PRECLINICAL DRUG
DEVELOPMENT TESTING FOR

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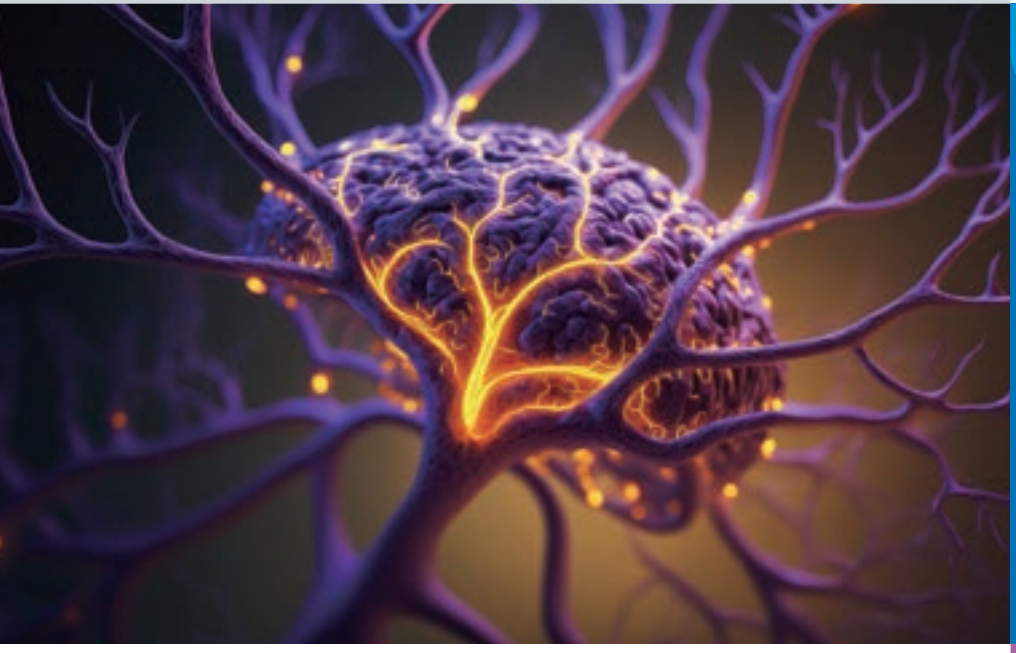
WuXi AppTec

# CENTRAL NERVOUS SYSTEM (CNS) DRUGS

## Shorten the Development Cycle with WuXi AppTec DMPK



Therapeutic Areas Series

# Unique Pharmacokinetics Evaluation System for Central Nervous System Drugs

Developing CNS drugs is challenging, with only an 8% success rate. Precise and rapid evaluation assays can help identify the most promising drug candidates faster, increasing the chances of success. To support this effort, WuXi AppTec DMPK has developed specialty capabilities for CNS, including:

- A distinctive "Funnel" model for *in vitro* assessment of brain penetration in CNS drugs.
- Various administration routes tailored to CNS research, such as lateral ventricle administration and intrathecal injection.
- Serial collection of cerebrospinal fluid (CSF) from rats and large animals.
- Precise separation and collection of approximately 20 distinct brain regions.
- Quantitative whole-body autoradiography (QWBA) for a comprehensive view of brain distribution.

We have deep expertise in researching small molecules, therapeutic proteins, oligonucleotides, and more, successfully supporting numerous CNS projects for global clients.

**Strengths**

- 15+ years of experience
- Distinctive "Funnel" model
- 6+ diverse CNS administration and sampling techniques
- Precise collection of 20 specific brain regions

**Features**

- Challenges in enhancing brain exposure
- Meticulous experimental operations
- Lack of a single reliable *in vitro* model of brain permeability

