State of the art

Opportunities and challenges in psychopharmacology

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This review addresses novel approaches for influencing the transcriptome, the epigenome, the microbiome, the proteome, and the energy metabolome. These innovations help develop psychotropic medications which will directly reach the molecular targets, leading to beneficial effects, and which will be individually adapted to provide more efficacy and less toxicity. The series of advances described here show that these once utopian goals for psychiatric treatment are now real themes of research, indicating that the future path for psychopharmacology might not be as narrow and grim as considered during the last few decades.

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Introduction

For millennia, humans have swallowed or inhaled substances with the purpose of putting their psyche in a comfortable zone, of adapting their psychological and bodily states to avoid suffering, and to regulate, metaphorically speaking, their *mental engine* in a manner suited to momentary environments and goals. Consumers of psychotropic medications (like those of addictive drugs) maintain this long-standing human tradition and effort.

From the 1950s onwards, several major psychotropic medications were discovered by serendipity. Antipsychotics were discovered from antihistaminergic medications, monoamine inhibitors from antituberculosis drugs,[1] and tricyclic antidepressants from a clinical trial of imipramine as an experimental antipsychotic.[2] The antipanic effect of antidepressants was isolated in an open trial where imipramine was given to all types of long-term hospitalized patients.[3] Some of the discoveries from the 1950s and 1960s were made on the basis of mechanistic hypotheses. Paul Janssen imagined that a molecule might be an antipsychotic if it inhibited the abnormal stereotypic movements due to (psychosis-inducing) amphetamines, and this led him to discover haloperidol, which did indeed inhibit these movements in rodents. Henri Laborit, who had studied chlorpromazine (CPZ) to dampen neurovegetative responses during anesthesia and surgery, later developed γ-hydroxy butyric acid (GHB, sodium oxybate) in order to augment the brain concentration of γ-aminobutyric acid (GABA), and stimulate the pentose pathway in glial cells for a brain-protecting effect. GHB did not increase GABA in the cerebrospinal fluid of laboratory animals,[4] but it was found to be useful in anesthesia, alcohol withdrawal, and narcolepsy. Levodopa was another illustration of how basic research led to useful treatments, with the discovery of dopamine in the brain, its loss in the striatum of Parkinson disease patients, and then the benefit of levodopa substitution.[5] The pharmacology and the behavioral and the biochemical effects of the first psychotropic medications were exhaustively studied in laboratory animals and membrane receptor-binding assays, with the development of many "me-too" molecules.

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Ongoing pharmaceutical research is oriented towards depression (developing rapid-acting antidepressants such as ketamine, psilocybin, kynurenine analogues), autism (microbiome research, influencing cellular chloride with bumetanide), schizophrenia (sigma2 receptor antagonists), insomnia (influencing orexin), and neurodegenerative disorders (influencing endogenous neuroprotective compounds, for example the Klotho proteins). Active and passive vaccination have been studied in addiction to cocaine or nicotine, as well as in Alzheimer disease.

As for new research opportunities, it has now become possible to measure rapidly and precisely lots of *things* and to explore what *comes out* as results, ie, various *fingerprints* of endogenous biological substance values. These hypothesis-free approaches open up the way to exploring variations in the genome, epigenome, proteome, or metabolome, and to link these to symptoms, clinical diagnosis, or therapeutic effect, as well as, for psychiatric disorders, to neuropsychology and neuroimaging findings. This review is about developments in psychopharmacology from the standpoint of the ongoing deciphering of biological networks which show abnormalities in psychiatric disorders. Several of these developments are further described in articles in this journal issue.

> Biologically based treatments should aim to prevent the occurrence of psychiatric syndromes in hereditarily or environmentally predisposed persons

Gene research

Genetic studies have allowed us to find associations between polymorphisms and a higher occurrence of given traits or disorders, as well as to identify the most significant polymorphisms among the many polymorphisms that characterize polygenic psychiatric disorders. They also clarified the unequal transmission of genes from both parents (the parent of origin effects), as in the case of autism.[6] Mosaicism, ie, the presence of genomic variants in only some neuronal or non-neuronal cells, can occur by mitosis abnormalities or through transposons. Transposons are retroviruses genetic codes that have been inserted into mammalian cells over evolution; they have lost the possibility to copy themselves into ribonucleic acid (RNA) and to insert themselves back as deoxyribonucleic acid (DNA) into genes. The possibility of making genetic analyses on single cells (Drop-Seq technology) enables single cell libraries and identification of clusters of genetically similar cells within a tissue.

