*Translating neuroscience research into new medicines is challenging, largely because of the complexity of the human brain. The critical factors involved in this process are considered, along with the future prospects.*



# Foundation review: Translational CNS medicines research

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**The major imperative of the pharmaceutical industry is to effectively translate insights gained from basic research into new medicines. This task is toughest for CNS disorders. Compared with non-CNS drugs, CNS drugs take longer to get to market and their attrition rate is greater. This is principally because of the complexity of the human brain (the cause of many brain disorders remains unknown), the liability of CNS drugs to cause CNS side effects (which limits their use) and the requirement of CNS medicines to cross the blood–CNS barrier (BCNSB) (which restricts their ability to interact with their CNS target). In this review we consider the factors that are important in translating neuroscience research into CNS medicines.**

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## Introduction

The successful translation of basic research results into safe and effective new medicines is the major goal of the pharmaceutical industry, which is made up of large (Big Pharma), medium (Biopharma) and small (Biotech) companies. This is particularly true for CNS medicines, which, compared to non-CNS drugs, take longer to get to market and have a lower probability of getting there [1,2]. In addition, most (if not all) CNS disorders are underserved by existing therapies, which are nearly all palliative only. A major contributing factor for the poor translation of neuroscience research into medicines is the high degree of complexity of the human CNS, particularly the human brain. Weighing on average 1.3 kg, the human brain consists of 100 billion neurons and a trillion glial cells arranged in an inter-connected network of circuits and subcircuits, with connectivity principally mediated through electrochemical transmission at its $10^{14}$ synapses. Because of this complexity, our knowledge of most brain disorders is largely rudimentary, which is a real challenge for the discovery of new CNS medicines since medicines research is intrinsically dependent upon a good understanding of disease biology. In addition, it is difficult to pharmacologically influence a single neuronal circuit or subcircuit, so some disruption of normal function often occurs with CNS medicines. In addition, the brain has a remarkably large demand for energy, which is delivered (in the form of oxygen and glucose in blood) through an intensely arborised network of blood vessels that permits near-instantaneous solute equilibration throughout the brain interstitial fluid (ISF). These blood vessels represent a further

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1359-6446/06/$ - see front matter  http://dx.doi.org/10.1016/j.drudis.2012.05.001

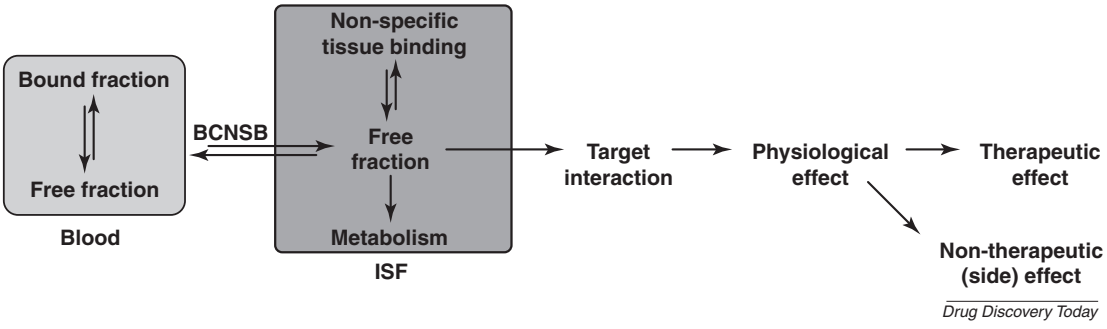


**FIGURE 1**

The major CNS compartments associated with CNS penetration and efficacy. The degree to which a drug binds to proteins (*e.g.* serum albumin, lipoprotein, glycoprotein and α, β, and γ globulins) in blood plasma has a major effect on its therapeutic efficacy. This is because only the unbound (or free) fraction is available for passive diffusion and exerts a pharmacologic effect. Thus, it is the free fraction that is metabolized or excreted (or both) and is available to permeate the BCNSB. The extent and rate of CNS permeation is determined by the physicochemical properties of a compound and its ability to act as a substrate for an ingress transporter. The concentration and duration of the compound in CNS interstitial fluid (ISF) is determined by several factors. These include the ability of the compound to act as a substrate for an egress transporter (principally P-glycoprotein), non-specific binding to tissue, egress into CSF and metabolism. Once in the ISF, it can interact with target proteins on plasma membranes (receptors and transporters) or target proteins within cells (mainly enzymes) and therefore evoke the desired physiological effect. The cumulative consequence of the effect of a neuroactive compound on its target protein leads to a physiological effect, which can result in therapeutic benefit or side effects (or both). *Abbreviations*: BCNSB: blood–CNS barrier; ISF: interstitial fluid.

challenge for translational CNS medicines research because, unlike blood vessels in every other part of the body (except the testes), their endothelial cells form tight junctions through the interaction of cell adhesion molecules. These joined endothelial cells, coupled with astrocytes, pericytes and macrophages, form a barrier that separates CNS ISF from blood. It is not just a physical barrier; it also represents a transport barrier, with specific transport mechanisms mediating the egress of compounds out of CNS ISF, and a metabolic barrier, with enzymes metabolizing molecules in transit across the blood–CNS barrier (BCNSB). The barrier function is not fixed, but can be modulated and regulated, both in physiology and in pathophysiology [3].

Once it has moved from the bloodstream to CNS ISF, a neuroactive compound (NAC) is available to interact with its molecular target, which is usually a protein, most commonly a membrane receptor or transporter, but enzymes too. With sufficient exposure at the appropriate concentration, the compound exerts a physiological effect (usually receptor antagonism or enzyme inhibition), which leads to a therapeutic effect. Non-therapeutic (or side) effects occur through interaction with other proteins, but can also be associated with action at the target protein. Ideally the therapeutic effects should be occurring at doses lower than those causing side effects. This difference is the therapeutic window and is also expressed as a ratio: the dose causing side effects/therapeutic dose [4] (Fig. 1).

