. Threatening stimuli automatically attract attention, even during subliminal exposure (very rapid presentation that cannot be consciously perceived) [142, 143]. Subliminal fear signals elicit activity in the brainstem region encompassing the superior colliculus and locus coeruleus, pulvinar and amygdala, and in fronto-temporal regions associated with orientation. This suggests that crude sensory input from the superior colliculo-pulvinar visual pathway to the amygdala may allow for sufficient appraisal of fear signals to innervate the locus coeruleus [143]. Amygdala activity is enhanced in response to both subliminal and supraliminal fear perception with greater right amygdala responses to subliminal fear, but left-sided responses to supraliminal fear. Cortically, subliminal fear is characterised by right ventral anterior cingulate activity and supraliminal fear by dorsal anterior cingulate and medial prefrontal activity. Although subcortical amygdala activity is relatively persistent for subliminal fear, supraliminal fear leads to more sustained cortical activity, suggest that preverbal processing of fear may occur via a direct rostral-ventral amygdala pathway without the need for conscious surveillance, whereas elaboration of consciously attended signals of fear may rely on higher-order processing within a dorsal cortico-amygdala pathway [144].

Incoming sensory signals of fear travel from thalamus to amygdala via two neural pathways: a direct subcortical route and an indirect pathway via the sensory cortex. In addition, the thalamo-cortico-amygdala pathways are subject to medial prefrontal modulation (MPFC). Here, the functional connectivity networks within thalamus, amygdala and sensory (inferior occipital, fusiform) cortices are modulated by the anterior cingulate cortex (ACC) and form part of a distributed neural system for fear perception. There is an inverse functional connectivity between occipito-temporal visual regions and the left amygdala, but a positive connectivity between these visual regions and the right amygdala, suggesting hemispheric specialization in the transfer of fear signals from sensory cortices to amygdala. ACC modulation of the thalamus-sensory cortex pathway presents a dorsal-ventral division. While the dorsal ACC shows a positive modulation of this pathway, the ventral ACC exhibits an inverse relationship. In addition, both the dorsal and ventral ACC show an inverse interaction with the direct thalamus-amygdala pathway. This suggests that thalamo-amygdala and cortical regions are involved in a dynamic interplay, with functional differentiation in both lateralised and ventral/dorsal gradients [145] and sustained phasic cortico-amygdala and autonomic responses may serve to prime the emotional content of fear signals, and differentiate them from initial stimulus novelty [146]. Life trauma tends to disrupt the normal pattern of medial prefrontal and amygdala regulation [147]. In people suffering from chronic anxiety, these disturbances (marked bilateral reduction in MPFC activity, in particular in right ACC and the ventromedial prefrontal cortex-structures linked to the experience and regulation of emotion, together with reduction in right amygdala response followed by enhance-

ment in left amygdala activity and bilateral anterior insula [148, 149] could be even more pronounced and aggravated by a poor capacity to disengage attention from threat. In fact, most studies on human anxiety indicate that an attentional bias toward threat is an essential component of anxiety, especially of dysfunctional anxiety [150–153]. It thus appears that dysfunctional anxiety could partly result from a vicious circle directly involving the patterns of medial prefrontal and amygdala regulation. An initial trauma could lead to disruption of the normal regulation pattern, directly leading to attentional bias toward threat which then further reinforces disruption of the amygdala-prefrontal regulation pattern, and so on. Indeed, fear circuit hyperactivation (exaggerated amygdala responsivity, diminished medial prefrontal cortex responsivity) has been observed to occur in an attention state involving focus on subjectively experienced fear [154].

3 The heterogeneity within and between the various clinical forms of anxiety

However, this is unlikely to be sufficient for full pathological progression.

Sex differences are apparent in the laterality and time course of fear perception in healthy individuals. In males, the right amygdala and autonomic arousal rapidly attenuate over time. By contrast, females show persistent bilateral amygdala responses, with a tendency towards greater left amygdala engagement over time. Females also show a greater general extent of amygdala response [155]. It appears that distinct physiological mechanisms (see section 3.5 below) might contribute to a lower threshold for vigilance to signals of danger in females, reflected in a profile of sustained amygdala-arousal interaction. Neuro-imaging suggests distinct prefrontal responses in individuals displaying dissociative and hyperarousal responses [156].

