Clinical research

New directions for drug discovery in psychiatric disease

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Although many new potential drug targets have been discovered subsequent to the cloning of the human genome and the discovery of most of the relevant receptors, the role of these receptors in psychiatric disease is still not clear. We argue that research into the disease process leading to new animal models that can be transposed to man is critical to drug discovery, and present an example of an animal model for schizophrenia using electroencephalography.

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The therapies for psychiatric disease have not been revolutionized in the last 10 years and no major new anxiolytics or antidepressants have appeared (although some interesting compounds are in development). The second generation of antipsychotics certainly allows better therapy, particularly in terms of cognitive aspects, but trade off improved tolerance, in terms of extrapyramidal side effects, for metabolic side effects (particularly weight gain) and, in some instances, cardiovascular issues.

Although there have never been as many recognized potential targets for drug therapy in psychiatric disease as at present, there has been no major progress in terms of marketed agents revolutionizing therapy. The partial cloning of the human genome now allows us to define the total number of receptors in the human genome (for example, about 48 nuclear receptors, about 750 receptors coupled to G proteins). This is a definitive statement defining the future, and perhaps eventually the limits, of drug discovery. One of us (M. Spedding) is chairman of the Nomenclature Committee for the International Union of Pharmacology (NC-IUPHAR), which has the mission of classifying these receptors. The sequences of the receptors coupled to G proteins (GPCRs) have now been defined, and the olfactory receptors and pseudogenes separated, leaving several hundred known or orphan receptors that may be drug targets. However, screening for agonists and antagonists, and then proceeding to clinical trials to test whether a certain hypothesis works, is one of the most expensive experiments known to man! Furthermore, the main reason for the failure of new drugs when they get into clinical trials is not pharmacokinetics or toxic side effects, but lack of efficacy *(Figure 1)*.

This lack of efficacy means that either the original hypothesis of why the drug should work in man was wrong or—and this is more likely—that the tests per-

formed in animals where the drug was active did not measure the same parameters as the tests in phase 1 or 2 clinical trials (which, in turn, may not reflect the disease situation). As there are now hundreds of potential targets from the human genome, and most compounds going into man appear not to be effective, what can be done? The response by much of the pharmaceutical industry is to push up screening of new targets by high-throughput testing of chemical libraries on new receptors (or other potential targets), eliminating targets that do not yield active results in disease models (frequently based on transgenic animals), and then taking promising compounds into the clinic for abbreviated "proof of concept" testing in man. However, it is not always possible to have proof of concept testing that reflects the situation in diverse patient populations.

An alternative approach is to benefit from the breakthroughs made in basic research in brain function over the last few years to study the pathology in man, and then construct new animal models which better mimic the disease state. It may then be possible to have animal testing and early clinical testing performed in very similar conditions, which will eliminate the risk of not testing for the same effect.
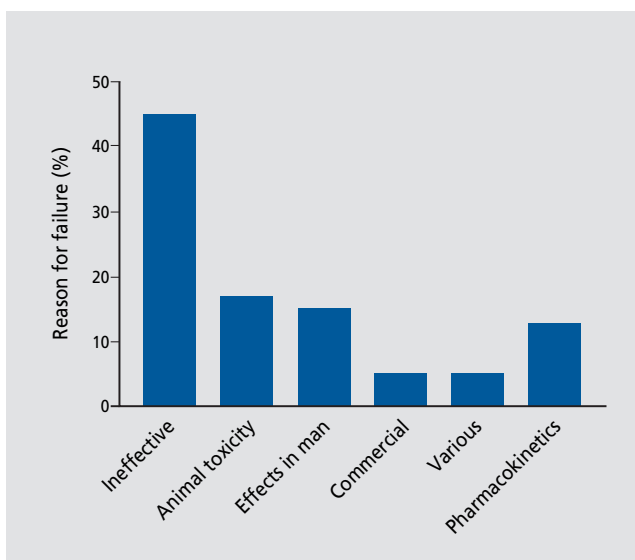

Figure 1. Reasons for stopping clinical development of 121 compounds from 7 British companies (B. Cox, personal communication).

