Original article

Receptor variants and the development of centrally acting medications

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The progressive changes in research paradigms observed in the largest pharmaceutical companies and the burgeoning of biotechnology startups over the last 10 years have generated a need for outsourcing research facilities. In parallel, progress made in the fields of genomics, protein expression in recombinant systems, and electrophysiological recording methods have offered new possibilities for the development of contract research organizations (CROs). Successful partnering between pharmaceutical companies and CROs largely depends upon the competences and scientific quality on offer for the discovery of novel active molecules and targets. Thus, it is critical to review the knowledge in the field of neuroscience research, how genetic approaches are augmenting our knowledge, and how they can be applied in the translation from the identification of potential molecules up to the first clinical trials. Taking these together, it is apparent that CROs have an important role to play in the neuroscience of drug discovery.

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Introduction

Analysis of the global burden of disease reveals that neuropsychiatric disorders (documented worldwide in 21 regions, which were subsequently divided into 195 countries) represent the largest cause of disability-adjusted life-years and are ranked second after the cardiovascular diseases.[1] With a prevalence of about 1%, schizophrenia represents a major burden, both in terms of suffering and economic impact.[2]

The World Health Organization estimates that depression is affecting as much as 4.4% of the global population, that 3.6% are suffering from anxiety disorder, and that the population of Alzheimer disease-affected patients is expected to grow from about 4% to 17% between 2020 and 2025. Fighting neuropsychiatric disorders therefore represents one of the many challenges in today's medical care.

The initial discovery made serendipitously in the 1950s of centrally acting compounds such as, for example, the benzodiazepines, and the observation that these molecules are active at the γ-aminobutyric acid (GABA) ionotropic receptors ($GABA_A$[3]) soon allowed the identification of newer molecules acting on GABAergic systems. Similarly, the first antipsychotic, chlorpromazine, which marked a turning point in the history of psychiatry, was subsequently found to act at the dopamine D_2 receptors.[4,5] Additional experiments revealed, however, that this molecule probably acts at many

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different targets ranging from G-coupled proteins such as the histamine receptors[6] to ligand gated ion channels such as the neuronal nicotinic acetylcholine receptors (nAChRs,[7-9]) or the glutamatergic N-methyl-D-aspartate (NMDA)receptors.[10,11]

Progress made in the understanding of the central nervous system has led to deeper investigations of the brain's precise circuitry. Decisive advances have been achieved in the elucidation of the mechanisms at play in the hippocampus related to cognitive processes such as learning and memory.[12] However, in spite of our current understanding of brain function, translation from animal models to human pathophysiology is still insufficient to overcome the barriers seen in the development of new active compounds.

CROs have an important role to play in the neuroscience of drug discovery

Difficulties in drug discovery are illustrated in the field of nAChRs, with the latest failure in a phase III clinical trial conducted with encenicline, which had shown positive outcomes both in animal models[13] and in clinical phase II.[14] That is, this quinuclidine derivative was found to be a powerful agonist at the human α7 nAChR displaying a large safety margin and proving efficacious in different animal models.[15] Although encenicline had shown improvements in cognitive impairments in schizophrenic patients versus placebo in the early clinical phase II,[14] administration of this compound to a larger population of schizophrenic patients did not yield significant improvements over the current treatments. Combined with the lack of success of other nAChR-specific molecules this caused a halt of the research in this particular field.[16] Similar situations have been seen for clinical trials for other central nervous system (CNS) disorders, such as fragile X syndrome, where the disruption in synaptic transmission believed to underlie the condition appeared to be well characterized preclinically, but in clinical trials molecules targeting G-protein coupled receptors (GPCRs) failed to show efficacy.[17] While it would go beyond the scope of this paper to review the multiple failures of compounds acting at a specific target, it is instructive to analyze how studies were designed and conducted and the intrinsic difficulties in targeting compounds for the CNS; instead, our focus here is on preclinical work and how a more in-depth understanding of the neurobiology that can be provided by specialist contractors may lead to future success.

From gene to function

Novel approaches in drug discovery encompass knowledge obtained from large genomic databases and correlations observed between genes and diseases. A good example was the identification of variants in the nicotinic receptor gene *CHRNA5* and risk of tobacco-smoking dependence.[18] This was further reinforced in different studies which also unveiled the broad interactions between multiple factors ranging from the gene promotor, cis-regulatory elements of gene expression, or multiple gene interactions.[19-21] Another example was the association between mutations in the γ2 subunit of $GABA_A$ receptor and a spectrum of epilepsy syndromes ranging from absence epilepsy to Dravet syndrome that provided a good starting point to identify the mechanisms underlying these pathologies.[22] To examine if and how compounds specifically targeting the $GABA_A$ receptors would prove beneficial it is first necessary to revisit our knowledge concerning these receptors.