## Target identification

Molecular targets for CNS drug discovery have been identified on the basis of: (i) observation of the effect of known compounds on behaviour; (ii) hypotheses derived from knowledge of pathophysiology; and (iii) a combination of (i) and (ii). The prototypic example of (i) is the discovery of the antipsychotic drug chlorpromazine which derived directly from the observation of the unexpected behavioural effects of the antihistamine promazine. The discovery that antipsychotic efficacy is mediated mainly through the antagonism of dopamine $D_2$ receptors led to the discovery and development of compounds with greater efficacy and reduced liability to cause side effects, particularly tardive dyskinesia. Another example of targets discovered through the effect of associated ligands on behaviour is benzodiazepine anxiolytic drugs. The prototypic example is chlordiazepoxide (Librium), which was made by adding a basic side chain within the tricyclic structure of chlorpromazine. It was found to have a tranquilising effect similar to that of chlorpromazine, but without the side effects. Its anxiolytic efficacy was subsequently discovered to be mediated through antagonism of $GABA_A$ receptors [5].

The first example of drugs discovered on the basis of disease pathophysiology came with the observation that Parkinson's disease is associated with reduced concentrations of dopamine and its major metabolite (homovanillic acid) in the striatum. This loss correlated with both cell loss from the substantia nigra (dopamine neurons project from this structure to the striatum) and two of the three cardinal symptoms of Parkinson's disease: tremor and lack of movement (akinesia). This led directly to dopamine replacement therapy, in the form of Carbidopa (L-DOPA and a an inhibitor of peripheral aromatic amino acid decarboxylase) and dopamine agonists, including apomorphine, bromocyrptine, lisuride, cabergoline, pergolide, pramipexole and ropinirole [6]. This new, and hypothesis-driven, approach to drug discovery gained further momentum with the discovery that Alzheimer's disease (AD) is associated with the loss of cholinergic neurons, which led to the development of drugs, particularly donepezil, rivastigmine and galantamine, to correct the deficit in cholinergic transmission [5,7].

An example of CNS drugs developed on the basis of both clinical observation and hypothesis is antidepressant medicines. The CNS efficacy of promazine led to the assessment of the CNS action of other antihistamines, which resulted in the discovery of imipramine and other tricyclic antidepressants. They were found to act by blocking the uptake of serotonin into serotonergic neurons. This, together with data showing that a drug used to treat tuberculosis, iproniazid, had efficacy in the treatment of chronically

depressed psychotic patients led to the hypothesis that defects in monoaminergic neurotransmission underlie depressive symptoms; iproniazid, is an inhibitor of the enzyme monoamine oxidase, which is involved in the catabolism of monoamine neurotransmitters [5,8].

Most CNS drugs target G protein-coupled receptors (GPCRs). There are more than 250 'known' GPCRs, for which the endogenous ligand has been identified, although receptors for which the endogenous ligand has not been identified are referred to as 'orphan' receptors, of which there are still more than 150 [9].

In addition, regulators of G protein signalling (RGS) proteins are emerging as potentially important drug targets, with the mammalian RGS protein family containing more than 20 members [10]. Another important drug target, which is benefiting from improved screening technologies, is ion channels. It is based on the discovery and successful commercialisation of drugs that modulate the activity of voltage-gated sodium, calcium and potassium channels, such as lamotrigine (Lamictal), nimodepine (Nimotop) and dalfampridine (Amprya/Famprya), respectively [11–13]. Another, less exploited, is ligand-gated ion channels, such as the NMDA receptor (*e.g.* memantine [14]). Transporter molecules have proven to be a rich vein for CNS drugs, particularly the 5-HT transporter. Selective serotonin re-uptake inhibitors (SSRIs) increase the interstitial concentration of serotonin by inhibiting its re-uptake into the presynaptic cell, thus making more serotonin available to bind to 5-HT receptors. They have established utility in the treatment of clinical depression; fluoxetine and paroxetine are the prototypic SSRIs. There are also established antidepressants, such as venlafaxine, which inhibit the uptake of both noradrenaline and serotonin [15]. A relatively small number of CNS drugs are enzyme inhibitors. These include inhibitors of acetylcholine esterase (*e.g.* donepazil, rivastigmine and galanthamine), monoamine oxidase (*e.g.* minaprine and selegiline for MOA-A and MAO-B, respectively) and catechol-*O*-methyl transferase (*e.g.* tolcapone and entacapone) (Table 1) [16–18].

The use of models carrying targeted mutations of genes, which have been postulated to be involved in the pathophysiology of particular diseases provides a useful approach for target validation. A commonly applied method is the generation of mice with respective genetic manipulations (*e.g.* gene knockout and inserting point mutations), and employs homologous recombination in embryonic stem cells (ES) to replace a wild-type gene with a modified one [19]. However, the utility of this approach for CNS disorders is limited as very few of them show Mendelian inheritance and some (such as traumatic brain injury) are caused entirely by environmental factors; most are a complex mix of genetic, epigenetic and the environmental factors.

Structural biology is making an increasingly important contribution to CNS drug discovery, with the expression, purification, and crystallization of protein targets, such as GPCRs [20]. Determination of the structure of drug targets greatly facilitates the identification of molecules (hits) which modulate the function of the biochemical target. Once this stage is complete, the process of transforming these into high-content lead series commences [21,22]. The resultant 'drug-like' leads are then further optimized into candidate drugs, which are then subjected to a battery of tests to demonstrate that they are likely to be safe and effective in human studies. This includes an integrated understanding of the pharmacokinetic (PK)–pharmacodynamic principles of exposure at the site of action, target binding and the demonstration of functional pharmacological activity [23]. Tests crucial for CNS drug candidates are considered below.