Genes and psychopharmacology research

Research findings have led to a growing relevance of genetic tests that guide the understanding of the prognosis and the choice of treatments of psychiatric disorders. Hypothesis-driven drug developments are ongoing at the level of gene regulation mechanisms. The DNA transcription factor PPAR-delta modifies directly or indirectly the transcription of several other genes or transcription factors. It might be a target to influence the oxidation of free fatty acids and the number of mitochondria. The transmembrane semaphoring 4D inflammatory protein increases the synthesis of inhibitory synapses, and suppresses seizures in animal models, making it a potential target in epilepsy treatment. Antisense oligonucleotides bind to normal or abnormal RNAs in order to achieve gene silencing or to modify alternative splicing (an antisense has been marketed for some genetic forms of Duchenne muscular dystrophy). In neurodegenerative disorders, antisense oligonucleotide research aims at lowering superoxide dismutase *(SOD1)* gene expression, at influencing the splicing of the apolipoprotein E receptor 2 *(ApoER2)*, at decreasing tau and other proteins that accumulate in these disorders. More than a century after the clinical description of Huntington disease, a genetic locus of the disease was identified on chromosome 4p16, and the excess of the protein huntingtin was then found. The core abnormality, ie, the polyglutamine repeats as DNA CAG triplets, is now being targeted in clinical trials using antisense molecules. The activation of human endogenous retroviruses (HERVs) described in schizophrenia and bipolar disorders[7] (antipsychotics could be responsible for part of this activation of HERVs) is being studied as a possible therapeutic target, and transposons offer a way towards gene editing. The different techniques of gene activation, inhibition, or editing are of paramount importance to construct models of neuropsychiatric disorders in laboratory animals and to study the pharmacology of candidate psychotropic medications.

Transcriptome research

The transcriptome is the total of the RNAs expressed from the genome. It gives a precise idea of changes in messenger RNAs (mRNAs), microRNAs (miRNAs) and other RNA synthesis, ie, it expands the information from gene polymorphism to the functional domain of DNA expression and RNA translation. Transcriptome research is of value for understanding central nervous system regulations and disorders. A transcriptome atlas of the brain has been published for two people,[8] and studying the transcriptome of single neurons opens up a new form of brain cartography in laboratory animals.[9] In a postmortem study, the transcriptome of prefrontal pyramidal neurons from the layers 3 and 5 showed differences in patients with schizophrenia compared with bipolar disorder patients, major depression, or persons with no psychiatric disorder.[10] It has become feasible to study clinical or biological transcriptional stimuli that confer susceptibility to both stressors and major psychiatric disorders.[11] Studies in rats have long aimed at solving aspects of human alcoholism, and this search continues with transcriptome analyses: chronic alcohol consumption results in hypermethylation of medial prefrontal cortex (mPFC) DNAs, persisting longer than the acute withdrawal period: 165 mRNA transcripts were modified at 3 weeks after withdrawal, 105 being upregulated and 60 downregulated; also 17 miRNA transcripts were upregulated and 24 downregulated, and these miRNA changes accounted for a greater part of the variance of post-alcohol neuroadaptation.[12] DNA hypermethylation is a key factor in a cascade leading to altered glutamatergic and brain-derived neurotrophic factor (BDNF) signaling within the network of interconnected structures such as the mPFC, the amygdala, the nucleus accumbens, the hippocampus, and other brain areas that govern changes in approach/avoidance, thus in alcohol intake.

Transcriptome and psychopharmacology research

Oligonucleotides can act as agonists *(mimics and agomirs)* or antagonists *(antagomirs)* of miRNA. The silencing of the brain miR-134 with an *antagomir* was efficacious against seizures in animal models.[13] While the first probable clinical application of miRNAs in psychiatry might be as biomarkers in schizophrenia, depression, and other disorders, research aimed at modulating endogenous proteins such as membrane neurotransmitters using *agomirs* or *antagomirs* will be of importance to increase the proportion of patients who respond to psychotropic medications, for example by rendering the serotonin transporter more sensitive to antidepressants.

Epigenome research

The epigenome describes the proportion of genes that are expressed (ie, the transcriptome) following experimental situations imposed on laboratory animals or by life events or disorders in animals or people.[14] Epigenesis is also central to normal development. There are two major mechanisms of epigenesis, DNA methylation and posttranslational structural changes of histones, the proteins around which DNA is wrapped. DNA methylation occurs by transfer of a methyl group from S-adenosyl methionine (SAM); histone modifications are through acetylation by more than 10 histone acetyltransferases (HATs), deacetylation by four major classes of histone deacetylases (HDACs), methylation (histone methyltransferases or HMTs) or demethylation (histone demethylases or HDMs), and also phosphorylation and dephosphorylation. The resultant covalent changes in histones influence DNA in the directions of gene expression or silencing. High-throughput techniques, like microarray and RNA sequencing, enable the measurement of changes due to epigenesis. For illustration, administration to gestating female rodents of vinclozolin, an endocrine disrupter, during sex determination induces different physical diseases in adults; the expression of genes in the hippocampus and the amygdala of these rodents studied in the third generation showed increases or decreases in the expression of many genes, representing a form of Lamarckian-type memory; the changes were specific to the brain structures and to the sex of the animal.[15] Epigenetic changes due to experiencing stress during pregnancy are associated with later mental dysfunctions in humans.[16] Not all epigenetic changes are stable over the lifetime.