In the case of the $GABA_A$ receptors, 19 genes encoding these proteins have been identified and their chromosome location determined. A single receptor results from the assembly of five subunits around an axis of symmetry formed by the ionic pore through which chloride anions will flow upon stimulation by the agonist. Depending on the chloride gradient, activation of $GABA_A$ receptors can yield either a hyperpolarization (inhibitory effect) or depolarization (stimulatory effect).[23] Given the multiple genes and the heteromer association in a single receptor complex this yields to a large repertoire of $GABA_A$ receptors with a distribution specific to certain brain areas. Understanding the receptor distribution is mandatory for the proper evaluation of the compound effects both in the in vitro and in vivo studies. The effects of compounds will tend to be determinant in signal transmission at postsynaptic receptors whereas they will have a more general modulatory activity at extrasynaptic receptors.

Following the determination of a suitable drug target, the design of the strategic procedure for the primary test can be determined. While expression of the receptors in a reconstituted system represents a suitable choice, details regarding the protein origin and sequence must be considered. Special

attention must be paid to the gene species as well as possible alternate splicing and variants. For example, it is known that two alternate splicing variants are expressed for the $GABA_A$ γ2 subunit and that physiological and pharmacological differences are observed between these two forms. In addition, the role of associated proteins and their effect should not be neglected.[24] Furthermore, since the functional outcome of $GABA_A$ receptor activation will depend upon the chloride gradient, this parameter must be kept in mind and studies should be conducted using the appropriate experimental paradigm.[23]

Recombinant receptor experiments conducted in cell lines or, for example, Xenopus oocytes, represent one of the possible first steps for testing compounds. While mammalian cells offer several advantages, different factors might also influence the outcome of the studies. First, the expression background of the cell must be appropriately verified, to confirm that they are not expressing the gene of interest by themselves, and whether there is an influence of related associated genes.[25,26] To appropriately mimic the heterozygosity of the human diploid gene conditions, expression of the mutated and normal gene must be conducted.[27,28]

Considering the multiple genes encoding for a given protein, for example the $GABA_A$ receptors, a typical drug discovery scheme must include suitable receptor combinations for screening and evaluation against other receptors or proteins that could cause side effects. In addition, gene splicings or variants must be evaluated, as must the appropriateness of the animal models that will be used in the validation and safety testing.

The selectivity of small molecules displaying agonistic or antagonistic properties being developed is often discussed. Development of positive or negative allosteric modulators that can enhance or reduce the intrinsic activity of the receptors is receiving special attention. Whereas benzodiazepines represent a classical example of positive allosteric modulators, other molecules such as neurosteroids are showing interesting alternatives.[29,30] The recent success of compounds developed by Sage Therapeutics in the case of certain forms of epilepsies or postpartum depression illustrates the advantages of positive allosteric modulators. Negative allosteric modulators targeted at specific receptor subtypes might also prove beneficial as shown in a recent study conducted at α5-containing receptors.[31]

Understanding the neuronal networks which contain the target receptor is the next level of complexity that must be addressed in the animal model. The receptor composition must be examined as precisely as possible. For example, recent work conducted in monkeys revealed that benzodiazepines caused a mild form of sedation mediated by α2/3 containing receptors versus a moderate/deep sedation caused by α1-containing receptors.[32] Indications, such as the recently approved brexanolone compound which is efficacious in postpartum depression and which acts at the extrasynaptic receptors such as those containing the α4β3δ subunits, require a model allowing differentiation from the compound targeting the α1β2γ2 receptors. Information about the localization of receptors in the different brain areas and furthermore at the cellular and subcellular level is determinant in understanding the receptor function in the native network. There too, designing a restricted number of experiments with the most appropriate model is expected to be greatly beneficial in the development pathway.

How genetics is modifying our thoughts about pharmacology

Cloning and sequencing of a number of genes encoding for determinant functions such as voltage or ligand-gated ion channels brought spectacular progress in biological sciences with, namely, the possibility to selectively express and probe the properties of a defined set of proteins. The development of sequencing technology led to the release of the first human genome draft sequence in 2001. Aimed at determining the sequence of human genes, this initial project took about 15 years to be completed. Enormous progress made both in the sequencing technology and computer sciences are now allowing the sequence analysis of an entire human genome in a matter of days. Major projects initiated by the 1000 Genomes Consortium and the Exome Aggregation Consortium will enable continued discovery of gene variants in relation to disease phenotypes. Moreover, in parallel, other projects have developed, including the Exome Sequencing Project or the Exome Aggregation Consortium.