Drug screening in vivo

If animal models are new, and reflect the disease better, then they may allow different compounds to be selected if final compound selection is performed in the disease model. Thus very different compounds will be chosen for drug development. The disease state may change the kinetics of receptor interactions or the multiple states of a receptor, meaning that screening in normal conditions may not be appropriate. From thermodynamics, changing affinity by 100- to 1000-fold (ie, a enormous change in structure–activity) may reflect a change in only one hydrogen bond between ligand and receptor, which is very difficult to predict on a molecular level. Thus, it is likely that conformational modifications in a disease state—if the target is really a causative agent in the disease process—would involve changes of such a magnitude. Indeed, switching the conformation of a receptor between *agonist* or *antagonist* states can change the affinity by more than a 1000-fold, entirely changing the structure–activity, because of changes in different binding pockets.[1-3] Thus, differences between receptor "states" can be more important than differences between types of receptor.[2]

It is thus clear then that screening in appropriate disease models, rather than on putative receptor targets under normal conditions, would lead to drugs better targeted toward the pathological events, and thus toward better treatment of the patient.

It is also important to ensure that the same measures can be made in animals as in clinical testing. This may be easily accessible in the cardiovascular system, but studies in the central nervous system (CNS) may require more indirect comparisons. However, some end points are amenable. We have studied electroencephalographic (EEG) techniques and extensively characterized means of transferring preclinical effects in conscious animals toward the same effects in man, as clinical EEG is a powerful means of defining the effects of drugs.

Which models may be used for schizophrenia?

Abnormalities in the neural circuits in the prefrontal cortex, which are involved in working memory, are the basis of the model of schizophrenia proposed by Goldman-Rakic,[4,5] and have been seen in imaging studies.[6] A robust reduction (>3.5 million) in the number of thalamic neurones innervating frontal regions has been

Clinical research

reported in subjects with schizophrenia.[7] Thus, the prefrontal cortex is a key area and the hippocampus is also important because the neurodevelopment model of schizophrenia indicates changes in its development.[8,9] Phencyclidine (PCP) is an *N*-methyl-D-aspartate (NMDA) antagonist that induces hallucinations in man. PCP and the structurally related molecule ketamine have been shown to exacerbate existing psychotic disorders in schizophrenics and to reactivate symptoms in remission.[10-17] Use of PCP in animals and of ketamine in man has been claimed to be the most valid model for schizophrenia today.[18]