## The blood–CNS barrier

The concentration of a drug in blood over time underpins its ability to interact with its target molecule and, in most instances, the time course in the plasma correlates well with the onset, intensity, and duration of therapeutic efficacy. Drugs or drug candidates can be administered by several different routes, but in most cases (notable exceptions include intravenous and intrathecal injections) it must cross several membranes before it reaches its site of action. Once absorbed into the bloodstream, bioactive compounds (BACs) are distributed to all parts of the body. But it is only the unbound (or free) fraction that can diffuse out of capillaries and into tissue. Once in tissue, particularly the liver, BACs are metabolised, generally in two phases: Phase I induces a chemical change (mostly oxidation, but reduction can also occur) that renders the drug more amenable to Phase II metabolism, which involves conjugation or synthetic addition of a large, polar molecule that renders the drug water soluble and thus ready for renal excretion. The normal consequences of this process of biotransformation are that the activity of the drug is lost as it is converted to an inactive metabolite; by contrast pro-drugs are converted to active metabolites.

In addition to solubility, permeability, metabolic stability and protein binding considerations, a NAC (and potential CNS medicine) also needs to have the correct physicochemical properties to permit movement through a structural and dynamic barrier that separates the blood from the CNS. This BCNSB exists because, unlike in most of the body, the cells that form the capillary walls are tightly sealed by cell adhesion molecules, such as claudin, occludin and adherens junction molecules. The BCNSB, which compromises the blood–brain barrier (BBB), the blood–spinal cord barrier and the blood–CSF barrier, limits the types of substances that can pass into the CNS. Thus, for example, penicillin, many chemotherapy drugs, oligonucleotides and most proteins cannot pass into the CNS, whereas other substances, such as alcohol, caffeine and nicotine, can. Thus, biologic drugs, which are too large to readily cross the BCNSB, have little utility in the treatment of most CNS disorders. The notable exception is multiple sclerosis where the efficacy of therapeutic proteins is not dependent on BCNSB permeation [24]. Some substances essential for the function of CNS cells, such as glucose and amino acids, do not readily permeate the barrier but instead enter through specific transport systems [25].

The selection of compounds with properties favourable for the movement of compounds from blood into the CNS has the potential to both increase the chances of candidates making it to market and reduce the time taken to get there [2,25,26]. Such selection is dependent on methods to assess the probability of compounds crossing the BCNSB in humans, some of which are described below.

### In vitro *studies*

There are several *in vitro* model systems to assess BCNSB permeation, including bovine and human brain endothelial cells co-cultured with astrocytes, immortalized brain endothelial cell lines,

**TABLE 1**

**Major generic CNS drugs**

| *Disease* | Generic name | Brand name | Mechanism of action |
|---|---|---|---|
| **ADHD** | Methylphenidate | Ritalin | NA and DA uptake blocker and releaser |
| **Alzheimer's disease** | Aricept | Donepazil | AChE inhibitor |
| **Amyotrophic lateral sclerosis** | Riluzole | Rilutek | Sodium channel blocker |
| **Anxiety** | Alprazolam | Xanax | $GABA_A$ receptor antagonist |
| | Dazepam | Valium, Diastat | $GABA_A$ receptor antagonist |
| **Depression** | Bupropion | Wellbutrin, Budeprion, Zyban | NA and DA uptake inhibitor |
| | Fluoxetine | Prozac | SSRI |
| | Venlafaxine | Effexor | SNRI |
| | Nortriptyline | Pamelor | NA inhibitor |
| | Citalopram | Celexa | SSRI |
| | Duloxetine | Cymbalta | SNRI |
| | Tazadone | Desyrel | SRI |
| | Amitriptyline | Elavil, Tryptizol, | SNRI |
| | Sertraline | Zoloft | SSRI |
| | Paroxetine | Paxil | SSRI |
| | Escitalopram | Lexapro | SSRI |
| | Mirtazapine | Remeron | A2 receptor antagonist |
| **Epilepsy** | Phenytoin | Dilantin, Phenytek | $GABA_A$ receptor antagonist |
| | Valproic acid | Depakote, Stavzor | $GABA_A$ receptor antagonist |
| | Carbamazepine | Tegretol | $GABA_A$ receptor antagonist |
| | Levetiracetam | Keppra | $GABA_A$ receptor antagonist |
| | Gabapentin | Neurontin | $GABA_A$ receptor antagonist |
| | Lamotrigine | Lamictal | Sodium channel blocker |
| | Topiramate | Topamax | $GABA_A$ receptor antagonist |
| | Pregabalin | Lyrica | $GABA_A$ receptor antagonist |
| **Insomnia** | Temazepam | Restoril | $GABA_A$ receptor antagonist |
| | Zaleplon | Sonata | $GABA_A$ receptor antagonist |
| **Migraine** | Sumatriptan | Imitrex | 5-$HT_{1B}$ and 5-$HT_{1D}$ agonist |
| **Parkinson's disease** | Apomorphine | Apokyn | $D_1$ and $D_2$ receptor agonist |
| | Trihexyphenidyl | Artane | MI muscarinic receptor antagonist |
| | Lisuride | Dopergin, Proclacam, Revanil | $D_2$, $D_3$ and $D_4$ and 5-$HT_{1A}$ and 5-$HT_{2A/C}$ receptor agonist |
| | Rasagiline | Azilect | MAO-A and MAO-B inhibitor |
| | Benztropine | Cognetin | ACh receptor antagonist |
| | Entacapone | Comtan | COMT inhibitor |
| | Selegeline | Eldepryl | MAO-B inhibitor |
| | Pramipexole | Mirapex | $D_2$, $D_3$ and $D_4$ receptor agonist |
| | Carpidopa/levodopa | Parcopa, Sinemet | DA precursor |
| | Bromocyrptine | Parlodel | $D_2$ receptor agonist |
| | Ropinirole | Requip | $D_2$, $D_3$ and $D_4$ receptor agonist |
| | Talcapone | Tasmar | COMT inhibitor |
| **Schizophrenia** | Seroquel | Quetiapine | Antagonist at DA, 5-$HT_{1A}$, adrenenergic, histamine and muscarinic receptors |
| | Clozapine | Clozaril | Antagonist at 5-HT and DA receptors |
| | Resperidone | Respiridal | Antagonist at DA, 5-HT, adrenaline and histamine (H1) receptors |
| | Olanzapine | Zyprexa | Antagonist at 5-HT and DA receptors |
| | Aripiprazole | Abilify | Partial agonist at $D_2$ and 5-$HT_{1A}$ receptors and antagonist at 5-$HT_{2A}$ receptors |