Generation of databases attempting to link a phenotype to a given set of mutations is, however, hampered by the number of possible mutations associated within a single gene and the understanding of the corresponding clinical phenotype. This is well exemplified in the case of geneti-

cally transmissible epilepsies such as autosomal dominant frontal lobe epilepsy (ADNFLE). Identified more than 20 years ago with the discovery of a first mutation in *CHRNA4*, the gene that encodes for the neuronal nicotinic acetylcholine receptor (nAChR), the clinical phenotype associated with this rare disease was thought to be sufficiently specific to be clinically homogenous.[33] However, knowing that the major brain nAChRs are hetero-pentamers composed of α4 and β2 subunits, it was immediately obvious that mutations in *CHRNB2* that encodes for the β2 subunit might also be at the origin of the ADNFLE phenotype. This hypothesis was subsequently confirmed by the characterization of a newly identified mutation in *CHNRB2*.[34,35] Since these initial descriptions, several mutations have been identified in *CHRNA4* and *CHNRB2*, confirming the association between the ADNFLE clinical phenotype and genotype. Importantly, patients suffering from ADNFLE are heterozygous for *CHRNA4* or *CHNRB2* indicating that the mutation must have a dominant effect. In vitro experiments conducted in different expression systems including recordings in *Xenopus* oocytes or transfected mammalian cells revealed that the mutations are causing multiple effects with differences associated with the specificity of the modified amino acids but that overall, they all caused an increase in the sensitivity to the natural ligand (acetylcholine).[28,36]

Introduction of these mutations in mouse or rat models offered additional information about the role of nAChRs in brain function and the association with a genetically transmissible epilepsy (reviewed in refs 37,38). When introduced in mice, *CHNRA4* mutation α4-S248F replicated some forms of epilepsies, but only in a mouse with a particular background.[37] This indicates that mutation of *CHRNA4* is probably insufficient to cause epilepsies but that additional factors are necessary, and this could explain the low penetrance of the clinical phenotype and detection of healthy obligatory carriers.

To date, carbamazepine remains the best treatment for ADNFLE but was shown to be efficacious only for certain mutations and, moreover, pharmaco-resistance observed in the clinic was documented underlying the role of brain plasticity in long-term treatment in humans. Another important lesson learned from the ADNFLE studies concerns the complexity of the clinical phenotype. While nocturnal epileptic episodes are unambiguous manifestations of the disease, additional factors must also be considered. Namely, while mutations in the *CHRNA4* gene are affecting only α4- and β2-containing receptors, alterations in the coding sequence of the accessory β2 subunit are expected to affect additional receptor subtypes such as α2β2 which is highly expressed in the frontal cortex. It is therefore not surprising to observe that *CHRNB2* mutations are probably associated with cognitive impairment.[29] Further development in the genetic analysis and correlation with clinical phenotype pointed to mutations in *CHRNA2*[38] and, surprisingly, in the sodium, activated potassium channel (Slack) *KCNT1*.[39]

As progress is made in the identification of genetic variants and the possible link with protein function, characterization of the possible alteration of the protein function and understanding of the overall cellular and network circuit physiology and pharmacology becomes a key pointer towards the development of novel molecules.

Common gene variants and complex disorders

Based on genetic approaches, one tendency is to focus attention on a single gene and conclude that presence or absence of a given variant will determine the overall phenotype. Whereas such an association exists in strongly correlated genetic variants, these remain rare cases illustrating the specificity of a given gene. However, the observation of an obligatory carrier in which the mutation is known to be present but not expressed clinically illustrates that in many cases the penetrance of the disease is incomplete. In other words, this suggests that presence of a given mutation can be the cause of a given disease but that it can be compensated for by other factors preventing the apparition of the clinical phenotype.

In a most recent study reported for a large cohort of 6987 children affected by global developmental delay and autism that were characterized both clinically and genetically, it was shown that 7.7% of the variance in risk was attributable to common genetic variations.[40] Moreover, this was further confirmed in an independent sample of 728 families from the same cohort in which a child and parents were analyzed; this revealed that the neurodevelopmental disorder is transmitted from the parents. In addition, the common-variant signal was significantly correlated with lower educational achievement and risk of schizophrenia.

These findings were further analyzed in the light of previous studies in which correlation between autism and other parameters such as height, birth weight, or cranial volume were examined and it was concluded that patients with monogenic disorders are affected by variants found in the general population. Altogether, these studies highlight the need for broader genetic screening and analysis of multiple variants that will account for a given clinical phenotype.