Drug-induced changes in EEG

The effects of PCP were not well characterized in the EEG of animals, and so we set up an animal model of the EEG effects of PCP. We had previously characterized more than 50 drug-induced changes in a model of EEG of prefrontal cortex, using the somatosensori-motor region as a control for effects on motor functions.[19,20] Chronically implanted EEG leads in the prefrontal cortex of conscious rats are used to obtain "fingerprints" of drug profiles over the range of 1 to 30 Hz[19,20] by subtracting the control EEG from the EEG spectrum in the
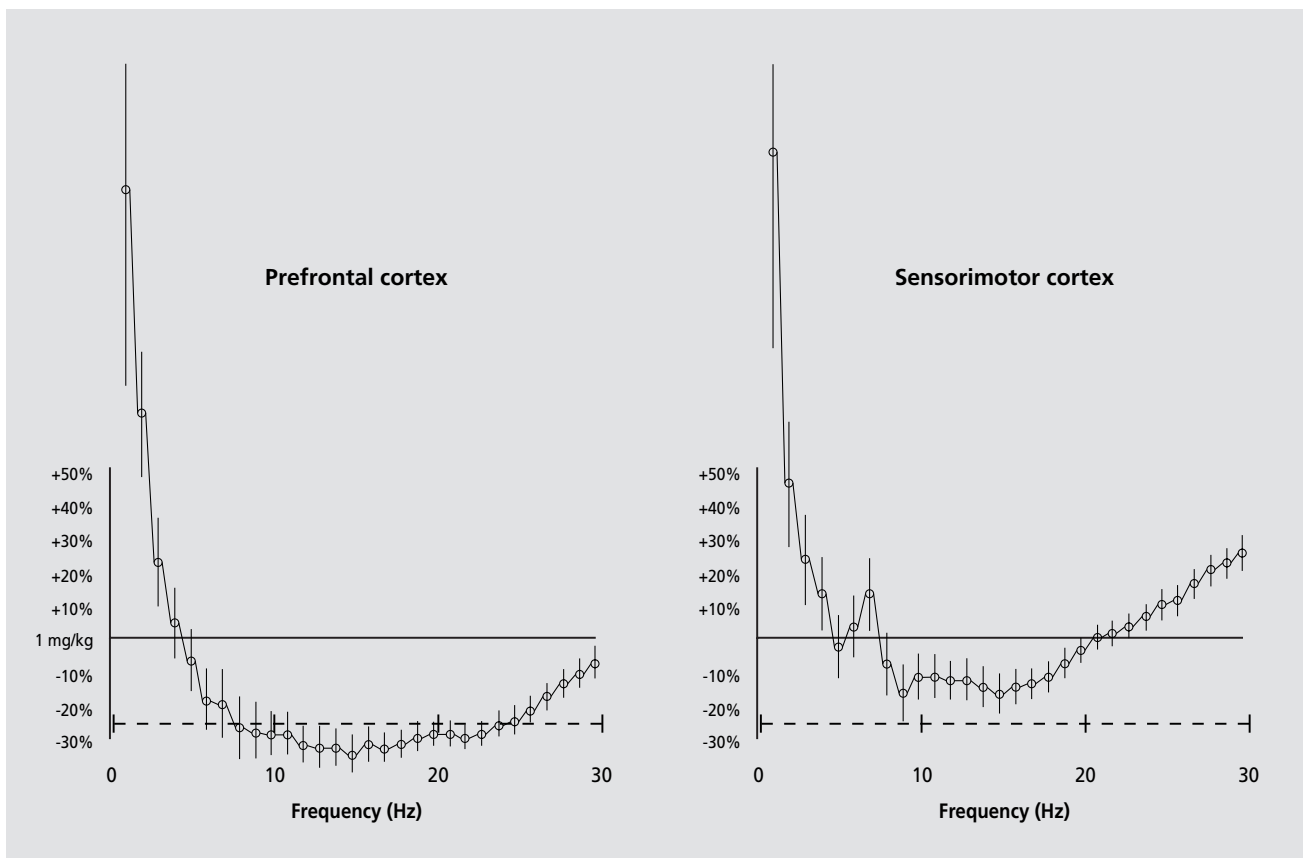

Figure 2. Dose–response effects of subcutaneous phencyclidine (PCP) 1 mg.kg⁻¹ on electroencephalographic (EEG) spectral power in the prefrontal cortex and sensorimotor cortex in conscious rats. The abscissa represents the EEG spectral component between 1 and 30 Hz. The horizontal line at zero indicates no change. The ordinate indicates the percentage change in the EEG power spectrum produced by drug administration, as a percentage of the EEG spectrum obtained with vehicle administration 24 h earlier. The increases in EEG power may be taken as a synchronization of EEG at the particular frequency and a decrease in power as a desynchronization. Because of local factors (electrode placement) synchronization of the EEG change yields larger percentage changes than desynchronization. Vertical bars for each point show 95% confidence intervals (n=6). Note the massive synchronization at low frequencies and the desynchronization at higher frequencies.

Reproduced from reference 21: Sebban C, Tesolin-Decros S, Ciprian-Ollivier J, Perret L, Spedding M. Effects of phencyclidine (PCP) and MK 801 on the EEGq in the prefrontal cortex of the conscious rats; antagonism by clozapine, and antagonists of AMPA-, alpha(1)- and 5-HT(2A)-receptors. *Br J Pharmacol*. 2002;135:65-78. Copyright © 2002, Nature Publishing Group.