Research on small molecules that have epigenetic effects is relevant to cancer, and to mental and physical health. One of these molecules is sodium butyrate; this short-chain fatty acid illustrates the complexity of physiology: aside from being an HDAC inhibitor, it serves as a major source of energy for intestinal cells, modifies intestinal permeability, is the ligand of G-protein coupled receptors (GPCRs, for example GPR109A, linked to the inflammasome), has anti-inflammatory effects, lowers glycemia, and finally is an essential part of the microbiota, because many gut bacteria

synthesize butyric acid.[17] These pleiotropic properties mean that butyric acid is among the molecules that are popular as over-the-counter food supplements, leading consumers who think of themselves as suffering from a "leaky gut" to jump to conclusions and hope that butyric acid will decrease a hypothetical endotoxin absorption.

Epigenome and psychopharmacology research

Early psychotropics are known to influence epigenesis: amitriptyline, imipramine, fluoxetine, escitalopram, and monoamine oxidase inhibitors have epigenetic effects, for example on the *BDNF* gene or its promoters. Haloperidol, sulpiride, risperidone, and clozapine also influence epigenesis, the latter through the influence on an *HMT* gene and secondarily on the enzyme glutamic acid decarboxylase (GAD), which transforms glutamate into GABA. Valproic acid is a potent HDAC inhibitor, with repercussions on tyrosine hydroxylase, GAD, possibly BDNF, and reelin.[18]

HDAC inhibitors can influence chromatin and inflammation among other effects if they bind to HDAC1, while if they bind to HDAC6, they then influence non-histone structures such as microtubules, and potentially the processes of aging. New HDAC inhibitors are therefore being developed taking into account their potency and their specificity for the different HDACs, of which there are around 20 members in four classes.[19] The approaches to influence the epigenome are promising[20,21] for example with proposals for new drugs to treat Alzheimer disease.[22]

MiRNA research

The microRNAs or miRNAs are a class of more than 1000 noncoding RNAs (ncRNAS) of about 20 nucleotides that regulate up to one half of the protein coding genes in mammals. Precursors of miRNAs are synthesized in the nucleus and then exported into the cytoplasm, where they are incorporated into a silencing complex bound to messenger RNA (mRNA) and inhibit protein synthesis in the cytoplasm. The miRNAs are influenced by many conditions aside from their gene polymorphism; for example they are dysregulated in germ-free mice, and these changes can normalize when mice are seeded with digestive flora. Administering antibiotics to rats to the point of suppressing the digestive flora also modifies miRNA synthesis; it is of note that these changes are also observed in the amygdala and the prefrontal cortex.[23] Postmortem tissue studies have suggested the upregulation of many and the downregulation of a lesser number of miRNAs in schizophrenia, bipolar disorders, and autism.[24] Certain miRNAs are involved in drug compulsive behavior.[25] Several of the miRNAs found to be dysregulated in these psychiatric disorders are involved in the building of neuronal cells, synapses, and glutamatergic or GABAergic systems. There are also long ncRNAs, that serve as receptacles that interrupt the action of miRNAs, thus the term "sponges."

miRNAs and psychopharmacology research

There exist several ways to inhibit miRNAs using low-molecular-weight inhibitors of specific miRNA synthesis, antisense oligonucleotides, or miRNA sponges that bind and block miRNAs.[26] Peripheral blood cells or plasma can be used to measure miRNA, a potential diagnostic tool in Alzheimer disease.[27] The miRNAs might be the intermediate mechanism that transduces the blocking of the serotonin transporter (SERT) by selective serotonin reuptake inhibitors (SSRIs) into downregulation of the 5-HT-1 receptor and of the SERT,[28] with ensuing increased serotoninergic transmission in prefrontal projection areas.

Mitochondria research

Mitochondria are involved in energy metabolism, intracellular calcium, free radical metabolism, and apoptosis. They support the final steps of energy production through oxidative phosphorylation, reactions coded by nuclear DNA as well as by mitochondrial genes (there are 37 of them, with 16 500 base pairs). It is within the mitochondria that the citric acid cycle (tricarboxylic acid cycle, Krebs cycle) transforms acetyl-CoA (from the catabolism of carbohydrates, proteins and lipids) by oxidation into adenosine triphosphate (ATP) and other small molecules. ATP can be interconverted into adenosine monophosphate (AMP) or adenosine diphosphate (ADP). The enzyme 5'-AMP-activated protein kinase (AMPK, to be distinguished from the cyclic-AMP dependent protein kinase) is a ubiquitous metabolic sensor of the ATP:AMP ratio which, under its activated form by a low ATP:AMP ratio, stimulates metabolic pathways that produce ATP and inhibits those consuming ATP. Mitochondria are transported from one intracellular location to another by microtubules and many proteins such as the kinesins and kinesins-related proteins, for example the protein disrupted in schizophrenia-1 (DISC1).