Analysis of the haplotype variations versus their pair diplotypes conducted in European population samples unveiled an immense diversity. On average, this corresponded to about 235 forms per gene with less than 15% having an autosomal dominant predominance.[41] Examples of genetic variants and the relevance of their analysis in drug treatment was already documented for cardiovascular treatment with the anticoagulant warfarin, and its resistance in specific patients, or the reduction-of-function variants in *CYP2C*[19], the enzyme responsible for the bioactivation of antiplatelet medication (clopidogrel).[42] The importance of gene variation in drug-related genes was further highlighted in another study suggesting the need for a closer examination both in clinical trials and day-to-day practice.[43]

The most recent pharmacogenomic analysis of GPCR drug targets conducted on data analyses from about 70 000 individuals revealed that genetic variations of the μ-opiod and cholecystokinin-A receptors could lead to altered or adverse drug response. Whereas the combination of multiple genetic variants might be complex to recapitulate in expression systems or animal models, it can be foreseen that analysis of the different key elements will lead to a better comprehension of the disease.

Translation from recombinant receptors to a functional network

Understanding the neuronal networks in which the target receptor is involved is the next level of complexity that must be addressed. The precise receptor composition must be examined as illustrated by the recent work conducted in animal experiments, which revealed a mild form of sedation mediated by the $GABA_A$ receptors containing α2/3 $GABA_A$ subunits versus a moderate/deep sedation caused by α1 subunit-containing receptors.[32] Similarly, symptoms thought to be associated with extrasynaptic receptors such as those containing the α4β3δ subunits require a model that differs from the compound targeting the α1β2γ2 subunit containing receptors. Information about the localization of the receptors in function of the brain areas and furthermore at the cellular and subcellular level is determinant to address the receptor role in the native network. There too, designing restricted numbers of experiments with the most appropriate model is expected to be greatly beneficial in the development pathway.

The outcome of modulating these molecular targets will be changes to the electrical signaling which underpins brain activity. Using electrophysiological approaches, it is possible to assess these changes from activity at the level of a single protein, through to measurements of synchronized activity between brain regions. In particular, it may be that developments in our ability to measure activity at the network level will benefit future neuroscience drug discovery. In patients, this activity can be measured noninvasively with electrodes applied to the scalp to record an electroencephalogram (EEG) or by magnetoencephalography (MEG). The EEG represents ensemble activity of neurons in the cortex, which in turn are being modulated by deeper brain structures. Such recordings revealed that the brain is highly synchronized into defined rhythms, or oscillations, at frequencies ranging from a few Hz to over 100 Hz, and these different rhythms are tightly linked to specific activities. These oscillations are thought to ensure coordination of neuronal activity both within defined structures, and across different brain regions. This synchrony is vital for cognitive function and for neurophysiological processes such as synaptic plasticity, which is essential for formation of new memories.[44] The EEG, and other techniques such as functional magnetic resonance imaging, have demonstrated that in CNS disorders the synchronized activity is disrupted.[45-47]

That reproducible disruptions in electrical signaling that can be measured in patients raises the prospect of development of functional biomarkers. It was recently reported that clinical trials making use of biomarkers in patient selection[48] had higher overall success probabilities than trials without biomarkers. This data set was across a range of trials, so not limited to CNS, and the situation was less clear for trials using biomarkers as a measure of the test compound's efficacy, but it does suggest that development of biomarkers is important.

The use of measures such as the EEG in clinical trials may be particularly attractive as the neuronal oscillations

recorded in humans are also present across species, and may be reproduced in preparations such as the in vitro brain slice.[49,50] As such the measurement of neuronal oscillations may provide a route for translational models for many CNS disorders.

The in vitro brain slice preparation provides a valuable preparation for the study of neuronal physiology: the synaptic connections that occur in vivo are maintained in the slice, neurones can easily be identified and accessed for recording, the external environment can easily be manipulated, and drugs can be applied and removed quickly.[51] This approach has been invaluable in research into neurophysiological processes, such as learning and memory, and the cellular activity that generates neuronal oscillations.

Brain slices prepared containing a region of interest, such as the hippocampus or prefrontal cortex, may be used to demonstrate that target engagement from a primary screen translates to the native target. This can be to study the effects on synaptic transmission, through to studying more complex network activity as illustrated in *Figure 1*. Here, gamma frequency oscillations are recorded in the CA3 region of the hippocampus. In addition to the potential translatability, these neuronal oscillations are particularly attractive for pharmacological studies as they require a fully functioning neuronal network, with the inhibitory systems intact, and the signal is stable for hours allowing application of compounds over a concentration range on the same slice, or application of different compounds.[52,53] In *Figure 1* the actions on the oscillations of amphetamine and guanfacine are illustrated. These are drugs that are known to modulate cognitive performance in animals and humans, albeit via different mechanisms, and have been used to treat conditions such as attention deficit-hyperactivity disorder (ADHD). Amphetamine is thought to exert its effects (at least in part) via dopaminergic mechanisms, whereas guanfacine is an α2A- adrenoceptor agonist. The data in *Figure 1* illustrate that both of these compounds modulate hippocampal circuit function, but in differing ways. Fully investigating the effects of putative medicines on neuronal function at the network level may be valuable in providing a functional signature to particular molecular targets.

