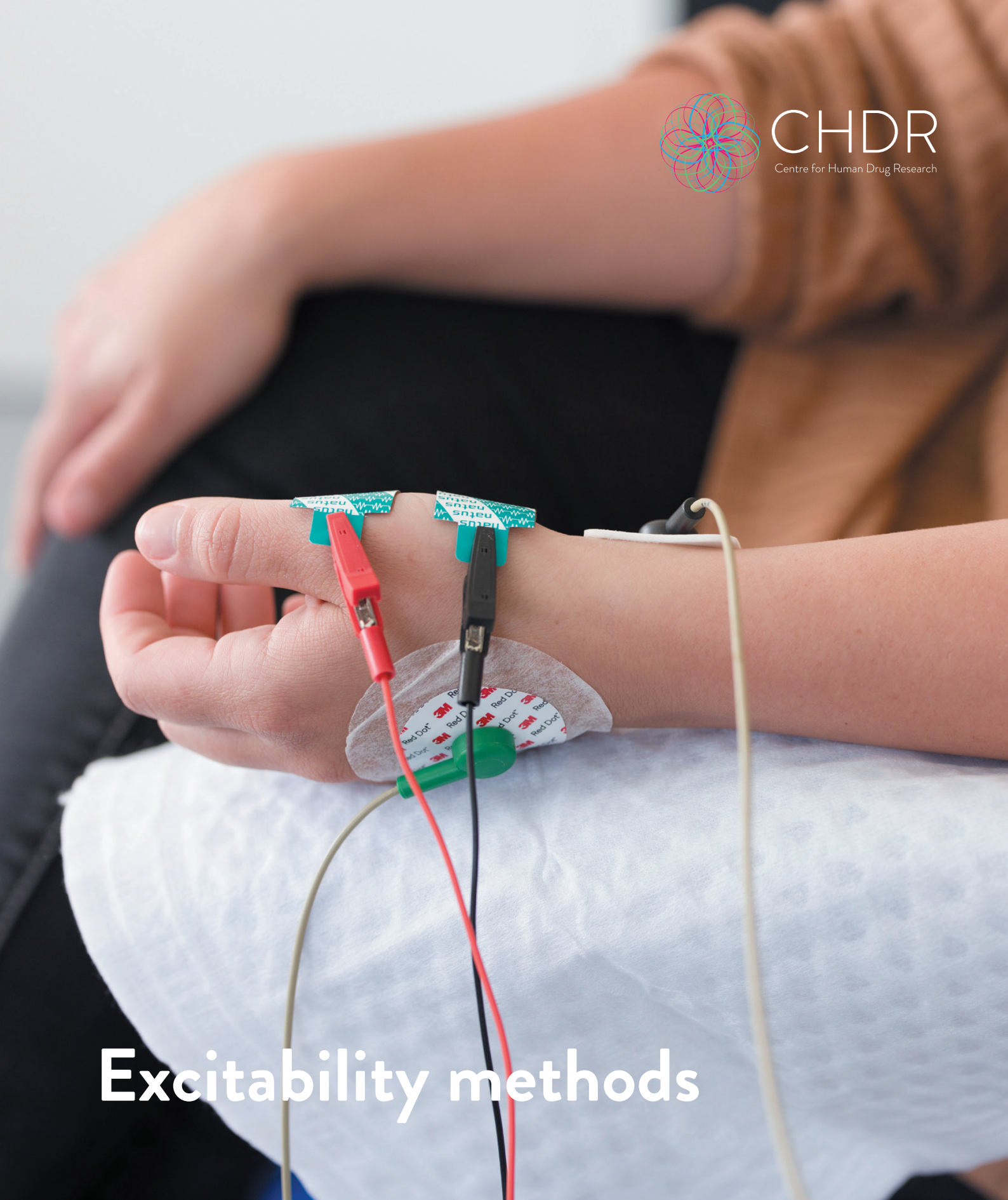


Excitability methods

What CHDR offers

- Transcranial magnetic stimulation (TMS) coupled with EMG and EEG for measuring corticospinal excitability
- Nerve excitability threshold tracking (NETT) of the motor and sensory nerves for measuring peripheral nerve excitability
- Muscle velocity recovery cycles (MVRC) for measuring muscle excitability

Excitability methods

CHDR offers a range of validated, in-house methods for measuring excitability. Such measures can serve as valuable pharmacodynamic biomarkers in early phase clinical trials, providing the means to establish proof-of-mechanism, target engagement and proof-of-concept for new pharmacological interventions targeting excitability.