*Abbreviations*: AChE: acetylcholinesterase; ADHD: attention deficit hyperactivity disorder; COMT: catechol-*O*-methyltransferase; DA: dopamine; NA: noradrenaline; MAO: monoamine oxidase; SNRI: serotonin and noradrenaline reuptake inhibitor; SSRI: selective serotonin reuptake inhibitor.

along with models using cells not derived from endothelial cells, such as Madin–Darby Canine Kidney (MDCK) cell lines. The key aspect of such assays is that they effectively and reliably predict the CNS penetration of NACs *in vivo*, so that they usefully inform structure–activity relationships. MDCK cells, which mirror the intact BCNSB with a high transmembrane resistance (in the range of 1800–2200 $\Omega/cm^2$) are a popular model because they have good predictive validity [27,28].

*In vitro* estimates of the unbound concentration of a NAC using brain slice uptake and brain homogenate binding provide a useful and rapid means to get an indication of *in vivo* ISF concentrations, measured using tissue microdialysis [29]. *In vitro* measurements of the unbound brain fraction may therefore provide a useful tool in the discovery and development of new brain medicines, including positron emission tomography (PET) studies [30].

### Ex vivo *studies*

*Ex vivo* studies provide a step between *in vitro* and *in vivo* assessment. *In situ* brain perfusion is the most common example and measures the rate of entry across brain endothelium *in situ* and is mostly suitable for both slow and fast brain-penetrating compounds. It involves catheterization of the common carotid artery in the anaesthetized animal (usually mice or rat), together with ligation of the external carotid artery. The brain is then perfused with physiological saline buffer containing the test substance and the brain removed for analysis once perfusion is complete. The brain is then removed for analysis and uptake (volume of distribution) determined [2].

Calculating log BB is another commonly used *ex vivo* method to assess BBB permeation. It is defined as the logarithm of the ratio of the concentration of a NAC in the brain and in the blood, measured at equilibrium, usually in rats following perfusion of blood from the cerebral vasculature [2]. Another, and similar approach, is measurement of the brain permeation of compounds by assessment of the rate of entry across brain endothelium *in situ*. It involves catheterization of the common carotid artery in the anaesthetized animal (usually mice or rat), together with ligation of the external carotid artery. Once perfusion is complete, the brain is removed for analysis and regional uptake [volume of distribution ($V_d$)] and $K_{in}$ determined [31].

Determination of receptor occupancy in the brain is an increasingly popular approach to indirectly assess the BBB permeation of NACs. It is a very powerful approach, largely because it can be applied to studies of both experimental animals (using receptor binding methods following systemic administration of a radiolabelled ligand) and humans, using both PET and single-photon emission computed tomography (SPECT) [32].

### In vivo *studies*

Tissue microdialysis is the most direct method for measuring the unbound brain fraction of a NAC and has been effectively applied to a wide variety of different molecules. It provides key information on the PK profile of a NAC in ISF, including measured parameters such as half-life, $C_{max}$, $T_{max}$ and total exposure. It also enables determination of calculated primary parameters, such as volume of distribution and clearance, which allows prediction of BBB influx and efflux rates for different brain regions [32,33]. The key aspect of measuring the concentration of a NAC in the ISF of experimental animals is its ability to predict its exposure in the human CNS, optimising clinical dosing since sampling ISF is not practicable in humans (Table 2).

The ISF drains into the CSF in the brain ventricles. In humans CSF is produced at a rate of 500 ml/day, from the choroid plexus and circulates from there through the interventricular foramina (foramen of Monro) into the third ventricle. It then travels through the cerebral aqueduct (aqueduct of Sylvius) into the fourth ventricle, where it exits through two lateral apertures (foramina of Luschka) and one median aperture (foramen of Magendie). It then flows through the cerebellomedullary cistern down the spinal cord and over the cerebral hemispheres [25].

Sampling ventricular CSF concentrations of a NAC provides a useful approach to assess the amount of systemically administered NAC in the brain of conscious animals by repeated sampling of CSF from the cisterna magna [32]. Such measures have been found to correlate well with pharmacodynamic (PD) readouts for both the anticonvulsant drug pregabalin and the 5-$HT_{1A}$ receptor antagonist WAY-100635 [2,34].

**TABLE 2**

**PK/PD studies in experimental animals and humans.**

| | Experimental animal | Human |
|---|---|---|
| [NAC] in blood | ✓ | ✓ |
| [NAC] in brain ISF | ✓ | ? |
| Receptor occupancy in brain | ✓ | ✓ |

### *Predicting the ability of NACs to permeate the BCNSB*

Several computer models have been established to predict the ability of NACs to permeate the BCNSB on the basis of molecular descriptors [35,36]. Key descriptors include the octanol–water partition coefficient (log *P*), molecular mass, polar surface area and hydrogen bonding properties (Table 3). Such models consider passive permeation only and so do not take account of either ingress or egress transport mechanisms.