Increased prefrontal activity reflects enhanced down-regulation of limbic arousal networks and dissociation (enhanced activation in the ventral prefrontal cortex for conscious fear, and in the bilateral amygdala, insula and left thalamus for non-conscious fear) is a regulatory strategy invoked to cope with high arousal. But this strategy appears to function only during conscious processing of threat [157].

In the case of generalized anxiety disorder (GAD), this mechanism clearly implicates emotional awareness. Individuals with GAD present significantly higher emotion regulation deficits than controls [158, 159] with heightened intensity of emotions, greater negative reactivity to emotional experience, increased avoidance of emotional experience, and less ability to self-soothe after negative emotions than controls [160]. However, they also show good emotion differentiation and use several emotion regulation strategies more often than controls [161]. But GAD and post-traumatic stress disorder (PTSD) are more strongly related to personality than other anxiety disorders. Here, negative reactivity and maladaptive emotion management appear to relate to a latent factor of emotion dysregulation. In contrast, heightened intensity of emotions apparently relates more strongly to dispositional emotion generation or emotionality [162]. As a result, GAD and PTSD appear to have more in common with major depression than with their anxiety disorder counterparts [163].

4 The neuroanatomy of emotional control

Emotion regulation relies on synergies between brainstem, limbic and cortical processes that promote the adaptive generation and regulation of affect, with prefrontal and cingulate regions inhibiting sub-cortical and cortical emotion processing systems in the cognitive control of emotional experience [164]. During fear conditioning, an activation of the anterior cingulate cortex is observed and, in case of trace fear conditioning, an additional activation of the hippocampus has been documented [165]. This suggests that the hippocampus may enable the storage of the spatiotemporal aspects of the fear experience, while the anterior cingulate cortex may permit to drive attentional resources toward the stimulus and to anticipate the occurrence of the fearful stimulus. During Pavlovian fear conditioning a conditioned stimulus (CS) is repeatedly paired with an aversive unconditioned stimulus (UCS). In humans, amygdala, anterior cingulate, and fusiform gyrus activity increased linearly with the CS-UCS pairing rate. In contrast, insula and left dorsolateral prefrontal cortex responses are larger during intermittently paired CS presentations relative to continuously and non-paired CSs [166]. This demonstrates two distinct patterns of activity in disparate brain regions. Amygdala, anterior cingulate, and fusiform gyrus activity parallel the CS-UCS pairing rate, whereas the insula and dorsolateral prefrontal cortex pattern appears to respond to the uncertainty inherent in intermittent CS-UCS pairing procedures. Intolerance of uncertainty is strongly related to pathological worry and with obsessive-compulsive disorder symptoms [167] and insula hyperactivity repeatedly observed in anxiety disorder individuals may therefore be directly linked to altered processes of uncertainty management [168]. During anticipation of fear, there is an activation of the prefrontal cortex, particularly of the orbitofrontal cortex, of the temporal area, and of the insulae [169–172]. When subjects are requested to try to self-generate emotions by re-experiencing past events, they show a decreased activation of the hypothalamus, of the posterior cingulate cortex, and of the orbitofrontal cortex and an increased activity in secondary somatosensory cortices, in the insulae, and in the hippocampus [173]. Interestingly, some of these modifications are observed in areas enabling the perception and the regulation of body internal states (somatosensory areas and insulae). Hence, in humans, subcortical (hypothalamus, amygdala, hippocampus) and cortical brain areas (prefrontal cortex, somatosensory areas, insulae, cingulate cortex) are engaged during fear or anxiety, clearly indicating that a plethora of neurophysiological mechanisms intervene in the process of emotion regulation (CNS) and its behavioural consequences (autonomic NS).