Measuring excitability

Signal transmission is a critical physiological process. For certain cells, this transmission is electrical in nature. Excitability is determined by how these cells regulate, generate, and conduct electrical stimuli. Excitability can be measured at a central (cortical excitability) or peripheral level (peripheral nerve and muscle excitability). A growing body of evidence suggests that abnormal excitability plays an important role in a wide variety of conditions including epilepsy, ALS, (neuropathic) pain, psychiatric disorders, polyneuropathy and muscle disorders.

CHDR offers validated methods that can be used as biomarkers to measure excitability at all three levels:

Corticospinal excitability:
Transcranial magnetic stimulation (TMS) coupled with EMG and EEG

Peripherical nerve excitability:
Nerve excitability threshold tracking (NETT) of the motor and sensory nerves

Muscle excitability:
Muscle velocity recovery cycles (MVRC)

These methods evaluate the excitability of the target tissue and shed light on the underlying mechanisms, including (dys)function of the specific receptors and ion channels that determine the excitability. Therefore, these methods can serve as valuable biomarkers in the development of pharmacological interventions that target these mechanisms. This yields deeper insights, enabling decision-making based on pharmacodynamics, as a complement to safety and pharmacokinetics.

CHDR has validated all three methods in a clinical setting, and we have published studies showing pharmacological effects of different compounds on excitability using each of these methods. Read more about each method on the following pages.

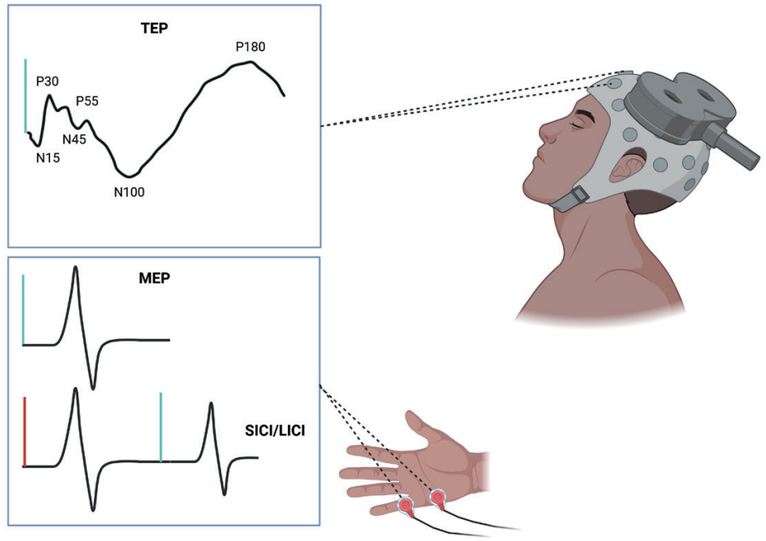
Choosing excitability methods for your study

Excitability methods promise deeper insights into drug action for a range of early-phase clinical trials:

- Early-phase trials of agents targeting neuronal hyper- or hypo-excitability observed in diseases such as depression, epilepsy or ALS could greatly benefit from including TMS and/or NETT, providing the opportunity to show proof-of-mechanism.
- Including NETT in a trial investigating an agent targeting sodium channels for neuropathic pain would provide more information on exact target engagement and dose selection.
- Including MVRC in a trial targeting muscle excitability with chloride- or sodium-channel compounds would help determine target engagement and dose.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) coupled with electromyography (EMG) and electroencephalography (EEG) can be used as a biomarker to study the clinical effects of drugs that are expected to affect cortical excitability. Both single-pulse and paired-pulse TMS stimulation protocols have been implemented at CHDR and can be customised according to study design. Abnormal cortical excitability is observed in various conditions including ALS, epilepsy and depression. Drug targets that would benefit from applying this biomarker include GABA, AMPA, glutamate and voltage-gated sodium channels.



Transcranial magnetic stimulation (TMS) combined with electro-encephalography is used to explore the cortical response using a TMS-evoked potential (TEP) (upper graph). TMS combined with electromyography is used to evaluate the motor-evoked potential (MEP) and short- and long intracortical inhibition (SICI/LICI) at the abductor digiti minimi (lower graph). Created in BioRender.com.

A novel biomarker with great potential

TMS-EMG – TMS targeted at the motor cortex results in motor evoked potentials (MEPs) in a peripheral muscle, which can be measured with EMG. Single-pulse TMS-EMG has the potential to demonstrate corticospinal excitability, while paired-pulse TMS-EMG focuses on cortical excitability by investigating the relative contribution of inhibitory and excitatory networks.

TMS-EEG – TMS combined with EEG allows direct and non-invasive measurement of the cortical response to TMS stimulation, reflected in a TMS-evoked potential (TEP). TMS-EEG makes it possible to stimulate and evaluate responses of brain areas outside the commonly targeted motor cortex, with a spatial resolution of around 10 mm and millisecond temporal resolution.