### *Ingress into the CNS*

CNS ingress through active transport is mediated through several transporter molecules that facilitate the ingress of solutes into the CNS. The most common transporter is that for glucose, which is not surprising given the huge demand that the brain has for energy. Glucose transporter 1 (GLUT1) is the most widely expressed isoform of the 13 GLUT family and its rate of transport of glucose is much higher than the facilitated transport of other solutes, such as lactate and amino acids [3,25].

Receptor-mediated endocytosis provides a mechanism for the selective uptake of macromolecules into the brain and occurs through receptors for the uptake of many different types of ligands, including transferrin, insulin, leptin and insulin-like growth factor [25].

### *Egress from the CNS*

In addition to effectively penetrating the BCNSB, NACs also need to have little or no interaction with several efflux transporters that ship exogenous substances out of CNS ISF. The therapeutic efficacy of several CNS drugs is constrained by the activity of such transporters, which constitute the family of ATP-binding cassette (ABC) transporters. The most abundant of these proteins is P-glycoprotein (P-gp), a 170 kDa transmembrane glycoprotein, which includes 10–15 kDa of N-terminal glycosylation. Another group of transporters is the organic anion transporting polypeptides, which mediate the egress of a wide spectrum of amphipathic transport substrates from the CNS, in addition to the ingress of drugs, such as opioid peptides. All of the CNS egress transporters, particularly P-gp, have low substrate specificity, making it difficult for computational methods to reliably predict ABC-transporter substrate properties of drug-like compounds. Nonetheless, the prospect for *in silico* pharmacological profiling of compound series for a liability of being a substrate for a CNS egress transporter would provide a very useful translational tool for CNS medicines research and good progress is being made on this front [37].

**TABLE 3**

**Target product profile of a CNS drug candidate**

| *Measure* | Target profile |
|---|---|
| **Potency at molecular target** | <10 nM |
| **Selectivity over other targets** | >30 |
| **Molecular weight** | <450 D |
| **Aqueous solubility** | >60 μg/ml |
| **$PK_A$** | Neutral or basic (7.5–10.5) |
| **Hydrophobicity** | Minimal ($c \log P < 5$) |
| **Nitrogen atoms** | At least one |
| **Number of linear chains outside of rings** | <7 |
| **Volume** | 740−970 $Å^3$ |
| **Polar surface area** | <70 $Å^2$ |
| **Solvent accessible surface area** | 460−580 $Å^2$ |
| **Hydrogen bond donors** | <3 |
| **Hydrogen bond acceptors** | <7 |
| **Molecular flexibility** | <8 rotatable bonds |
| **Protein binding** | $K_D$ for serum albumen binding <30 μM |
| **Metabolic stability** | High (>80% remaining after one hour) |
| **CYP P450 enzyme inhibition** | <30% at 30 μM |
| **CYP2D6 metabolism** | Minimal |
| **CYP3A4** | No induction |
| **Human P-glycoprotein** | No or minimal substrate activity |
| **MDCK permeability** | High: $P_{app}$ >20 × $10^{-6}$ cm/s<br>Moderate: $P_{app}$ 2–20 × $10^{-6}$ cm/s |
| **PKs** | %F/$Cl_p$/MRT<br>Excellent: >50/<25% $Q_H$/two to four hours<br>Viable: %F 10–50/25–75% $Q_H$/0.5–2 hours |
| **Model of the target disorder** | Clear therapeutic efficacy |
| **CNS PK–PD relationship** | A meaningful correlation |
| **Therapeutic ratio over side effect liability (*e.g.* motor coordination)** | >10 |
| **Therapeutic ratio over hERG $IC_{50}$ (using the free plasma concentration of the therapeutic dose)** | >30 |

Data partly derived from [67] and [22]. $Q_H$: hepatic clearance; MRT: mean residence time, which is the average amount of time that a BAC spends in the bloodstream.

## CNS PDs

Once in CNS ISF, a NAC is available to interact with its target, which is usually a receptor, transporter or enzyme. With sufficient exposure at the appropriate concentration, the compound exerts a physiological effect (usually receptor antagonism and transporter of enzyme inhibition) which leads to a therapeutic effect. Non-therapeutic (or side) effects occur through interaction with other proteins (off-target), but can also be associated with action at the target protein. Ideally the therapeutic effects should be occurring at doses lower than those causing side effects.

Measurement of the PD profile of a neuroactive drug candidate is an increasingly important aspect of CNS drug discovery, particularly if it is able to link experimental and human studies. In experimental animals it is possible to measure the concentration of second messengers in ISF or ventricular CSF, along with NAC-induced changes in behaviour. Such measures can then be linked to the PK profile of the compound and a PK–PD relationship established. An essential requirement of such a relationship is that the NAC enters the CNS ISF compartment at a concentration and duration sufficient to evoke the desired therapeutic effect. This is difficult to measure directly in humans, so a PK profile in the blood and brain ISF of experimental animals can be used with the PK profile in human blood to infer the concentration of NAC in human brain ISF (Table 2). Thus, tissue microdialysis can be used to determine the concentration of chemical messengers that change in response to the action of a NAC on its molecular target. This includes both first messengers (neurotransmitters and hormones) and second messengers [cAMP, cGMP, inositol trisphosphate (IP3), diacylglycerol (DAG) and nitric oxide (NO)] [32].