Much of the preclinical electrophysiology, both in vitro and in vivo, has been conducted with single electrodes; however, in recent years technologies have been developed that allow recording from multiple sites, both in vitro, and in vivo.[54] These approaches generate massive data sets that not only allow study of the network activity, but simultaneously sample the activity of the individual neurones underlying this activity. Through application of spike-sorting algorithms, it is possible to separate the recording data into streams from specific cell types and demonstrate how the activity of individual cells correlates with the overall network activity. In combination with other technologies, such as optogenetics.[55-57] These approaches will be of great value to neuroscience drug discovery. Application of these approaches will allow in-depth knowledge of the actions of different compounds on neuronal function, of relevance both for how a compound may control the disease, and also to aid understanding of side effects.

A further development that may aid generation of more predictive data is the recording of electrical signaling from human neuronal tissue[58]: it is possible to prepare brain slices from tissue removed from patients undergoing elective neurosurgery. As with tissue from rodents, the human brain slices allow study of compound effects at the cellular and network level. Of course, these tissues come from patients who will be medicated and have an ongoing medical condition, so use of the tissue may not be applicable to all CNS disorders, but for conditions such as epilepsy it is possible to reproduce the network activity that underlies the patient's conditions in vitro. These approaches will facilitate a greater understanding of the condition and may be valuable in the evaluation of new treatments.

Addition of D-amphetamine to the bathing medium (10μM) resulted in changes in the power spectrum recorded in a rodent brain slice, such that there was an overall increase in power and the peak in the spectrum shifted from the gamma range to the 10-20 Hz ("beta") range. This was associated with the occurrence of high-amplitude sharp wave activity. Addition of guanfacine (10μM) to the bathing medium had little effect on the peak frequency in the power spectrum, but decreased the gamma-frequency power to 66% of control.

Conclusions

Rationalization in drug development has become a mantra in large pharmaceutical companies, and it is often thought that it is more efficient to collaborate with (and even in given cases to acquire) smaller biotechnology companies