presence of the test drug 24 hours later. We reported that activation of noradrenergic and dopaminergic receptors causes a decrease in EEG power (desynchronization), whereas inhibition of these two systems increases EEG power (synchronization).[19,20] Decreases in EEG power in this model are induced by agents which increase vigilance, such as modafinil.[19,20] Interestingly, nearly all the antipsychotic agents that we tested (clozapine, haloperidol, and risperidone) increased theta/alpha1 power (peaks between 7 and 8 Hz),[19-21] indicating an impact on cortical processes in the prefrontal cortex, as theta rhythm is involved in memory processes and neuronal plasticity. We found that antipsychotic agents (haloperidone, chlorpromazine, risperidone, clozapine, and olanzepine) increased theta frequencies (about 8 Hz) in the rat prefrontal cortex. Theta rhythm is 4 to 7 Hz in man and 3 to 12 in rats (usually 7±2 Hz), and is increased by movement, implying a role in motor function: the

faster a rat runs, the faster the theta rhythm. Theta appears during rapid eye movement (REM) sleep.

Theta rhythm is used to create a unit of cell assemblies, across the brain, in phase, working on a common problem. Thus, theta also has the capacity to separate assemblies that are working on different problems. Thus, the fact that antipsychotic agents increase the probability of theta rhythm in the prefrontal cortex indicates direct effects in cognitive processes.

In contrast, while studying the effects of the propsychotic NMDA antagonist PCP, to our surprise, we found massive increases in EEG spectral power at low frequencies (<4 Hz) and a powerful reduction in power (desynchronization) at 6 to 30 Hz. The model was simple: implantation of transcortical electrodes in the prefrontal and sensorimotor cortices with analysis of EEG power spectra between 1 and 30 Hz over 3 hours. The effects of the vehicle, administered on the first day, was
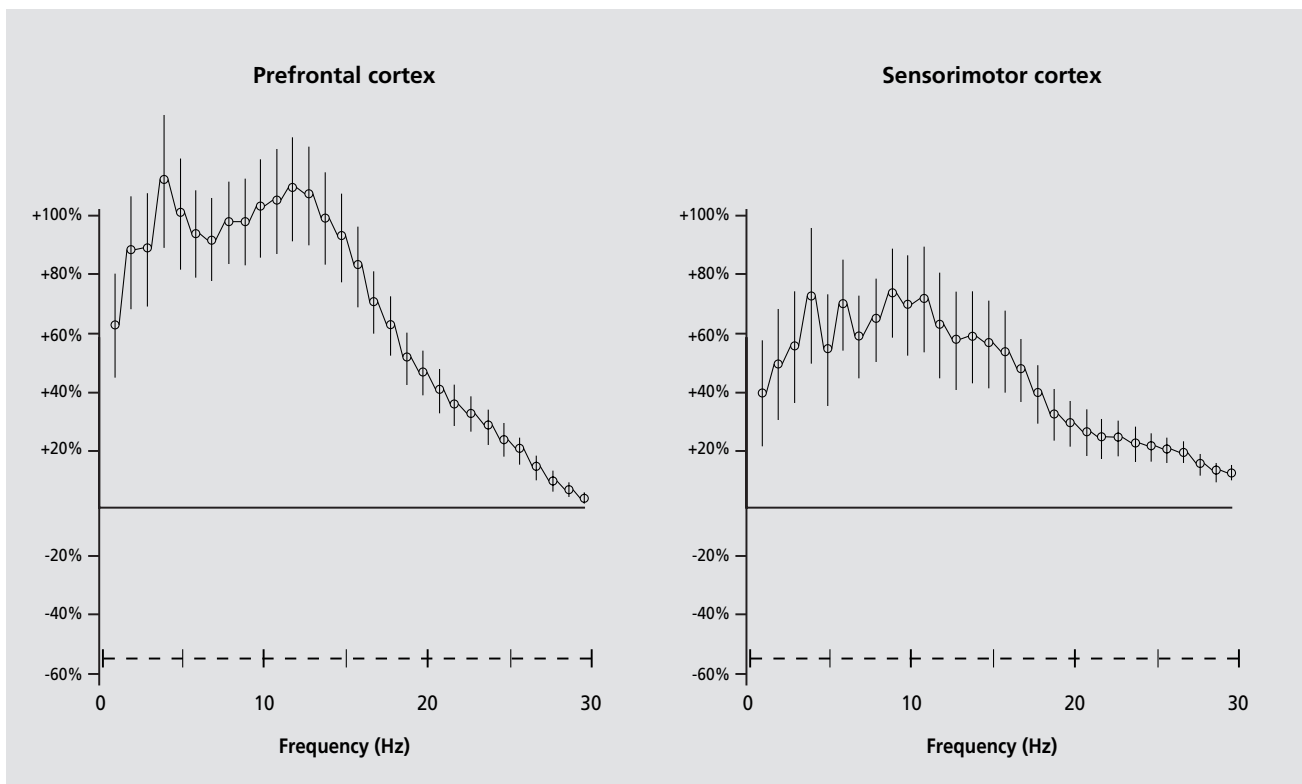