Another complementary approach is to measure PK indirectly by assessment of receptor occupancy in experimental animals following systemic dosing of radiolabelled molecules that bind to receptors or transporters. This permits receptor or transporter occupancy to be determined. This approach can also be applied to the intact human brain by use of imaging methods, such as PET and SPECT with antagonists and partial agonists; full agonists can be therapeutically effective at low levels of receptor occupancy. PET uses ligands containing short-lived positron emitting isotopes ($^{15}O$, $^{11}C$, $^{18}F$, $^{76}Br$), whereas SPECT uses lower energy γ-emitting isotopes ($^{123}I$, $^{99m}Tc$). PET is more sensitive and versatile and

enables scatter correction to be performed; SPECT is less expensive and, thus, more widely available as it does not rely on a local cyclotron for production of isotopes [38]. PET imaging has been extensively employed to determine receptor occupancy of a variety of different ligands in both humans and non-human primates and rats [32,39,40]. Such studies provide valuable proof-of-concept data and, along with the PK data described in Table 2, help guide dose selection and duration for Phase I and II clinical trials. However, it only works well for antagonists and partial agonists since full agonists can be therapeutically effective at low levels of receptor occupancy.

Other approaches to provide a PD readout of drug candidates in the clinic include magnetic resonance imaging (MRI) functional magnetic resonance imaging MRI (fMRI) and electroencephalography (EEG). fMRI utilises the paramagnetic properties of oxygenated and deoxygenated haemoglobin to assess changes in blood flow in the brain associated with neural activity [41], whereas EEG uses scalp electrodes to measure electric fields in the brain [42].

These imaging techniques have all benefited from improvements in technology (which has increased resolution) and increasing computing power, which has greatly enhanced data handling capacity and capability. Neuroimaging has contributed to CNS translational research in several other ways:

(i) Understanding the biological basis of brain disorders, especially with the use of PET and MRI, particularly fMRI [41].

(ii) Monitoring the evolution of chronic neurologic disorders so that patients experiencing breakthrough disease (*e.g.* clinically isolated syndrome for multiple sclerosis) which is identified by transient clinical features coupled with the visualisation of MS lesions (plaques) in the CNS [43].

(iii) Detecting a disease in individuals with no clinical symptoms, such as early Parkinson's disease, detected by observing loss of nigro-striatal neurons *in situ* using PET, and early AD, detected by visualisation of amyloid plaques, again using PET [44–47].

(iv) Improved diagnosis to increase patient homogeneity in clinical trials. Examples include:
   a. The use of MRI to distinguish between hemorrhagic and ischemic stroke to ensure clot-busting drugs (such as Altepase) are not administered to patients with a clot protecting a ruptured blood vessel [48].
   b. The use of diffusion and perfusion weighted MRI to visualize the penumbra and thus identify stroke patients most likely to respond to neuroprotective therapy [49].
   c. Distinguishing AD from other forms of dementia using PET and [$^{11}$C]PIB to visualize amyloid and [$^{18}$F]deoxyglucose to visualize pyramidal cell loss [46].
   d. The use of MRI has gained widespread acceptance in the diagnosis of MS, and for obtaining key data to aid prognosis early in the course of the disease. It is also employed to assess breakdown of the BBB by use of contrast agents, such as gadolinium [43,46,50].

(v) As surrogate endpoints of clinical efficacy. The best example of this is the use of MRI to measure the efficacy of immunomodulatory drug candidates by visualising the number of plaques in the brains of people with MS [43]. MRI has also been used to assess the neuroprotective efficacy of compounds by assessment of brain volume [51].

(vi) Assessment of the PKs of a NAC in the CNS, especially using PET [32].

(vii) Assessment of the PD effect of a NAC by measuring: receptor occupancy using receptor specific PET or SPECT ligands [32].

Biochemical measurements can also be used to get PD readouts of drug action. The BCNSB makes it difficult to use biomarkers in blood as a reliable measure of CNS function, with the exception of neuroendocrine challenge tests [52]. Lumbar CSF provides a more direct measure of CNS function but it still suffers from being some distance from the brain and so its reliability as a biomarker is sometimes questionable.

## Experimental models of CNS disorders

An important, but not necessarily essential, component of CNS translational research is the use of experimental models. Numerous models of CNS disorders have been established and used to both understand pathophysiology and to aid the drug discovery process. Key requirements are that the models have strong phenomenological similarities (face validity), similar pathophysiology (construct validity) and that they effectively predict therapeutic efficacy in humans (predictive validity) (Table 3). Varying degrees of construct validity has been established in most models, but predictive validity has been a more challenging goal to achieve, largely because of the absence of clear therapeutic efficacy in the clinic with established utility to reliably predict therapeutic efficacy. In transgenic models of AD, for example, mice expressing one or more human genes with mutations known to contribute to AD pathology, produce some, but not all, of the pathological hallmarks of this disease; plaques are evident but not tangles and neurodegeneration [53]. Predictive validity has not been established because no drug candidate targeting amyloid or tau (the critical proteins involved in plaques and neurofibriallary tangles, respectively) has shown efficacy in the clinic [54] (Table 4).

## Clinical studies

A typical target product profile of a CNS drug candidate is shown in Table 3. Once selected, along with one or more back-up candidates, it enters preclinical development, which aims to establish the safety profile of the compound. Typically, both *in vitro* and *in vivo* tests will be performed. *In vitro* studies aim to establish the toxicity profile of a compound, including assessment of long-term carcinogenicity, toxic effects on mammalian reproduction and metabolism, using tissues from several species. *In vivo* studies, in both a rodent and non-rodent species, aim to establish the PK profile of the compound and link this information to the PD response of the compound and its toxicity profile. The No Observable Effect Levels determined from the toxicokinetic studies is then used to determine initial Phase 1 clinical trial dosage levels on a mass API per mass patient basis. Most preclinical studies must adhere to Good Laboratory Practise and ICH Guidelines to be acceptable for submission to regulatory agencies, such as the Food and Drug Administration in the United States and the European Medicines Agency.