5 The molecular neurophysiology of anxiety

A large number of studies have indicated that the serotonergic (5-HT) system plays an important role in the pathophysiology of anxiety disorders and involvement of the noradrenergic system has also been postulated. Serotonergic neurons in the central nervous system impinge on many other neurons and modulate their neurotransmitter release. Presynaptic 5-HT heteroreceptors have effects on axon terminals of central cholinergic, dopaminergic, noradrenergic, or γ -aminobutyric acid-ergic (GABAergic) neurons while GABAergic interneurons expressing 5-HT het-

eroreceptors regulate acetylcholine, dopamine, or noradrenaline release. In vitro studies on slices or synaptosomes and in vivo microdialysis experiments have shown that 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, and/or 5-HT₄ heteroreceptors mediate this modulation. 5-HT_{1B} receptors on neocortical cholinergic, striatal dopaminergic, or hippocampal GABAergic axon terminals are examples of release-inhibiting 5-HT heteroreceptors; 5-HT₃ receptors on hippocampal GABAergic or 5-HT₄ receptors on hippocampal cholinergic axon terminals are examples of release-facilitating 5-HT heteroreceptors. GABA released from GABAergic interneurons upon activation of facilitatory 5-HT receptors, e.g., 5-HT_{2A} or 5-HT₃ receptors, mediates inhibition of the release of other neurotransmitters such as prefrontal neocortical dopamine or neocortical acetylcholine release, respectively. Conversely, attenuated GABA release in response to activation of inhibitory 5-HT heteroreceptors, e.g., 5-HT_{1A} or 5-HT_{1B} receptors, on GABAergic interneurons is involved in paradoxical facilitation of hippocampal acetylcholine and striatal dopamine release, respectively. Such 5-HT heteroreceptors are considered potential targets for appropriate 5-HT receptor ligands which, by enhancing the release of a relevant neurotransmitter, can compensate for its hypothesized deficiency in distinct brain areas such as, for example, the impaired release of hippocampal or neocortical noradrenaline in major depression [174]. Furthermore, dopamine (DA) and acetylcholine (ACh) appear to play opposing roles in the nucleus accumbens in the control of GABA output systems for approach and avoidance. Contrary to DA, which fosters approach, ACh release is a correlate or a cause of meal satiation, conditioned taste aversion and aversive brain stimulation. ACh may also counteract excessive DA-mediated approach behaviour as revealed during withdrawal from drugs of abuse or sugar when the animal enters an ACh-mediated state of anxiety and behavioural depression. Accumulating evidence indicates that ACh is important in the inhibition of behaviour when extracellular DA is high and in the generation of an anxious or depressed state when DA is relatively low [175]. However, the synergistic action of 5-HT plus phasic cholecystokinin (CCK) apparently activates a circuit that simultaneously limits DA and release ACh in the accumbens [176]. Apart from these neurotransmitter systems there is now increasing evidence that the GABA system is important in the pathophysiology of anxiety disorders.

Three major types of GABA receptors have been identified so far: GABA_A, GABA_B and GABA_C receptors. Whereas GABA_A and GABA_C receptors belong to the family of ligand-gated ion channels, GABA_B receptors are transmembrane receptors coupled with G-proteins that activate second messenger systems. The rapid inhibitory action of GABA is mediated mostly through GABA_A receptors. The GABA_A receptor subtypes are composed of five subunits and are formed by temporal and spatial regulation of subunit α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , ρ 1–3, and π expression in brain regions and/or by cellular regulation of assembly to pentameric receptor complexes [177–179]. GABA_A ergic drugs, such as benzodiazepines, barbiturates, and volatile anesthetics, enhance the actions of GABA [180]. There is an extraordinary heterogeneity in the distribution of GABA_A-receptor subunits, as evidenced by

abrupt changes in immunoreactivity along well-defined cytoarchitectonic boundaries and by pronounced differences in the cellular distribution of subunits among various types of neurons. Thus, functionally and morphologically diverse neurons are characterized by a distinct GABA_A-receptor subunit repertoire. Multiple experiments have identified 12 subunit combinations in defined neurons. The most prevalent combination is the triplet $\alpha 1/\beta 2,3/\gamma 2$, detected in numerous cell types throughout the brain. An additional subunit ($\alpha 2$, $\alpha 3$, or δ) sometimes is associated with this triplet, pointing to the existence of receptors containing four subunits. The triplets $\alpha 2/\beta 2,3/\gamma 2$, $\alpha 3/\beta 2,3/\gamma 2$, and $\alpha 5/\beta 2,3/\gamma 2$ are also identified in discrete cell populations. The prevalence of these seven combinations suggests that they represent major GABA_A-receptor subtypes. Five combinations also apparently lack the $\beta 2,3$ -subunits, including one devoid of $\gamma 2$ -subunit: $\alpha 1/\alpha 2/\gamma 2$; $\alpha 2/\gamma 2$, $\alpha 3/\gamma 2$; $\alpha 2/\alpha 3/\gamma 2$ and $\alpha 2/\alpha 5/\delta$ [181].