An important component of the mitochondria is cardiolipin (CL), a lipid from the inner membrane of mitochondria that plays a role in mitochondrial enzyme functions. When CL is oxidized, losses in energy metabolism occur and inflammatory processes are stimulated, up to apoptosis. Of note is the fact that CL antibodies are found increased in syphilis, schizophrenia, and several other diseases.

A series of metabolic intermediates generated from energy metabolism, the Krebs cycle, and the respiratory chain, for example α-ketoglutarate, acetyl-CoA, and reactive oxygen species, enter the nucleus and influence the expression of proteins by interacting directly with DNA or with RNA transcription factors, and by longer-term changes due to epigenesis. Exposure to stressors induces mitochondria to produce more energy, modulates stress hormone secretion, and increases nutrient intake.[29]

Grouping small molecules, or metabolites, defines the metabolome, a phenotypic description of cell biology and energy metabolism. The separation of these molecules and purification from proteins and lipids is done using gas or liquid chromatography followed by measurements by nuclear magnetic resonance or spectrometry, replacing innumerable separate assays. The metabolome can be centered on one molecule and its biological network, for example nicotinamide adenine dinucleotide (NAD) and its phosphorylated form,[30] or on the metabolism of tryptophan, tyrosine, purine, and methionine in affective disorders,[31] or on the short-chain fatty acid propionic acid as a marker of mitochondrial dysfunction in autism,[32] or a series of serum markers that distinguish between schizophrenic patients and normal controls, or between depressed and nondepressed cardiac patients.[34]

An illustrative well-documented example of metabolomic research is the work done on the kynurenine pathway (metabolism of the essential amino acid tryptophan in glial cells) that produces quinolinic acid, a neurotoxic endogenous selective agonist at NMDA receptors, and kynurenic acid, a NMDA antagonist with neuroprotective effects. Inflammation linked to infection modifies the kynurenine pathway, and a high ratio of quinolinic acid to kynurenic acid in the blood is associated with clinical depression.[35] Kynurenine-like molecules change miRNA expression in the hippocampus and this could be one of the mechanisms of their rapid antidepressant effect in preclinical studies.

Mitochondria abnormalities have mainly been linked to muscular and neurodegenerative diseases and other rare diseases due to mutations or deletion of one or more genes of the respiratory chain. But mitochondria changes have also been found in depression,[36] schizophrenia,[37] and bipolar disorder,[38] adding to the list of biological abnormalities in psychiatric disorders.

Mitochondria and psychopharmacology research

Research on mitochondria pharmacology is of relevance to psychiatry. Several medications stimulate the mitochondria respiratory chain of mitochondria and/or decrease free radical production; the antidepressant effect of ketamine, a voltage-dependent N-methyl-D-aspartate receptor (NMDAR) non-competitive antagonist, might be mediated through influence on energy metabolism and the respiratory chain, as reflected in the metabolome.[39] Paths to influence the buildup, intracellular trafficking, and functioning of mitochondria have been studied in order to develop drugs protecting these organelles; to evaluate these drugs, tests measure the number and functionality of mitochondria in circulating blood cells.[40] Changes in energy metabolism being central to acute or chronic kidney disorders, modalities to protect mitochondria have been searched for by nephrologists[41] using peptides that bind to CL. One such compound efficacious in animal models of renal failure is considered a potential candidate against Alzheimer's disease.[42] Another target is carnitine palmitoyltransferase I (CPT1), an enzyme from the outer membrane of mitochondria involved in the uptake of fatty acids into the mitochondria and that promotes fatty acid oxidation. Also, activating the mitochondrial transcription factor A via the protein peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1) promotes biogenesis of mitochondria rather than mitophagy.