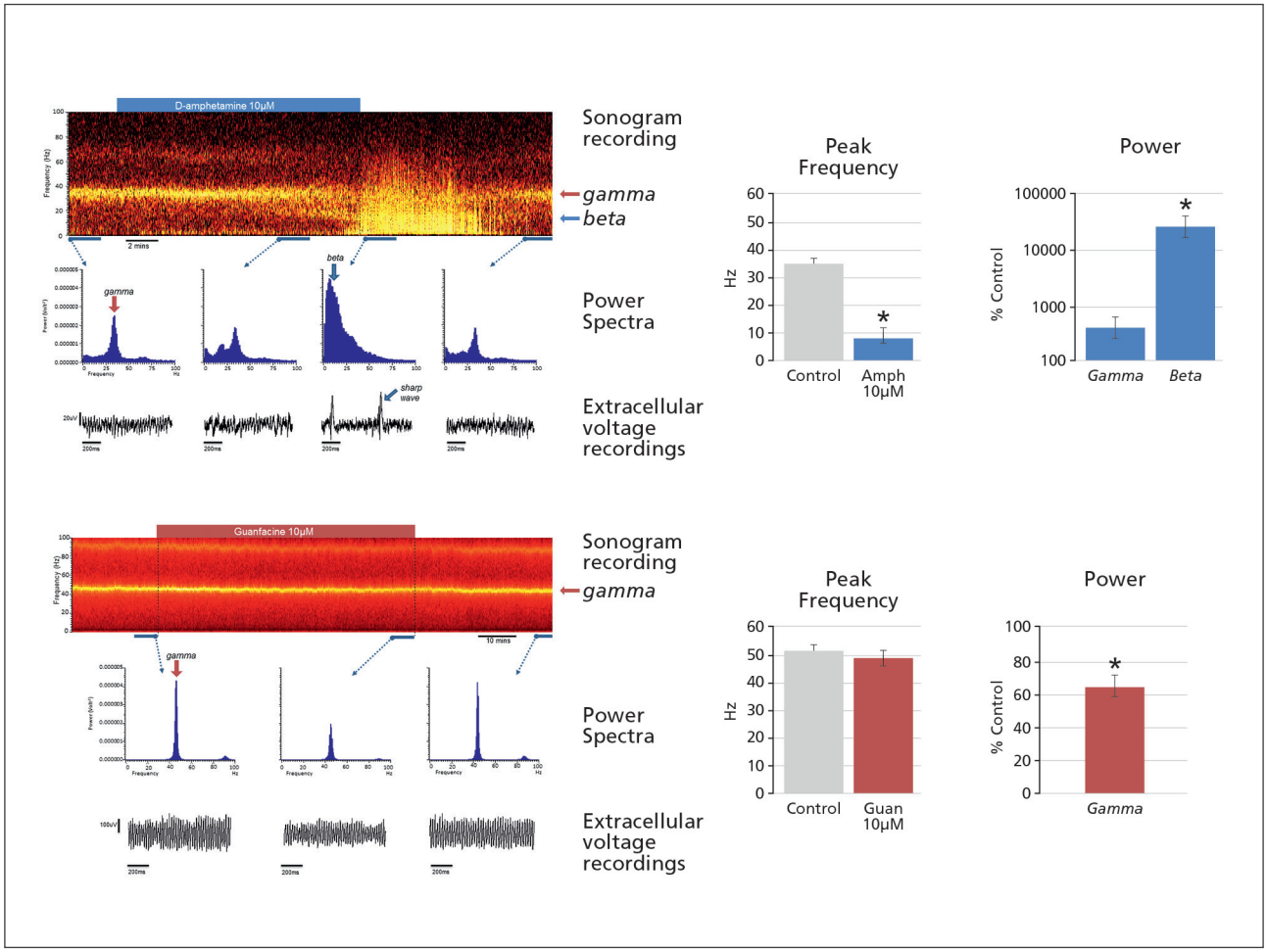

Figure 1. **Modulation of gamma oscillations by drugs used in ADHD.**

presenting an adequate potential rather than establishing in-house a complete development process. Cuts in internal research work forces have been observed in many companies while small biotechnology companies are burgeoning. The lack in internal resources of small biotechnology companies and reduced forces in larger groups has favored the development of specialized CROs. To be successful, CROs need to produce high-quality science while maintaining technologies and internal knowledge at the forefront of the scientific research.

Progress made in genetics combined with deeper analysis of brain circuits has shown the need for highly specialized scientists that can contribute in appropriate groups to exploring and understanding the effects of compounds in the brain at the highest level. Analysis of drug effects range from the molecular to the neural circuit level, including the potential influence of genetic variants. In view of these multiple requirements in drug discovery, and the need for highly specialized skilled scientists and specific equipment at each step of drug development, the collaboration between pharmaceutical companies and CROs will probably grow stronger. This can make a significant contribution to minimizing time and cost of the development of new therapies. ■