Figure 3. Dose–response effects of subcutaneous clozapine (0.2 mg.kg⁻¹) expressed as percentage change of electroencephalographic (EEG) spectral power in the prefrontal cortex and sensorimotor cortex of conscious rats at each frequency between 1 and 30 Hz. Vertical bars represent 95% confidence intervals (n=6).

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Clinical research

subtracted from the effects of the drugs, administered on the second day, allowing an EEG power spectrum of the effects of the drugs. The results were published by Sebban et al[21] in 2001, and are summarized here:

- The EEG effects of the propsychotic agent PCP showed that PCP (0.1-3 mg.kg^{-1}, subcutaneous) *(Figure 2)* caused a marked dose-dependent increase in EEG power in the frontal cortex at 1 to 3 Hz, with decreases in power at higher frequencies (9-30 Hz). MK801 (0.05-0.1 mg.kg^{-1}, intraperitoneal [IP]) caused similar effects, but with lesser changes in power.
- In contrast, the noncompetitive AMPA (amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) antagonists GYKI 52466 and GYKI 53655 increased EEG power over the whole power spectrum (1-10 mg.kg^{-1}, IP).
- Clozapine, an atypical antipsychotic agent (0.2 mg.kg^{-1}, subcutaneous) synchronized the EEG (peak 8 Hz) *(Figure 3)*. The 5-HT$_{2A}$ antagonist M100907 specifically increased EEG power at 2 to 3 Hz at low doses (10 and 50 µg.kg^{-1}, subcutaneous), whereas at higher doses (0.1 mg.kg^{-1}, subcutaneous) the profile resembled that of clozapine.
- Clozapine (0.2 mg.kg^{-1}, subcutaneous), GYKI 53655 (5 mg.kg^{-1}, IP), prazosin (0.05 and 0.1 mg.kg^{-1}, IP), and M100907 (0.01 and 0.05 mg.kg^{-1}, subcutaneous) antagonized the decrease in power between 5 and 30 Hz caused by PCP (1 mg.kg^{-1}, subcutaneous), but not the increase in power at 1 to 3 Hz in prefrontal cortex *(Figure 4)*.

Conclusion

Thus, clozapine, supposedly the best antipsychotic agent available, apart from its limiting side effects, clearly increased theta rhythm in prefrontal cortex, indicating beneficial effects on cognition. Clozapine also partially antagonized the effects of PCP on EEG, but only the desynchronization. These results clearly show that the effects of PCP on EEG can be used a model for schizophrenia, which may be transposable to man. The profiles of compounds screened for activity in this model are allowing new therapies for schizophrenia to be developed, particularly if all the effects of PCP may be antagonized. ❏