Drugs or vitamins which are said to protect from oxidative stress have to be tested to establish how specific domains of mitochondria physiology can become a target of neurodegenerative disorder treatment.[43] The synthetic ubiquinone MitoQ, an antioxidant that specifically targets mitochondria, has been shown to decrease some markers of aging in animals, as well as to improve memory in a mouse model of Alzheimer disease, but with a toxic effect of mitochondria swelling.[44] The plant polyphenol resveratrol acts on sirtuins, a group of protein desacetylases, and this activates AMPK; it has shown a series of beneficial effects in animal

models of aging or Alzheimer disease. Its effects were studied in a 52-week placebo-controlled clinical trial on Alzheimer disease patients, where it showed little to no clinical improvement.[45] HDAC inhibitors are being developed as cognitive enhancers, taking into account their specificity for classes of HDACs.[46]

Protein research

Many marketed psychotropic drugs have a major selective mode of action, on an enzyme, a receptor (agonists, antagonists, inverse agonists, or allosteric modulators), a transporter, or another protein. This one medication/one protein model has been fruitful over the years. However, the approach has its limits; for example, it was hoped that acetylcholinesterase inhibitors would be efficacious in Alzheimer disease, but clinical results remained inferior to those with dopaminergic agents in Parkinson disease. This is because, aside from the defect in acetylcholine, there are other neurotransmitter changes, abnormal accumulation of amyloid and tau proteins, innate immune and inflammatory mechanism activation, changes in mitochondria, and miRNA dysregulations.[47] From the observation of this pleiotropic pathophysiology of Alzheimer disease, treatments aiming at multiple targets were proposed,[48] but no combination of compounds, studied under the optimistic label of *rational polypharmacy*, has yet been recognized as clinically effective (combinations of compounds challenge the feasibility of identifying dose/response curves for each of their constituents).

Proteins and psychopharmacology research

Influencing enzymes and other proteins by small molecules has been a main focus of research on proteins in psychopharmacology, and research on known G-GPCRs and orphan ones (ie, those for which no endogenous ligand has been identified), on membrane transporters, ion channels, microtubule transporters and growth factors, oligonucleotides, and other proteins will continue to be a mainstream domain for psychotropic medications development. Proteins are important targets of treatment because biological abnormalities in psychiatric and other disorders generally lead to altered proteins. A focus of research is preventing or decreasing the abnormal proteins that accumulate in several neurodegenerative and psychiatric disorders: research aims at inhibiting the synthesis of such abnormal brain proteins, at facilitating their clearance by active or passive vaccination, at influencing their tridimensional folding and protein/protein interactions. Proteomic, and metabolomics, analyses will lead to the discovery of biomarkers guiding the treatment of psychiatric disorders.[49]

Microbiome research

A *Human Microbiome Project* (HMP) was started in 2008 by the National Institute of Health in the USA, with the goal of genetically identifying all small organisms that symbiotically inhabit our skin, digestive tract, and other organs. Humans have diets that go from plant-based to animal-based and this modifies their digestive tract microbiota.[50] Bacteria synthesize short-chain fatty acids, such as butyrate, that influence immunity and penetrate into the brain, and there are many bidirectional exchanges from gut to brain in health and in disease. The microbiota modulates proteins important for brain plasticity, and this might be relevant for normal neurodevelopment, as well as for depression, schizophrenia, or cognitive decline in aging. Stressors or infections lead to changes in the microbiota and in the intestinal barrier, enabling endotoxins to reach the bloodstream, with possible psychiatric repercussions.[51] Digestive bacteria metabolize drugs and influence hepatic drug metabolism enzymes. Clozapine alters the microbiota, which might be one of the mechanisms of side effects such as obesity or vascular accidents.[52]

Microbiota and psychopharmacology research

Influencing the microbiota might add to the other possibilities of manipulating neurotrophic factors such as BDNF and proteins involved in plasticity, with beneficial effects on neurodegeneration and cognition.[53] Changes in diet, administration of supplements, of probiotics (live bacteria delivered orally in acid-resistant galenic forms) and prebiotics (long-chain carbohydrates that serve as nutrients for bacteria) have been proposed as therapies for irritable bowel syndrome, autism, and other psychiatric syndromes. These treatments might be made more efficacious on the basis of precision medicine, with metagenomic microbiome analysis and specific probiotics or prebiotics (rather than the first-generation preparations that moderately influence the microbiota). Fecal microbiota transplantation, first studied against severe bacterial infection by *Clostridium difficile*, is being evaluated in ongoing double-blind trials of anxious or depressed patients.

Neuroimaging research

Structural and functional neuroimaging techniques can identify groups of subjects who have psychiatric disorders in comparison with normal controls (with more accuracy when patients are severely affected). They enable us to study the metabolic activity of small brain regions, as well as connections between regions (the connectome). Functional MRI maps of neural circuits offer a fine analysis of neuronal networks involved in specific brain functions and of the components of clinical syndromes such as anxiety, depression, or psychosis.[54]

Neuroimaging and psychopharmacology research
Neuroimaging can explore structural and functional changes that are induced by biological or nonbiological treatments, for example connectivity alterations in given networks after antidepressants, electroconvulsive therapy, transcranial magnetic stimulation, or psychotherapy in depression. Neuroimaging technologies make it possible to measure the concentration of some endogenous substances, and to describe the in vivo time course of biochemical changes along with brain tissue concentration of the active drug or its metabolites. Ligands or tracers for positron emission tomography (PET) neuroimaging enable the quantification of 5-HT transporters, 5-HT2a receptors, and dopamine receptors and transporters, as well as the proportion of their occupancy under psychotropic agents.