# Preclinical DMPK Strategy for CNS Drugs

| Assay type | Screening stage | | Regulatory submission stage |
|---|---|---|---|
| | Tier 1 | Tier 2 | IND |
| *In vitro* permeability | Funnel model | P-gp substrate evaluation | P-gp substrate evaluation |
| | | BCRP substrate evaluation | BCRP substrate evaluation |
| *In vitro* metabolic stability | Liver microsomal stability | Microsome/hepatocyte metabolite identification | Liver microsomal stability, hepatocyte stability, metabolite identification |
| | Hepatocyte stability | | |
| | Brain homogenate stability | | |
| Protein binding | Plasma protein binding | / | Plasma protein binding |
| | Brain homogenate protein binding | | Brain homogenate protein binding |
| Drug interaction | Metabolism related | CYP enzyme phenotyping study | CYP enzyme phenotyping study |
| | | CYP enzyme inhibition | CYP inhibition |
| | | CYP enzyme induction | CYP induction |
| | Transporter related | Transporter substrate evaluation | Optional |
| | | Transporter inhibition evaluation | |
| *In vivo* PK | Mouse and rat brain PK (plasma, CSF, and brain) | Dog and monkey brain PK (plasma and CSF) | Single and multiple dose PK, *in vivo* tissue distribution, metabolism, and excretion |

Selecting the right CNS drug candidates requires precise *in vitro* models, well-defined parameters, and effective screening strategies. That's why we've developed a comprehensive CNS drug evaluation platform. Our stepwise screening approach enables rapid, accurate, and efficient assessments, helping you identify candidates with optimal brain permeability and stability—accelerating your path to success.

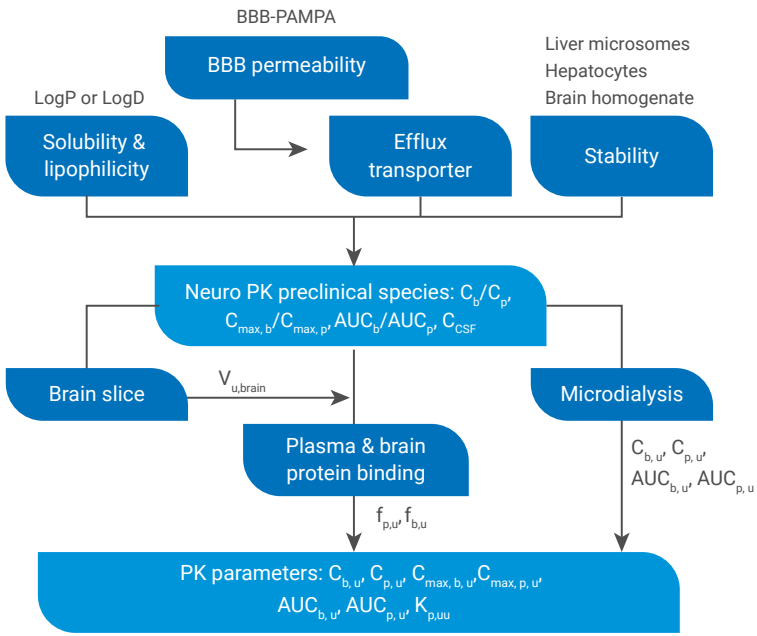



Figure 1. A robust drug-screening platform enables comprehensive DMPK assessment of CNS drugs at WuXi AppTec DMPK

# WuXi AppTec DMPK CNS Drug Platform

## ▶"Funnel" model for *in vitro* brain permeability evaluation of small molecules

This model (Figure 2) accurately differentiated 10+ CNS drugs that could penetrate the brain and 14-drugs with poor passive diffusion ability and/or as substrates for efflux transporters among 24 commercially available drugs. (Figure 3).