Our work with TMS-EMG and TMS-EEG

Together with the Clinical Neurophysiology (CNPH) Group of the University of Twente – one of our closest collaborators in the field of cortical excitability testing – we have evaluated the effects of levetiracetam, valproic acid and lorazepam on cortical excitability in healthy volunteers. Our findings indicate that these drugs show significant effects on cortical excitability as measured by single-pulse and paired-pulse TMS-EMG and TMS-EEG.[1]

Further clinical trials at CHDR involving TMS include a study of TMS-EMG as a possible biomarker for AMPA receptor modulation[2], and an investigation of ketamine effects on TMS-EMG and TMS-EEG, examining changes in excitability that might be related to the anti-depressant effect of ketamine. We are also studying the effects of levetiracetam on TMS-EMG and TMS-EEG in patients with epilepsy, evaluating whether TMS can be used as a biomarker for efficacy of treatment in these patients.

Technical specifications

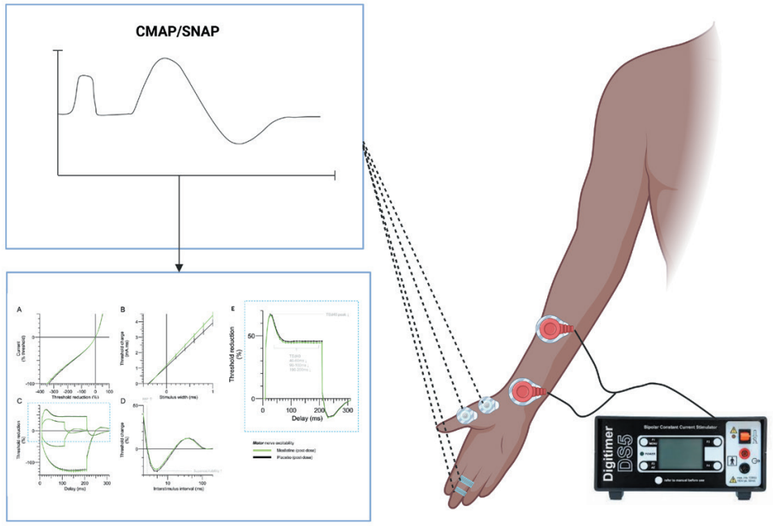
- MagPro R30 with MagOption stimulator (MagVenture GmbH, Hückelhoven, Germany)
- MCF-B65 butterfly coil (2x75mm) (MagVenture GmbH, Hückelhoven, Germany)
- 32-channel TMS compatible EEG system (EEG amplifier: TMSi, Oldenzaal, the Netherlands; EEG caps: ANT Neuro, Enschede, the Netherlands)
- NeuroCenter software (Clinical Science Systems, Leiden, the Netherlands)

[1] Ruijs, T. Q., Heuberger, J. A., de Goede, et al. Transcranial magnetic stimulation as biomarker of excitability in drug development: A randomized, double-blind, placebo-controlled, cross-over study. *British Journal of Clinical Pharmacology*. 2022; 88(6); 2926-2937.

[2] O'Donnell, P., Dijkstra, F. M., Damar, et al. Transcranial magnetic stimulation as a translational biomarker for AMPA receptor modulation. *Translational Psychiatry*. 2021; 11(1); 1-10.

Nerve excitability threshold tracking (NETT)

Nerve excitability threshold tracking is a technique used to measure the excitability of the peripheral motor or sensory nerves. Using a standardised and validated stimulation protocol, it is possible to assess a range of nerve properties, such as resting membrane potential and sodium and potassium channel conductance. NETT also detects nerve excitability abnormalities in conditions such as ALS, neuropathic pain and polyneuropathy. Drug targets that could benefit from applying this biomarker include potassium channels and voltage-gated sodium channels.



Nerve excitability threshold tracking (NETT) uses electrical stimulation of the median nerve (red electrodes) to measure the amplitude of the compound muscle action potentials (CMAP) at the abductor pollicis brevis and the peak-to-peak amplitude of the sensory nerve action potentials (SNAP) at digit three (upper graph). A stimulation paradigm is used to evaluate different excitability properties (lower graph shows NETT recording). Created in BioRender.com.

Broad insight into nerve properties

NETT is performed at CHDR using QTRAC software. The standard stimulation protocol, TRONDNF, includes four paradigms to interrogate the nerve. These include the strength-duration relationship, recovery cycle, threshold electrotonus and current-threshold relationship. The latent addition paradigm can also be implemented. Measurements are supervised and performed by specialised staff at CHDR, who have received training from world experts in the field of NETT.