Prediction is an important field for neuroimaging. An early prediction of neurodegeneration is possible through quantification of brain β-amyloid, tau proteins, or level of HDAC activity.[55] Relapse after withdrawal in case of methamphetamine addiction can be predicted by neuroimaging.[56] In a trial of 70 depressed patients receiving escitalopram, sertraline, or venlafaxine the evaluation of their personal history of early life stressor exposure and the measurement of their amygdala response to happy and to fearful faces gave an area under the curve for the receiver operating characteristic (ROC) curve of 0.92 for predicting functional remission, while it was 0.70 when taking into account age, years of education, baseline depression severity, and depression episode duration.[57] Depressed patients with smaller hippocampal volumes have a worse prognosis for both remission during antidepressant treatment and duration of the remission.[58] The accuracy of prediction should be further studied before these techniques are adopted for individual patients in the clinical setting.[59]

Technical innovations

A series of new techniques enable further study of brain physiology and biochemistry, and serve as tools to discover novel psychiatric treatments.

Induced pluripotent stem cell and organoids
Animal or adult human somatic cells can be induced back to pluripotent stem cells, and from these specific mature cells can be obtained and organized in two-dimensional or three-dimensional structures (organoids) that mimic aspects of whole-organ organization. This offers a tool for ex vivo omics studies and effects of treatments,[60,61] for example the pharmacological influences on tissue differentiation. Work is ongoing on autism[62] and other developmental disorders, for example Rett syndrome and Williams syndrome, as well as on neurodegenerative disorders.[63] The induced pluripotent stem cell (iPSC) can be studied in single individuals,[64] as can organoids. Animal research is testing brain stem cell transplantation, for example of GABAergic interneuron progenitors to facilitate plasticity.[65]

Chemogenetics
Chemogenetics enable activation or deactivation specifically of one type of neuronal or non-neuronal cell within a given network, while the living animal is experiencing an inferred behavioral state (perceptions, emotions, stress, etc) or expressing behaviors. To this end, a series of proteins and enzymes are engineered in order to become responsive to small molecules. Among these proteins, GPCRs have been modified, under the name of designer receptors exclusively activated by designer drugs (DREADDs). They are inserted into a given cell population using tools such as viral infection. DREADDs enable study of neurotransmitter GPCRs in their conditions of fully active to fully inactive, depending on ligands, receptor proteins heterodimer formation or interaction with β-arrestins (β-Arr—endogenous proteins serving as negative regulators of GPCRs). Small molecules that only bind to DREADDs can be administered for exploratory purposes, and ultimately as therapeutic agents in humans.[66]

Optogenetics
With optogenetics, light-sensitive molecules such as pumps or channels are virally introduced into cells; the infected cells can then be influenced by light. For example, channelrhodhopsin 2 mediates an excitatory cation current when blue light (delivered by fiberoptic cable) hits the cell, while halor-

hodopsin generates an inhibitory current under yellow light. These rhodopsins and other molecules in development are named *actuators* since they influence cells, while *indicators* are molecules that indicate the level of activation of cells by emitting light.[67] Optogenetics, just like chemogenetics, allows study of the functional anatomy of brain functions and higher cognition in ways previously not possible.[68] Illustrative findings are the exploration of the series of individual cells activated when a laboratory animal is exposed to a distinctive odor, of the fact that turning on and off the dopaminergic neurons in the ventral tegmental area of mice induces manifestations related respectively to depression and relief thereof,[69] or that repeated stimulation of the cortex and the striatum induces lasting manifestations akin to obsessive-compulsive disorder.[70]

Gene editing

The discovery of the clustered regularly interspaced short palindromic repeats (CRISPR) with its associated Cas nuclease (CRISPR/Cas9) came from work on how bacteria defend themselves from viral attacks by eliminating viral genes: CRISP/Cas9 is an endonuclease enzyme that cuts DNA pieces at a location specified by a RNA guide, opening a new way towards reverse engineering of genetic.[71] The CRISPR/Cas9 techniques are used ex vivo, for example on cultures of induced pluripotent stem cells, on which a copy of the gene CHD8 associated with autism can be knocked out in order to study biochemical networks that might be related to autism.[72] CRISPR/Cas973 could lead to more specific corrections than agents that stimulate or inhibit enzymes and other proteins involved in epigenesis, and have only a partial corrective therapeutic effect. CRISP/Cas13 is another CRISP that edits RNAs. Limitations to gene editing (aside from laboratory difficulties linked to the CRISPR/Cas9 or Cas13 techniques) are that psychiatric disorders are multigenic, making it necessary to develop tools that modify *(repair)* many genes in order to have a clinically useful effect, and of doing this early in fetal development. Also, the high rate of de novo genetic changes is a constraint towards finding ways of developing gene therapies uniquely suited for one or a few persons. Finally, gene editing raises more ethical questions than other approaches mentioned in this review.