Several studies have demonstrated that patients with panic disorder have a dysfunction of the GABA_A receptors or altered brain GABA concentrations (or both). Practically all effects of the benzodiazepines result from their actions on the ionotropic GABA_A receptors in the central nervous system. Benzodiazepines do not activate GABA_A receptors directly but they require GABA. The $\gamma 2$ subunit, one of the major subunits in the brain, is necessary for the expression of the full benzodiazepine pharmacology. The main effects of benzodiazepines are sedation, hypnosis, decreased anxiety, anterograde amnesia, centrally mediated muscle relaxation and anti-convulsant activity. In addition to their action on the central nervous system, benzodiazepines have a dose-dependent ventilatory depressant effect and they also cause a modest reduction in arterial blood pressure and an increase in heart rate as a result of a decrease of systemic vascular resistance [182]. However, many drug effects on GABA_A receptor function have been shown to depend on subunit combinations (subtypes) and even on critical amino acids in specific subunits [183].

In the rat, dependence and withdrawal syndromes elicited by the benzodiazepine tranquilizer chlordiazepoxide (CDPX) result from a decreased ratio of initial chloride flux rate to desensitization rate, caused by an increase in desensitization of cerebral cortex fast desensitizing GABA_A receptors. In rats made tolerant to CDPX, the rate of GABA_A receptors desensitization in the absence of CDPX is enhanced 3-fold below saturation with GABA, and the dependence of this rate on GABA concentration changes from a sigmoid to a hyperbolic curve. The contribution of this effect in vivo depends on desensitization making a contribution to signal termination [or the fraction of receptors that are inactive (desensitized)]. In naïve rats, the total signal due to this receptor form does not depend much on GABA concentration or the presence of CDPX because increased channel opening is counterbalanced by increased desensitization. In contrast, the total signal of this receptor from tolerant rat is significantly increased by CDPX or increased GABA concentration [184].

However, glucocorticoid hormones (corticosterone, cortisol, progesterone) down-regulate the expression of GABA_A receptors in the hippocampus, the striatum and the hypothalamus [185] and increase the attenuation of post-synaptic 5-HT autoreceptors 1A

and 1B, leading to decreased 5-HT re-uptake [186], and thereby to increased 5-HT signalling [187] thus increasing anxiety while inhibiting the therapeutic effects of buspirone [186].

While explaining the pro-anxiety effects of cortisol, these data also shed a new light upon the so-called "5-HT hypothesis" (low 5-HT promotes anxiety and aggressive behaviour [188]). It would appear instead that it is increased 5-HT-mediated neuro-transmission that is associated with increased anxiety and aggressive behaviour while increased serum glucocorticoid concentration might serve to facilitate active coping behaviour in a threatening situation [189]. But cerebral steroid metabolism also plays a significant role. Patients with panic disorder have increased concentrations of GABA agonistic 3α -reduced neuroactive steroids in association with a decrease of the antagonistic 3β -reduced stereoisomer [190, 191]. However, in patients with panic disorder, changes in neuroactive steroid composition have been observed opposite to those seen in depression [190]. This change in neurosteroids composition might serve as a counter-regulatory mechanism against the occurrence of spontaneous panic attacks. Indeed, during panic induced experimentally by lactate or CCK-tetrapeptide (CCK-4), patients with panic disorder had a significant decrease in GABA agonistic 3α -reduced neurosteroids and a corresponding increase in the antagonistic 3β -reduced isomer relative to healthy controls [192] where no such changes in neuroactive steroid concentrations could be observed with the exception of $3\alpha,5\alpha$ -tetrahydrodeoxycorticosterone and allo-tetrahydrodeoxycorticosterone [190]. These changes in neuroactive steroids during experimentally induced panic might result in decreased GABAergic tone, which may contribute to the pathophysiology of panic attacks [193].