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References

1. Global analysis of neurological disease: burden and benefit. *Lancet Neurol.* 2017. https://doi.org/10.1016/S1474-4422(17)30338-1.
2. Dykxhoorn J, Hollander AC, Lewis G, Magnusson C, Dalman C, Kirkbride JB. Risk of schizophrenia, schizoaffective, and bipolar disorders by migrant status, region of origin, and age-at-migration: A national cohort study of 1.8 million people. *Psychol Med.* 2018;5:1-10. doi: 10.1017/S0033291718003227. [Epub ahead of print].
3. Möhler H. Endogenous benzodiazepine site peptide ligands operating bidirectionally in vivo in neurogenesis and thalamic oscillations. *Neurochem Res.* 2014;39(6):1032-1036.
4. Ban TA. Fifty years of chlorpromazine: A historical perspective. *Neuropsychiatr Dis Treat.* 2007;3(4):495-500.
5. Seeman P. Dopamine D2 receptors as treatment targets in schizophrenia. *Clin Schizophr Relat Psychoses.* 2010;4(1):56-73.
6. Mailman RB, Murthy V. Third generation antipsychotic drugs: Partial agonism or receptor functional selectivity? *Curr Pharm Des.* 2010;16(5):488-501.
7. Ashoor A, Lorke D, Nurulain SM, et al. Effects of phenothiazine-class antipsychotics on the function of α7-nicotinic acetylcholine receptors. *Eur J Pharmacol.* 2011;673(1-3):25-32.
8. Giraudat J, Dennis M, Heidmann T, Chang JY, Changeux JP. Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: Serine-262 of the delta subunit is labeled by [3H]chlorpromazine. *Proc Natl Acad Sci U S A.* 1986;83(8):2719-2723.
9. Grinevich VP, Papke RL, Lippiello PM, Bencherif M. Atypical antipsychotics as noncompetitive inhibitors of alpha4beta2 and alpha7 neuronal nicotinic receptors. *Neuropharmacology.* 2009;57(2):183-191.
10. Barygin OI, Nagaeva EI, Tikhonov DB, Belinskaya DA, Vanchakova NP, Shestakova NN. Inhibition of the NMDA and AMPA receptor channels by antidepressants and antipsychotics. *Brain Res.* 2017;1660:58-66.
11. Sacchi S, Novellis V, Paolone G, et al. Olanzapine, but not clozapine, increases glutamate release in the prefrontal cortex of freely moving mice by inhibiting d-aspartate oxidase activity. *Sci Rep.* 2017;7:46288.
12. Moser EI, Moser MB, McNaughton BL. Spatial representation in the hippocampal formation: A history. *Nat Neurosci.* 2017;20(11):1448-1464.
13. Hayward A, Adamson L, Neill JC. Partial agonism at the α7 nicotinic acetylcholine receptor improves attention, impulsive action and vigilance in low attentive rats. *Eur Neuropsychopharmacol.* 2017;4:325-335.
14. Keefe RS, Meltzer HA, Dgetluck N, et al. Randomized, double-blind, placebo-controlled study of encenicline, an alpha-7 nicotinic acetylcholine receptor agonist as a treatment for cognitive impairment in schizophrenia. *Neuropsychopharmacology.* 2015;40(13):3053-3060.
15. Stoiljkovic M, Leventhal L, Chen A, et al. Concentration-response relationship of the α7 nicotinic acetylcholine receptor agonist FRM-17874 across multiple in vitro and in vivo assays. *Biochem Pharmacol.* 2015;97(4):579-589.
16. Rollema H, Bertrand, Hurst S. Nicotinic agonists and antagonists. In: *Encyclopedia of Psychopharmacology.* Berlin, Heidelberg, Germany: Springer; 2014.
17. Berry-Kravis EM, Lindemann L, Jønch AE, et al. Drug development for neurodevelopmental disorders: Lessons learned from fragile X syndrome. *Nat Rev Drug Discov.* 2018;17(4):280-299.
18. Bierut LJ, Stitzel JA, Wang JC, et al. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry.* 2008;165(9):1163-1171.
19. Haller G, Kapoor M, Budde J, et al. Rare missense variants in CHRNB3 and CHRNA3 are associated with risk of alcohol and cocaine dependence. *Hum Mol Genet.* 2013;23(3):810-819.
20. Smith RM, Alachkar H, Papp AC, et al. Nicotinic alpha5 receptor subunit mrna expression is associated with distant 5' upstream polymorphisms. *Eur J Hum Genet.* 2010;19(1):76-83.
21. Bierut LJ. Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. *Trends Pharmacol Sci.* 2010;31(1):46-51.
22. Kang JQ, Macdonald RL. Molecular pathogenic basis for GABRG2 mutations associated with a spectrum of epilepsy syndromes, from generalized absence epilepsy to dravet syndrome. *JAMA Neurol.* 2016;73(8):1009-1016.
23. Knoflach F, Hernandez M, Bertrand D. GABAA receptor-mediated neurotransmission: Not so simple after all. *Biochem Pharmacol.* 2016; 115:10-17.
24. Maher MP, Matta JA, Gu S, Seierstad M, Bredt DS. Getting a handle on neuropharmacology by targeting receptor-associated proteins. *Neuron.* 2017;96(5):989-1001.
25. Matta JA, Gu S, Davini WB, Lord B, Siuda ER, Harrington AW, Bredt DS. NACHO mediates nicotinic acetylcholine receptor function throughout the brain. *Cell Rep.* 2017;19(4):688-696.
26. Gu S, Matta JA, Lord B, Harrington AW, Sutton SW, Davini WB, Bredt DS. Brain α7 nicotinic acetylcholine receptor assembly requires NACHO. *Neuron.* 2016;89(5):948-955.
27. Bertrand D, Terry AV. The wonderland of neuronal nicotinic acetylcholine receptors. *Biochem Pharmacol.* 2017;151:214-225.
28. Steinlein OK, Hoda JC, Bertrand S, Bertrand D. Mutations in familial nocturnal frontal lobe epilepsy might be associated with distinct neurological phenotypes. *Seizure.* 2012;21(2): 118-123.
29. Carver CM, Reddy DS. Neurosteroid structure-activity relationships for functional activation of extrasynaptic δgabaa receptors. *J Pharmacol Exp Ther.* 2016;357(1):188-204.
30. Seljeset S, Laverty D, Smart TG. Inhibitory neurosteroids and the GABAA receptor. *Adv Pharmacol.* 2015;72:165-187.
31. Kawaharada S, Nakanishi M, Nakanishi N, et al. ONO-8590580, a novel GABAAα5 negative allosteric modulator enhances long-term potentiation and improves cognitive deficits in preclinical models. *J Pharmacol Exp Ther.* 2018;366(1):58-65.
32. Duke AN, Meng Z, Platt DM, et al. Evidence that sedative effects of benzodiazepines involve unexpected gabaareceptor subtypes: Quantitative observation studies in rhesus monkeys. *J Pharmacol Exp Ther.* 2018;366(1):145-157.
33. Steinlein OK, Mulley JC, Propping P, et al. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet.* 1995;11(2):201-203.
34. De Fusco M, Becchetti A, Patrignani A, et al. The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat Genet.* 2000;26(3):275-276.
35. Phillips HA, Favre I, Kirkpatrick M, et al. CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. *Am J Hum Genet.* 2001;68(1):225-231.
36. Eggert M, Winterer G, Wanischeck M, Hoda JC, Bertrand D, Steinlein O. The nicotinic acetylcholine receptor alpha 4 subunit contains a functionally relevant SNP haplotype. *BMC Genet.* 2015;16:46.
37. Boillot M, Baulac S. Genetic models of focal epilepsies. *J Neurosci Methods.* 2016;260:132-143.
38. Becchetti A, Aracri P, Meneghini S, Brusco S, Amadeo A. The role of nicotinic acetylcholine receptors in autosomal dominant nocturnal frontal lobe epilepsy. *Front Physiol.* 2015;6:22.
39. Kim GE, Kronengold J, Barcia G, et al. Human slack potassium channel mutations increase positive cooperativity between individual channels. *Cell Rep.* 2014;9(5):1661-1672.
40. Niemi MEK, Martin HC, Rice DL, et al. Common genetic variants contribute to risk of rare severe neurodevelopmental disorders. *Nature.* 2018;562(7726):268-271.
41. Hoehe MR, Church GM, Lehrach H, et al. Multiple haplotype-resolved genomes reveal population patterns of gene and protein diplotypes. *Nat Commun.* 2014;5:5569.
42. Roden DM, Wilke RA, Kroemer HK, Stein CM. Pharmacogenomics: The genetics of variable drug responses. *Circulation.* 2011;123(15): 1661-1670.
43. Schärfe CPI, Tremmel R, Schwab M, Kohlbacher O, Marks DS. Genetic variation in human drug-related genes. *Genome Med.* 2017;9(1):117.
44. Latchoumane CV, Ngo HV, Born J, Shin HS.