Big data and e-health

Genetics and other hypothesis-free research generate huge amounts of data. To give only one example, a worldwide program for the study of detailed neuroanatomy changes in depression resulted in a publication by 20 research teams working out the detailed MRI data and clinical status of a total of 10 105 persons.[74] Preclinical drug design often includes analysis of different omics, and clinical trials and precision medicine are more and more guided by omics and big data, as well as artificial intelligence. This is a welcome evolution in psychiatry.

Nanotechnology

Nanoparticles of 1 to 100 nm are used as drug transporters or as facilitators of drug action in the fields of oncology, infectious diseases, and a few other fields. Liposomes are spherical carriers that can contain lipophilic or hydrophilic substances and improve the bioavailability of these substances at digestive, cellular, and blood-brain barrier membranes. Polymer drug conjugates, for example with polyethylene glycol (PEG) can protect drugs of high molecular weight (enzymes, miRNAs, growth factors, etc) from enzymatic degradation and enhance their entry into cells having somewhat leaky membranes such as cancer or atheromatous cells. Depot antipsychotics already exist with crystalline nanostructures and preclinical research on psychotropic medications that can be PEGylated is ongoing. Nanotransporters could direct therapeutic agents into intracellular locations, an opening towards the area of subcellular pharmacology of organelles, selective synapses, and dendrites. A risk of nanomaterials is their accumulation in the liver, the lung, or the bone marrow.

Discussion

Treatments in psychiatry aim at decreasing mental symptoms (objective variable) and suffering (subjective variable) by compensating abnormalities when endogenous rebalancing mechanisms have failed. Psychotropic medications have improved the lives of many, but psychiatric syndromes affecting millions (psychosis, depression, anxiety, addiction, and other disorders) are often resistant to treatment. Moreover, these medications are useless or have very limited efficacy in neurodevelopmental disorders or dementia. Severe structural defects during brain development or later in life cannot be cured, but only be partly compensated for, while prenatal genetic, epigenetic, or other metabolic abnormalities could theoretically be corrected early or later in life.

The innovations mentioned in this review are a fascinating confirmation of the complexity of brain physiology, as well as of the pleiotropic modulating effects on biological pathways of endogenous and exogenous substances. They open up paths towards the next level, or next generation, of biomarkers of the links between clinical states and neural circuits and transmitters that will guide diagnostic procedures, the discovery of new therapies, and the choice of existing ones. Of relevance is the fact that some biomarkers measured in circulating blood cells, for example gene expression, can quite directly reflect the changes within the nervous system.[75] These novel approaches can be coupled, as in imaging genomics, where cognitive and high-resolution neuroimaging data are collected in parallel with DNA, miRNA or epigenome sequencing; for example, the amount of GABA-producing gut bacteria measured in fecal transcriptome was negatively correlated with brain imaging signatures of depression in humans.[76]

It might be that some future therapies will have record effect sizes, as did the first historical antibiotics, to the point where they will not have to be compared with a placebo or to other drug- on non-drug treatments. However, predicting which of these promising innovations will enter clinical use is uneasy, since only clinical trials will finally attest their clinical usefulness or lack thereof. In order to search for ongoing innovative trials, clinicians have access to the exhaustive information provided by the United States National Library of Medicine. As of December 2018 the Internet portal clinicaltrials.gov lists 2010 studies in Alzheimer disease, 2966 in schizophrenia, and 3910 in mood disorders.[77]

A selection of goals for the future of psychopharmacology is in *Table I*, leaving aside the very question of which goals are utopian, versus which will be found to have clinical efficiency.

- Increase the efficacy of pharmacological treatments for common psychiatric disorders.
- Prevent the occurrence of psychiatric disorders or change their course with disease-modifying treatments, rather than provide only symptomatic therapy.
- Establish what pathological traits or biological abnormalities are responsive to treatment, dependently or independently of clinical classifications.
- Discover predictive biomarkers (genes, epigenes, miRNAs, proteins, microbiome, neuroimaging data, etc) that explain important proportions in the variance of clinical status and of the response to treatment.
- Set up innovative methodologies for clinical trials in the fields of evidence-based psychiatry, precision psychiatry and predictive psychiatry.
- Set up longitudinal studies based on omics analyses coupled to clinical and neuroimaging data comparing periods of acute symptoms with remission, for each patient.
- Identify how drugs, environmental plastic residues, agrochemical substances, and other pollutions alter brain development, cognition, and mental health; determine how to diagnose these alterations clinically and through paraclinical tests; explore preventive and therapeutic approaches against these threatening situations.
- Disseminate the use of clinical tests and assays that identity subsets of highly responsive patients, as well as patients at risk of adverse drug events.
- Inform physicians about relevant biological and clinical findings which enable them to write rational drug prescriptions.