With the successful completion of formal preclinical studies, the drug candidate enters the first phase of testing in human subjects in a trial of 20–100 healthy volunteers. This is designed to assess the safety, tolerability, PKs, and PDs and includes single and multiple ascending dose studies, along with an assessment of

**TABLE 4**

**Experimental models of CNS disorders**

| *Disorder* | Prototypic model(s) | Key features of human disorder and predictive power | Refs |
|---|---|---|---|
| **Attention deficit hyperactivity disorder** | Spontaneously hypertensive rat (SHR) and 6-hydroxydopamine-lesioned (6-OHDA) animals | Some clinical aspects reproduced but predictive power not established | [68] |
| **AD** | Transgenic mice expressing mutated human genes | Some key aspects of pathology reproduced but predictive validity not established because of the absence of drugs with clear neuroprotective efficacy | [53,69] |
| **Amyotrophic lateral sclerosis** | Transgenic mice expressing mutated *SOD1* gene | Motor neurodegeneration coupled with locomotor deficits, progressing to hyper-reflexia, paralysis and premature death | [70] |
| **Anxiety** | Several models established, including the conditioned emotional response and fear-potentiated startle | Good predictive power for benzodiazepine anxiolytics but not for other classes of drug | [71] |
| **Chronic pain** | Several models, including models of neuropathic pain | Key aspects reproduced but the predictive power of the models not yet clearly established | [72] |
| **Depression** | The forced swimming test | Good predictive validity [73] | |
| **Epilepsy** | Several models | Important aspects of partial seizures, generalized seizures and status epilepticus reproduced with good predictive validity | [74] |
| **Huntington's disease (HD)** | YAC128 transgenic mice expressing full-length human HD gene with 128 CAG repeat | Reproduces molecular, cellular and clinical features of HD | [70] |
| **Insomnia** | Several models based on disruption of the sleep-wake cycle by stress of NACs | Key aspects reproduced but predictive validity yet to be clearly established | [75] |
| **Migraine** | Trigeminovascular nociceptive activation and mechanically induced cortical spreading depression | Altered modulating trigeminal sensory processing and migraine aura reproduced and some predictive validity | [76,77] |
| **Multiple sclerosis** | Several models of experimental autoimmune encephalomyelitis have been established in both rodents and primates | Acute monophasic, relapsing–remitting and chronic progressive CNS inflammation reproduced | [78] |
| **Parkinson's disease** | Reserpine treatment in rodents and MPTP induced lesions of dopaminergic neurons in primates | Key aspects of pathophysiology and clinical features reproduced with good predictive validity but predictive power has not been fully established. Existing models may not be relevant to neuroprotective agents. | [79]. |
| **Schizophrenia** | Numerous models | Aspects of schizophrenia reproduced in different models but, overall, their predictive power is not reliable | [80] |
| **Stroke** | Middle cerebral artery occlusion | Pathophysiology and clinical impairment reproduced but predictive validity not established because of the absence of drugs with clear neuroprotective efficacy | [81] |
| **Traumatic brain injury** | Several models, including the control cortical contusion (CCC) model | Key aspects of the pathophysiology and clinical outcome reproduced by predictive validity difficult to assess in the absence of drugs with proven clinical efficacy. However, therapeutic hypothermia showed efficacy in the CCC model and humans. | [82–84] |

the effect of food on its PK profile for orally administered compounds. Once complete, Phase II trials can commence using larger groups (20–300). They are designed to assess how well the drug works, in addition to continuing Phase I safety assessments in a larger group of volunteers and patients. Phase II studies are sometimes divided into an assessment of dosing requirements (Phase IIa) and assessment of therapeutic efficacy (Phase IIb).

With successful completion of Phase II studies, a drug candidate enters Phase III trials, which are randomized controlled multicentre trials on large patient groups (300–3000 or more depending upon the disorder under investigation). These are aimed at being the definitive assessment of how effective the drug is in comparison with placebo or the current gold standard. The latter hurdle is becoming increasingly challenging as more CNS drugs become generic (Table 1); the patents of yet more CNS drugs are due to expire in the near future, including drugs for the treatment of Alzheimer's disease (Rivastigmine, Galantamine and Namenda) and multiple sclerosis (Avonex, Betaseron, Rebif and Copaxone) [7,24].

Because of their size and comparatively long duration, Phase III trials are the most expensive, time-consuming and difficult trials to design and run, especially in therapies for chronic medical conditions. Typically, at least two successful Phase III trials, demonstrating the safety and efficacy of a drug candidate are requires in order, to obtain approval from the appropriate regulatory agencies.

Assessment of the safety and efficacy of a drug candidate, in both experimental animals and humans, is an essential requirement for approval to market the drug. However, the probability of making it to market is lower at Phase I, II and III for CNS drug candidates than for drugs targeting other conditions, such as cancer and cardiovascular disease [2].

CNS translational research aims to improve the probability of success of CNS drug candidates. To achieve this, it is important to identify the factors contributing to the high attrition rate of CNS drug candidates. Such factors include:

(i) Failure to cross the BCNSB
The dosing regimen in humans is usually guided by the PK profile in blood, which may well be different from the PK profile in CNS ISF. Therefore, measuring the free fraction of a NAC in the ISF of experimental animals usefully informs the dosing regimen used in the clinic, and so reduces the likelihood of false positive data (*i.e.* lack of efficacy because of insufficient exposure to the target molecule in the CNS). There are examples of compounds that have completed Phase III clinical trials, but failed to show efficacy simply because they were not able to cross the BCNSB, including the antioxidant Cerovive and the NMDA receptor antagonist Gavestinel, both of which were being developed as neuroprotective drugs for stroke [4,55]. The use of tissue microdialysis to establish the PK profile of such compounds in the brain would have established that exposure to their respective targets in the brain was probably not consistent with therapeutic efficacy. Similarly receptor occupancy studies using PET in both experimental animals and humans would provide a good PD readout and establish the ability of the NAC to enter CNS ISF (Table 2).