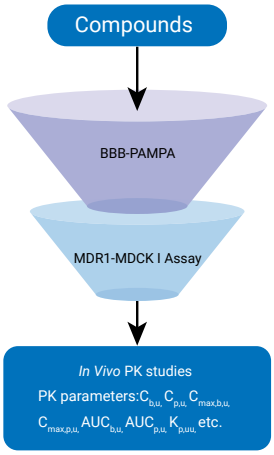



Figure 2. "Funnel" model for evaluating CNS drugs in the brain

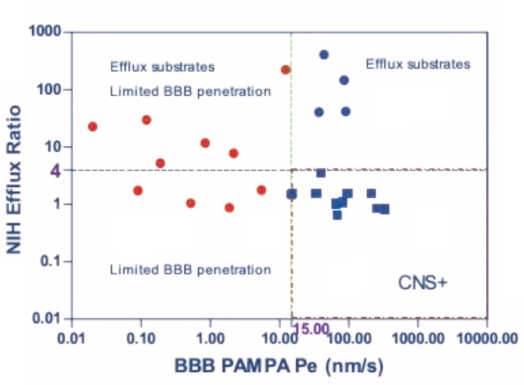


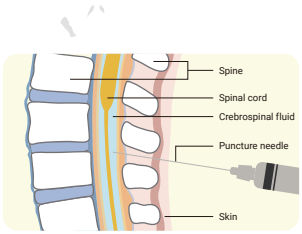

Figure 3. The "funnel" model successfully identified 10+ CNS drugs (blue squares) from a pool of 24 commercially available drugs

## ▶Brain administration routes

In addition to conventional administration routes, advanced methods for direct brain delivery, such as lateral ventricle administration and intrathecal injection, have been developed for rodents and large animals. WuXi AppTec DMPK reports a success rate of over 90% for intrathecal injections in monkeys, exceeding the upper limit of the 30-90% success rates reported in the literature.

Brain stereotaxic instrument

Schematic diagram of lateral ventricle administration

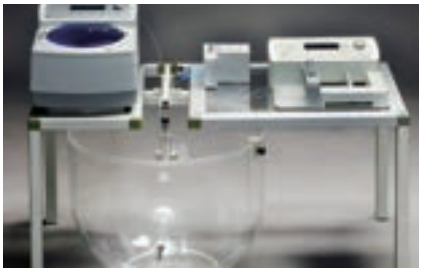



Site of intrathecal injection

# WuXi AppTec DMPK CNS Drug Platform

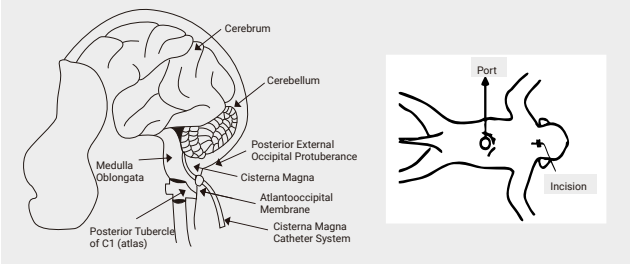
## ▶Microdialysis technology

Microdialysis in rodent and large animals helps to obtain $K_{p,uu}$ directly *in vivo* without performing *in vitro* protein binding assays.



## ▶Serial CSF collection

The cannulation or puncture models of both rodents and large animals, including rats, monkeys, and dogs, enable serial CSF collection, which reduces animal number and individual variation.