Table I. Future developments in psychopharmacology.

Research context

Innovations in psychopharmacology are sought in a context of previous failures in central nervous system drug discovery and development, with the disengagement of major pharmaceutical industries from research in psychiatry. In parallel, startups were created covering many aspects of neuroscience (see *Box* for list) to which major companies outsource research. Given startups search for drug already on the market that could be repositioned or repurposed by the discovery of new indications, with the incentive to pharmaceutical industries of a lesser overall cost of drug development. Investors, venture capital funds, and major pharmaceutical industries actively support these startups; they often acquire those showing promising results in order to develop their patents opportunities or, rarely, to block a competitor in their own field of marketing.

Prescribers' education

While knowledge on brain physiology and on the exact mode of action using elaborate neurobiological and

- **Addex Therapeutics** (new drugs against addiction)
- **Almylam Pharmaceuticals** (microRNA research)
- **Arigos Biomedical** (freezing organs without the damage of crystallization)
- **Atomwise** (artificial intelligence for drug discovery)
- **Brain Resource** (depression and clinical care)
- **Compass** (psilocybin against depression)
- **GeNeuro** (human endogenous retroviruses)
- **HiQScreen** (pharmacology and electrophysiology of ligand-gated channels)
- **Ionis Pharmaceuticals** (antisense oligonucleotides)
- **Microbiota** (metagenomics microbiome and personalized medicine)
- **Minerva Neurosciences** (new drugs discovery)
- **Mitochondrial Therapeutics Consulting** (mitochondria research)
- **Naurex** (NMDA antagonists)
- **Neurochlore** (bumetanide in autism)
- **Ocean group** (senescence)
- **PharmaBiome** (fecal transplantation)
- **Pherin Pharmaceuticals** (neuroactive steroids administered nasally in anxiety disorders)
- **RaNA Therapeutics** (long non-coding RNAs)
- **SENS Research Foundation** (senescence)
- **Seres Health** (microbiome and dysbiosis)
- **VistaGen** (NMDA antagonists)

and many others…

Box. Examples of neuroscience startups.

pharmacological techniques is an inescapable starting point towards innovations in psychopharmacology, such knowledge remains of moderate to limited relevance to clinicians. Clinicians know the indications of antidepressants, anxiolytics, or antipsychotics, and they also group psychotropic drugs according to their modes of action, ie, dopaminergic antagonists, SSRIs, antihistaminergics, etc. This classification based on receptograms and enzymograms is already complex, with unsolved questions. For example, why are sulpiride or amisulpride, which selectively antagonize D2 and D3 dopamine receptors, almost as efficacious in psychoses as is the multi-target molecule clozapine, which antagonizes a great number of neurotransmitter receptors, among other modes of action. Clinicians understand that even SSRIs cannot be labelled as a monotherapy, since the specific blockade of the 5-HT transporter has innumerable downstream effects, notably on BDNF and on the respiratory chain of mitochondria.[78] Novel therapies characterized by a single target might also have wide-ranging downstream consequences. Explaining the primary targets and downstream effects of future psychotropic medications will ask for educational efforts directed towards physicians. These efforts should concern the relations between clinical signs or symptoms and changes in homeostasis that will have been proven to validly explain why a person is no more mentally healthy. Until then, psychotropic medications will continue to be chosen on the basis of clinical aspects easily amenable to evaluation, together with a limited list of laboratory tests.

Prediction and prevention

Since psychiatric symptoms have such deleterious consequences on the lives of patients, preventing their first occurrence is of paramount importance for public health. In an ideal world, biologically based treatments should aim to prevent the occurrence of psychiatric syndromes in hereditarily or environmentally predisposed persons. A society where psychiatrically asymptomatic citizens are asked or commanded to give access to their personal life and biological data to state or private institutions in order to be tested for a large array of disease predispositions and potential treatment responses is satisfying from an individual point of view, if leading to longer healthy life expectancy. One can imagine a distant future when clinicians will ask for the assay of omics to determine biological correlates of their patient's depression or other psychiatric disorders, to guide the choice of which miRNAs and other endogenous molecules to block or activate, to predict the occurrence of side effects and to follow up changes in omics as markers of treatment success. This will be a great improvement on the present syndrome-oriented choice of psychotropic medications, with their limited efficacy and with our difficulty to predict their risks. ■

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