6 The key role of glial populations

Far from being no more than silent supportive cells of neurons, glial cells are dynamic partners participating in brain metabolism and communication between neurons.

Astrocytes constitute the largest glial population in the mammalian brain. Evidence suggest that they are involved in provision of metabolic substrates for neurons [194], maintenance of the extracellular ionic environment and pH [195], uptake of neurotransmitters [196] and regulation of synaptic strength and plasticity and provide a pathway for synaptic cross-talk [197]. They interact with one another to form large networks propagating Ca²⁺ waves [198], and associate with neurons, oligodendrocytes and endothelia [199]. Astrocytes extend ezrin and radixin-containing thin lamellate processes into the neuropil, in particular around synapses, where they can modulate synaptic function or mediate glial-neuronal communication [200]. The structural and functional properties of these processes suggest that they represent a separate astroglial compartment [201]. Thus, parts of the brain (such as the hippocampus) are divided by astrocytes into separate compartments, each one the sole domain of an individual astrocyte [202, 203]. Glial cells physically take up part of the extracellular K⁺ extruded by neurons during the depolarizing phase thus spatially buffering local increases in extracellular K⁺ [204]. Furthermore, propagation of Ca²⁺ waves through networks of astrocytes, via the release of signalling molecules >>

▷▷ such as ATP, affects the activity of distant neurons integrated in different neuronal circuits [205–207].

Interferon-alpha (IFN- α) is produced by astrocytes and dendritic cells [208, 209] and IFN- α therapy in the treatment of several viral diseases and cancers produce significant neuropsychiatric and neurotoxic adverse events, including depression and anxiety [210]. In the rat, acute IFN- α increases serotonin turnover in prefrontal cortex and increases dopamine turnover in hippocampus [211] whereas *in vitro*, glucocorticoids and IFN- α increase the tightness of the blood-brain barrier [212]. In transgenic mice expressing IFN- α from astrocytes, *in vivo* and *in vitro* electrophysiological studies revealed impaired neuronal function and disturbed synaptic plasticity with pronounced hippocampal hyperexcitability probably linked with abnormal calcium metabolism and cholinergic neurons dysfunctions [213]. Arginine vasopressin (AVP), released from the CNS and leading to increased tightness of the blood-brain barrier, plays an important role in regulating several aspects of CNS functions including aggression, anxiety, and cognition. AVP induces glutamate release from astrocytes isolated from the cerebral cortex and hippocampus. AVP-induced increase in glutamate release and $[Ca^{2+}]_i$ is brought about by two distinct subtypes of V(1) receptors (V(1a) and V(1b)). V(1b) receptors are predominantly expressed in astrocytes isolated from the hippocampus and V(1a) receptors are solely expressed in astrocytes isolated from the cerebral cortex [214]. Unanticipated reductions in the density and number of glial cells are reported in fronto-limbic brain regions in major depression and bipolar illness. Moreover, age-dependent decreases in the density of glial fibrillary acidic protein (GFAP) (mature astrocytes) and levels of GFAP protein are observed in the prefrontal cortex of younger depressed subjects [215]. Factors such as stress, excess of glucocorticoids, altered gene expression of neurotrophic factors and glial transporters, and changes in extracellular levels of neurotransmitters released by neurons may modify glial cell number and affect the neurophysiology of depression.

7 Molecular aspects of neuroglial functions in relation to anxiety

The S100 proteins comprise a family of Ca^{2+} binding proteins of at least 21 members. They are differentially expressed in a variety of cell types and tissues and are thought to play unique roles, although they share a high degree of sequence homology and expression overlap. S100A1 is involved in the cytoarchitecture of the brain, in learning and memory, and in avoidance–approach behavior. S100A1 is found in the hippocampus, cerebral cortex and amygdala, and partially co-localises with the GFAP astrocyte marker in the stratum radiatum of the hippocampus. S100A1 knock out (KO) mice develop well and their brains present with normal morphology. Astrocytes and neurons of S100A1KO mice do not differ from those of Wt mice regarding shape, distribution and density. In the water maze, S100A1KO mice perform equally well as Wt, implying that S100A1 is not involved in spatial learning and memory. However, in avoidance–approach tests, predominantly male S100A1KO mice show reduced anxiety-like responses and enhanced explorative activities [216]. Hence, S100A1 plays a role in modulating innate fear and exploration of novel stimuli.