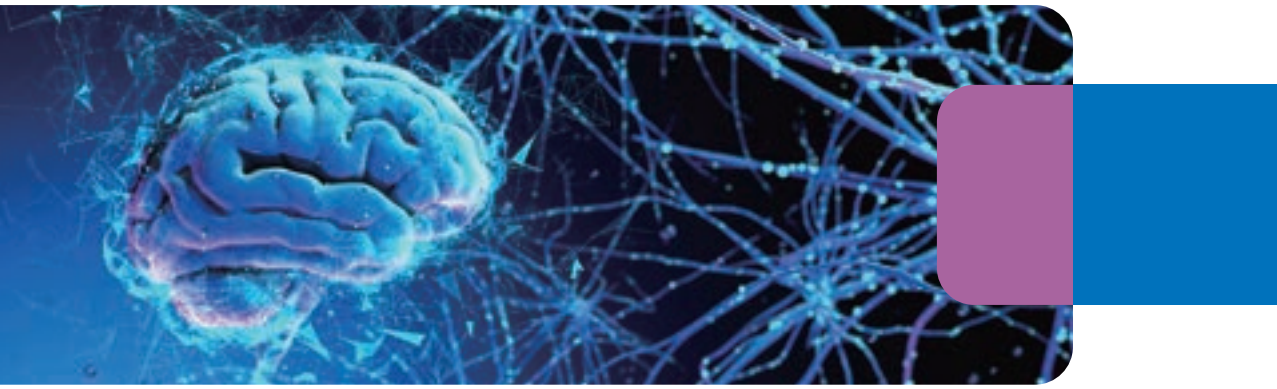

Position of the cistern magna cannulation catheter and port

## ▶Precise brain region collection

Approximately 20 brain regions can be precisely collected. Drug distribution and exposure in each brain region can be evaluated individually.

| Species | Olfactory bulb | Cerebral cortex | Thalamus | Hypothalamus | Hippocampus | Corpus striatum | Diencephalon | Cerebellum | Pons | Medulla oblongata | Substantia nigra | Brainstem | Dorsal root ganglion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rat | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Mouse | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Monkey | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | - | ✓ | ✓ |
| Dog | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | - | ✓ | ✓ |

| Species | Dura mater | Caudate nucleus | Amygdaloid body | Cingulate cortex | Gray matter in the cortex | White matter in the cortex |
|---|---|---|---|---|---|---|
| Monkey | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Dog | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |



# Case Study Brain microdialysis of acetaminophen in rats

We employed a combination of brain microdialysis and intravenous blood microdialysis (Figure 4) to analyze data from the same animal, allowing us to obtain free drug concentrations in both the brain and plasma, as well as unbound AUC and $K_{p,uu}$. The results from this microdialysis technology were compared with conventional *in vivo* and *in vitro* methodologies.

The results indicated that the free drug concentrations obtained via the intravenous blood microdialysis method closely aligned with those from the serial blood sampling method after correction with the plasma fraction unbound obtained from the *in vitro* plasma protein binding assay (Figure 5). The free unbound brain-blood AUC ratio was calculated based on the concentrations of the free compound in brain and plasma (Figure 6); the validation ratio (0.774) was similar to the data reported in the literature (0.823)[2]. The application of microdialysis can simultaneously monitor free drug concentrations in brain and blood from the same animal, and unbound AUC and $K_{p,uu}$ can also be calculated.

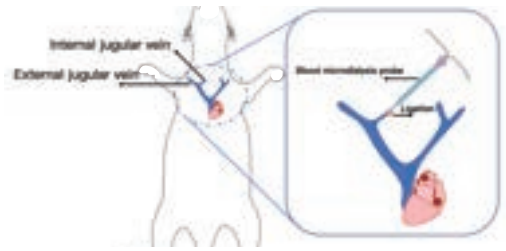



Figure 4. Schematic diagram of intravenous blood microdialysis

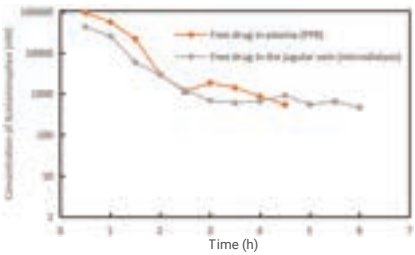



Figure 5. Comparison of free drug concentration in plasma obtained via the serial blood sampling and the microdialysis method

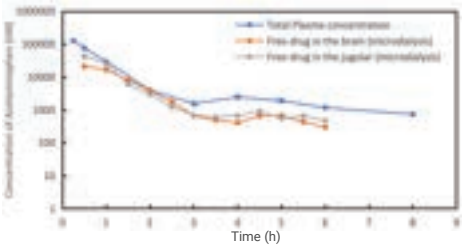



Figure 6. Brain microdialysis in combination with intravenous blood microdialysis

References

[1] Stepankova K, Jendelova P, Machova Urdzikova L. Planet of the AAVs: the spinal cord injury episode. Biomedicines. 2021 May 28;9(6):613.

[2] Sauernheimer C, Williams KM, et al., J Pharmacol Toxicol Methods. 1994 Nov;32(3):149-54.

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