Thalamic spindles promote memory formation during sleep through triple phase-locking of cortical, thalamic, and hippocampal rhythms. *Neuron.* 2017;95(2):424-435.
45. López-Sanz D, Bruña R, Garcés P, et al. Functional connectivity disruption in subjective cognitive decline and mild cognitive impairment: A common pattern of alterations. *Front Aging Neurosci.* 2017;9:109.
46. Nimmrich V, Draguhn A, Axmacher N. Neuronal network oscillations in neurodegenerative diseases. *Neuromolecular Med.* 2015;17(3):270-284.
47. Grent-'t-Jong T, Gross J, Goense J, et al. Resting-state gamma-band power alterations in schizophrenia reveal E/i-balance abnormalities across illness-stages. *Elife.* 2018;7. doi:10.7554/eLife.37799.
48. Wong CH, Siah KW, Lo AW. Estimation of clinical trial success rates and related parameters. *Biostatistics.* 2018;20(2):273-286.
49. Huberfeld G, Blauwblomme T, Miles R. Hippocampus and epilepsy: Findings from human tissues. *Rev Neurol (Paris).* 2015;171(3):236-251.
50. Bazelot M, Teleńczuk MT, Miles R. Single CA3 pyramidal cells trigger sharp waves in vitro by exciting interneurones. *J Physiol.* 2016;594(10):2565-2577.
51. Crepel F, Dhanjal SS, Garthwaite J. Morphological and electrophysiological characteristics of rat cerebellar slices maintained in vitro. *J Physiol.* 1981;316:127-138.
52. Sathyasaikumar KV, Tararina M, Wu HQ, et al. Xanthurenic acid formation from 3-hydroxykynurenine in the mammalian brain: Neurochemical characterization and physiological effects. *Neuroscience.* 2017;367:85-97.
53. Sathyasaikumar KV, Stachowski EK, Wonodi I, et al. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophr Bull.* 2011;37(6):1147-1156.
54. Buzsáki G, Tingley D. Space and time: The hippocampus as a sequence generator. *Trends Cogn Sci.* 2018;22(10):853-869.
55. Magnin E, Francavilla R, Amalyan S, et al. Input-specific synaptic location and function of the alpha5 GABAA receptor subunit in the mouse CA1 hippocampal neurons. *J Neurosci.* 2018;39(5):788-801.
56. Mendrela AE, Kim K, English D, et al. A high-resolution opto-electrophysiology system with a miniature integrated headstage. *IEEE Trans Biomed Circuits Syst.* 2018;16. doi:10.1109/TBCAS.2018.2852267.
57. Anderson HE, Weir RFF. On the development of optical peripheral nerve interfaces. *Neural Regen Res.* 2019;14(3):425-436.
58. Connor B, Firmin E, McCaughey-Chapman A, Monk R, et al. Conversion of adult human fibroblasts into neural precursor cells using chemically modified mrna. *Heliyon.* 2018;4(11):e00918.