Prefrontal cortex

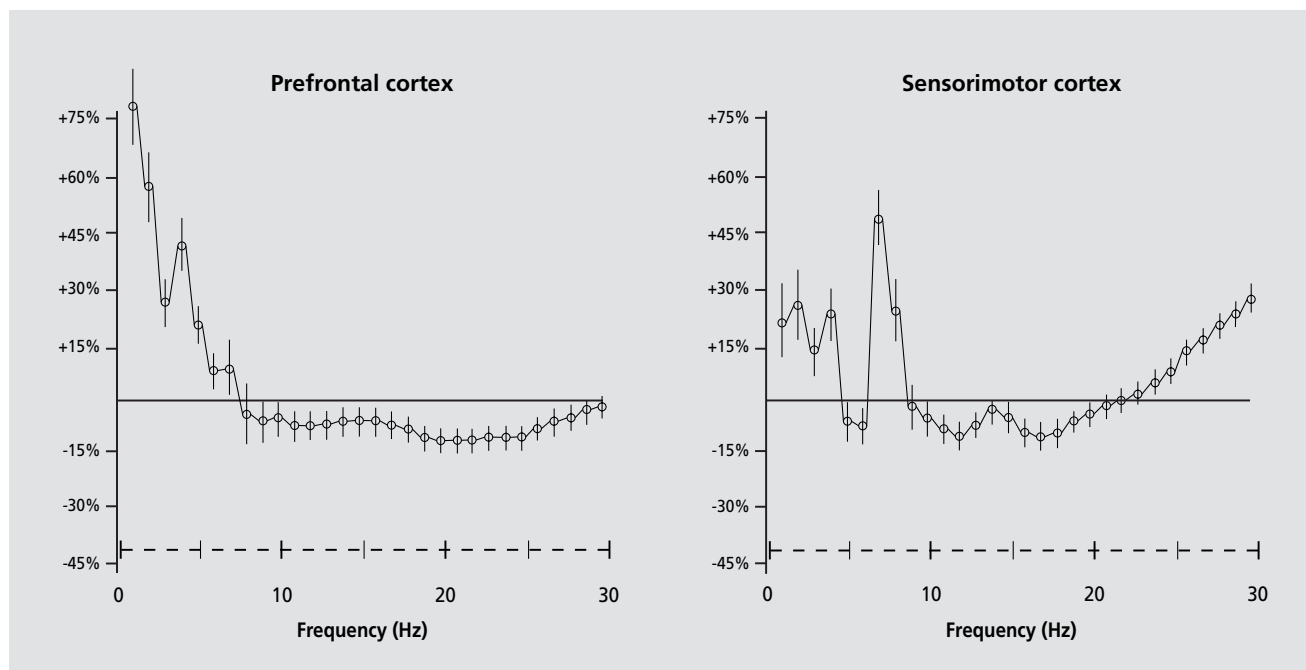**Sensorimotor cortex**

Figure 4. Coadministration of subcutaneous phencyclidine (PCP) (1 mg.kg^{-1}) and clozapine (0.2 mg.kg^{-1}), expressed as percentage change of electroencephalographic (EEG) spectral power in the prefrontal cortex and sensorimotor cortex of conscious rats at each frequency between 1 and 30 Hz. Vertical bars represent 95% confidence intervals (n=6).

Reproduced from reference 21: Sebban C, Tesolin-Decros S, Ciprian-Ollivier J, Perret L, Spedding M. Effects of phencyclidine (PCP) and MK 801 on the EEGq in the prefrontal cortex of the conscious rats; antagonism by clozapine, and antagonists of AMPA-, alpha(1)- and 5-HT(2A)-receptors. *Br J Pharmacol*. 2002;135:65-78. Copyright © 2002, Nature Publishing Group.

Nuevas tendencias para el descubrimiento de fármacos en la enfermedad psiquiátrica

El papel de los receptores en la enfermedad psiquiátrica aun no está aclarado, aunque recientemente se han descubierto nuevas posibilidades en el uso de los fármacos como consecuencia de la clonación del genoma humano y del descubrimiento de la mayoría de los receptores importantes. Nosotros argumentamos que la investigación en los procesos patológicos que conducen a nuevos modelos animales que se pueden traspasar al hombre es crítica en el descubrimiento de fármacos y presentamos un ejemplo de un modelo animal para la esquizofrenia utilizando la electroencefalografía.

Les voies de l'innovation thérapeutique en psychiatrie

Le clonage du génome humain a permis de découvrir de nombreuses cibles potentielles nouvelles pour les médicaments et de mettre en évidence la plupart des récepteurs qui leur sont associés. Cependant, le rôle de ces derniers dans la maladie psychiatrique reste obscur. Il est clair, en ce qui nous concerne, que la recherche sur les processus morbides conduisant à de nouveaux modèles animaux transposables à l'homme est essentielle pour la découverte de médicaments. À ce titre, nous proposons un exemple de modèle animal pour la schizophrénie utilisant l'électroencéphalographie.

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