(ii) Heterogeneity in the patient population
Because of the complexity of CNS disorders, many CNS clinical trials have examined a non-homogenous patient population. For example, in trials to assess the efficacy of potential neuroprotective drugs for stroke, some patients have a large cortical infarction, whereas others have a lacunar infarction, which has a completely different prognosis [56]. Removal of patients with lacunar stroke therefore increases the power of clinical trials for ischemic stroke. A further refinement of the stroke population has been achieved on the basis of comparing diffusion and perfusion weighted MRI data to distinguish stroke patients with a 'penumbra', which are probably amenable to neuroprotective intervention, from those without a penumbra, who are less likely to respond to pharmacotherapy.

(iii) The dose or dosing schedule is suboptimal
The importance of the dosing schedule to the efficacy of a drug to treat a CNS disorder is illustrated by Altepase, recombinant tissue plasminogen activator. It has been approved for the treatment of ischaemic stroke but efficacy requires treatment initiation within three hours of stroke onset. The time constraint is applied because the proportion of patients with full recovery decreases with the time taken to commence treatment post stroke; although some significant benefit persisted for treatment initiation of 3–4.5 hours [57]. In addition to thrombolytic agents for stroke, there have also been a large number of clinical trials to investigate the neuroprotective efficacy of drug candidates, predominantly NMDA receptor antagonists, but without success [58]. An analysis of both efficacy and side effect liability of several such compounds in experimental animals established that many had very poor therapeutic ratio [4]. It seems likely that this contributed to the lack of clinical efficacy, because reducing the dose to avoid unwanted side effects led to the administration of subtherapeutic doses. Other factors that have reduced the probability of observing therapeutic efficacy with neuroprotective drug candidates is that the compounds were not given soon enough after the stroke or TBI or that the treatment duration was not sufficient.

(iv) The challenge of demonstrating neuroprotective efficacy in chronic neurodegenerative disease
Neuroprotective agents that slow the progress of neurodegenerative change in chronic diseases, such as AD, Parkinson's disease and multiple sclerosis, have the potential for a major impact. However, no such drug has yet made it to the market [59]. Several clinical trials have failed to show neuroprotective efficacy, particularly for AD [54]. In such studies, it is probably best to commence treatment soon after symptoms emerge, largely because substantial neurodegenerative change occurs before the emergence of symptoms. An example of a good neuroprotective study is a randomized, double-blind, placebo-controlled clinical trial of the herbal product *Ginkgo biloba* in individuals with mild cognitive impairment, which is probably a precursor to AD [60]. The study included 3069 community dwelling participants aged 72–96 years and had a median follow-up of 6.1 years [61]. Although twice daily dosing of *Ginkgo biloba* failed to attenuate cognitive decline, the study provides a touchstone for the assessment of neuroprotective agents in chronic neurodegenerative disease.

## CNS side effects

A final challenge is the fact that brain medicines have a high propensity to cause CNS side effects [2]. This derives either from activity at the target receptor or enzyme or from activity at other receptors or enzymes. It is therefore essential that lead compounds have a good therapeutic ratio, particularly for drugs that will be used chronically. Several tests are used to establish if drug candidates have a liability to cause CNS side effects. They are largely based on assessments of locomotor activity and include, measures of motor coordination (with the rotarod test), a structured observation test (the Irwin screen), and measures of spontaneous motor activity [4,62].

## Concluding remarks

Neuroscience continues to shed light on the biochemistry and physiology of the CNS, in addition to how the functioning intact brain generates a complex repertoire of thoughts, emotions and behaviours. This knowledge serves to improve our understanding of the biological basis of CNS disorders and thus provides the foundation for the discovery of new CNS medicines [59].

Disorders of the CNS represent the largest area of unmet medical need, with more than 1.5 billion people affected worldwide. It represents a massive market (worth $100 billion in 2009) and is set to grow considerably in the years ahead. This is because the

incidence of many CNS diseases (such as AD, Parkinson's disease and stroke) increase exponentially after age 65 and the population of the world is getting older, with those aged 65 or more increasing in number by 1 billion between 2000 and 2050 [2]. Another factor is the inexorable increase in the number of people who are overweight, especially the obese [63].

Although the need for new CNS medicines is large and is set to grow substantially in the years ahead, many companies are moving away from this sector. This is largely because of the high risk of failure associated with CNS medicines research and the longer clinical phase and approval time for CNS drugs compared with other therapeutic categories [64].

Understanding the challenges of CNS medicines research, as described above, will increase the probability of new medicines emerging to treat disorders of the CNS.

Neurodegenerative disorders constitute a major unmet medical need in the CNS arena, so effective neuroprotective drugs are likely to have a major impact. However, the development of such medicines for acute brain injury was not successful and the challenge for chronic neurodegenerative disease is even greater. For CNS disorders not associated with neurodegeneration, such as schizophrenia, depression and anxiety, the challenges are mainly associated with demonstrating clinical efficacy over and above existing therapies, many of which are now generic (Table 1).

New economic realities, coupled with the gap between the cost of R&D and the productivity of the pharmaceutical industry is forcing a major reappraisal of the ways and means by which the industry discovers and develops new medicines [65]. This most acute in the CNS sector, where both the risk of failure and time to market is greatest. However, the medical need is high and is set to increase substantially in the decades. Therefore, it is the sector that has the highest potential for growth. The challenge is to improve the translation of neuroscience knowledge into CNS medicines, which may require new business constructs with wider stakeholder involvement [66].

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