The N-myc downstream-regulated gene (NDRG) family consists of four proteins: NDRG1, NDRG2, NDRG3, and NDRG4 in mammals.

They are intracellular proteins associated with stress response, cell growth, differentiation, synapse formation, and axon survival. Following adrenalectomy, hippocampal NDRG2 mRNA increases in response to glucocorticoids. NDRG2 is predominantly expressed in neurogenic regions of the adult brain and, in these regions, NDRG2 is localized to GFAP-positive astrocytes or radial glia. In one of these regions, the subgranular zone of the dentate gyrus, NDRG2 expression is decreased after adrenalectomy, and is restored to sham-operated levels by corticosterone, indicating that it is under positive regulation by glucocorticoids *in vivo* [217]. Chronic treatment with a tricyclic antidepressant (imipramine) and a selective serotonin reuptake inhibitor (sertraline) reduces the expression of NDRG2 mRNA and protein in the rat frontal cortex. Repeated electroconvulsive treatment also significantly decreases NDRG2 expression in this region of the brain [218].

Agmatine, an endogenous neuromodulator, appears to be principally produced by astrocytes, although neurons also synthesise it. Agmatine is packaged into synaptic vesicles and released upon neuronal depolarisation. It has high affinity for several transmembrane receptors, such as α_2 -adrenergic, imidazoline I(1) and glutamatergic NMDA receptors. In addition to activity at these receptors, agmatine irreversibly inhibits neuronal nitric oxide synthase and downregulates inducible nitric oxide synthase. The effects of injected agmatine in animals include anticonvulsant-, antineurotoxic- and antidepressant-like actions. Intraperitoneal or intracerebroventricular injections of agmatine rapidly elicit antidepressant-like behavioural changes in the rodent forced swim test and tail suspension test. Intraperitoneal injections of agmatine into rats and mice also elicit acute anxiolytic-like behavioural changes in the elevated plus-maze stress test. In an animal model of acute stress disorder, intraperitoneal agmatine injections diminish contextual fear learning. Furthermore, intraperitoneal injections of agmatine reduce alcohol and opioid dependence by modifying behaviour in a rat conditioned place preference paradigm [219].

5 - SYSTEMS BIOLOGY APPLIED TO COGNITIVE DISORDERS

1 The model building rationale

The necessarily brief survey above provides some indication of the density and extent of the integrative effects that constitute the main mechanisms associated with cognitive dysfunctions such as pathological anxiety.

Phenotypes and behaviour depend on the integrated effects of multiple signalling pathways and molecules, genetic polymorphism and environmental stimuli. This is true for all biological systems, from individual cells all the way to organisms.

In the classical approach to systems biology, it is indispensable to construct databases that are as exhaustive as possible. Furthermore, it is just as vital that the contents of these databases be exploited with the highest possible flexibility [220, 221]. This results in strategies for updating, archiving, indexing, text-mining, information mapping etc. of ever increasing complexity.

But in fact, how are these databases actually constructed? It was first necessary to impose filters at the data collection stage. Can one have the least idea of what could constitute a good filter, as

opposed to a bad one, when, at this stage, it is impossible to define the types of information that will be required in fine? Indexing strategies had then to be implemented. Can one have the least idea of what could constitute a good strategy, as opposed to a bad one, when, at this stage, it is impossible to define the types of relationships that will have to be eliminated? But that is not all. The information entered in such a database is always incomplete, biased and partly erroneous. The "true" is thus intermixed with the "uncertain", without possibility to eliminate the "false" and even less to determine in which context the "true" may become "false".

But what happens if all information is regarded as suspect? Not only the databases need not be exhaustive anymore, but their contents, which are largely subject to caution, do not present the least analytical importance. What becomes analytically important are the indices attached to these databases. Not because these indices could be credible per se, but because they will allow the very rapid production of a large number of hypotheses that will systematically be subjected to destruction attempts, using the multiple crosschecks allowed by the redundancies in the scientific literature as archived in the public databases. A false hypothesis is very easy to destroy and the elements that allow its destruction can then be used to build a new, more solid hypothesis, and so on iteratively, until a hypothesis that cannot be refuted has been obtained. This does not mean that this hypothesis is correct. It merely means that this hypothesis, supported by multiple information intersects, must be seriously considered. Undestroyed hypotheses are then merged into meta-hypotheses and the iterative destructive process is applied anew, eventually leading to a very detailed biological model that clearly suggests what should be investigated, how, where, when and why.

This model-building process, functioning on the basis of negative selection of working hypotheses, embodies three major operating principles. First, biological systems are characterized by integrative, non-linear mechanisms (human genome = less than 3×10^4 genes, transcriptome = over 2×10^5 entities, proteome = about 10^6 individual components, etc.). This implies that any analytical approach tending to linearity by approximations will only generate artefacts. Second, published information, be it under the form of text, images, charts, sequence, etc. is always incomplete (to an unknown extent), biased (in an unknown manner and to an unknown extent) and erroneous (to an unknown extent). This implies that analytical approaches based upon positive selection (information is considered valid) will necessarily generate incoherencies. Thirdly, the functions of biological components are context-dependent [222–224]. This implies that, within biological systems, events dictate to contexts how to distort, contexts dictate to components how to behave and components dictate to events how to arise. This, in turn, means that analytical treatments in terms of components must be abandoned. It becomes necessary to adopt a relativistic and event-driven analytical approach. This allows to very rapidly reconstruct events that define contexts distortions, which, in turn, define component-associated behaviours and functions, leading to the inhibition of specific events while favouring the arousal of other specific events and so on, iteratively. The net result is a biological model, entirely supported by a

very large body of well identified published information, that very clearly suggests coherent mechanisms for hitherto unexplained observations and that describes in detail the conditions allowing the development of a biological phenomenon, the mechanisms associated with its progression, the molecular/biochemical features that characterise these changes (i.e. the biomarkers) and the means whereby it could be prevented or alleviated.

However, it is important to realize that such a model can only be an approximation of biological reality. Furthermore, the more complex the reality attached to the model, the coarser the model will be. It is therefore indispensable that such a model be confronted to the biological reality it is supposed to represent. The data arising from these experimental verifications can then be re-injected into the model, correcting errors and miss-directions. This approach results in a situation where biological investigations proceed at a rapid pace with a hitherto unachievable success rate.

6 - CONCLUSION

The domain of neurological disorders is subject to intense research activities. Therefore, considerable amounts of new information constantly accumulate. For example, in schizophrenia a recent analysis revealed high probability candidate genes involved in GABA neurotransmission (GABRA1, GABBR1, and GAD2), glutamate neurotransmission (GRIA2), neuropeptide signalling (TAC1), synaptic functions (SYN2 and KCNJ4), myelin/glia function (CNP, MAL, MBP, PLP1, MOBP and GFAP), and lipid metabolism (LPL) [225]. Bipolar (manic-depressive) and related disorders, on the other hand, appear strongly associated with DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa), PENK (preproenkephalin), and TAC1 (tachykinin 1, substance P), suggesting that more primitive molecular mechanisms involved in pleasure and pain may have been recruited by evolution to play a role in higher mental functions such as mood [226].

While these findings could explain the EEG gamma band abnormalities detected in schizophrenia, they do not help to shed light upon the mechanisms involved. Furthermore, there is still no convincing evidence that a crucial druggable molecular component exists which, if targeted, would yield medications with efficacies greater than those currently available and are thought to exert their main antipsychotic effect through antagonism of dopamine D2 receptors [227]. It appears, instead, that drugs which interact with a multiplicity of molecular targets are likely to show greater efficacy in treating the core symptoms of schizophrenia [228] and some of the above pathways suggest possible avenues for augmentation pharmacotherapy of schizophrenia with other existing agents, such as benzodiazepines, anticonvulsants and lipid modulating agents.

Hence, it remains amply evident that without predictive functional models sufficiently detailed so as to enable the precise identification, in mechanistic terms, of events leading to pathological consequences, thereby identifying the relevant biomarkers associated with these events together with the modes of intervention most likely to prevent or alleviate the problems, the above suggestions shall remain just that. Mere suggestions. ●

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