Our work with NETT

The NETT method has been clinically validated at CHDR in a study investigating the effects of two sodium channel blockers — mexiletine and lacosamide — on threshold tracking of motor and sensory nerves in 18 healthy subjects. Treatments were compared to placebo in a randomised three-way cross-over design, with measurements being performed at baseline and at two post-dose timepoints. Both drugs showed a multitude of clear significant effects on NETT parameters versus placebo. These effects are in line with reduced excitability of the peripheral motor and sensory nerves.[3]

We have also performed further clinical trials using threshold tracking, such as a study of the effects of retigabine and riluzole on axonal excitability in patients with ALS.[4]

Technical specifications

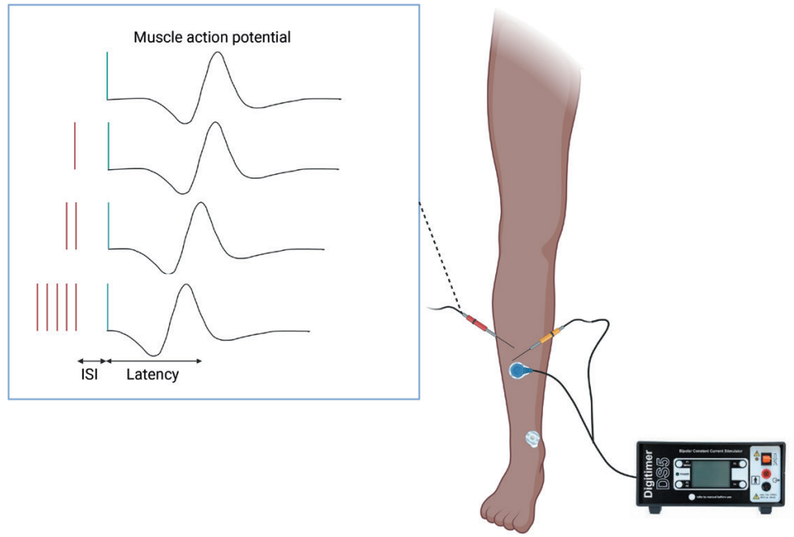
- Digitimer DS5 electrical stimulator
- Digitimer D440-2 EMG amplifier
- Quest Scientific Hum Bug Noise Eliminator
- GenTherm Norm-O-Temp 111W hyperthermia system with accessory hoses
- QTRAC software (UCL Institute of Neurology, London, UK) including TROND protocol

[3] Ruijs, T. Q., Koopmans, I. W., de Kam, et al. Effects of mexiletine and lacosamide on nerve excitability in healthy subjects: A randomized, double-blind, placebo-controlled, crossover study. *Clinical Pharmacology & Therapeutics.* 2022; 112(5):1008-1019.

[4] Kovalchuk, M. O., Heuberger, J. A., Sleutjes, et al. Acute effects of riluzole and retigabine on axonal excitability in patients with amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled, crossover trial. *Clinical Pharmacology & Therapeutics.* 2018; 104(6):1136-1145.

Muscle velocity recovery cycles (MVRC)

Muscle velocity recovery cycles (MVRC) is a technique used to assess the excitability of muscle cells, independent of neuromuscular transmission. Using a standardised and validated stimulation protocol, MVRC can be used to determine a range of muscle excitability properties, such as relative refractory period and the physiological depolarising afterpotentials (early and late supernormality). Abnormal muscle cell excitability can be detected in neuropathies, myotonias and channelopathies. Drug targets that would benefit from applying this biomarker include chloride and sodium channels.



For muscle velocity recovery cycles (MVRC), electrical stimulation of the tibial anterior muscle fibres using a needle electrode (yellow) creates a muscle action potential, which is recorded using a second needle electrode (red). The latency of the muscle action potential is measured after single test pulses (blue vertical line), and 1, 2 or 5 conditioning pulses (red vertical lines) followed by a test pulse (blue vertical line) at different interstimulus intervals (ISI). Created in BioRender.com.

High-quality, validated assessments

At CHDR, MVRC is performed using QTRAC software. We perform recovery cycles with one, two and five conditioning stimuli, as well as the frequency ramp protocol. Measurements are supervised and performed by specialised staff at CHDR, who have been trained by world experts in the application of MVRC technique.

Our work with MVRC

The MVRC method has been validated at CHDR in a study investigating the effects of mexiletine (a voltage-gated sodium channel blocker and oral lidocaine analogue) on MVRC in 12 healthy subjects. Mexiletine was compared to placebo using a randomised two-way cross-over design, with measurements being performed at baseline and at two post-dose timepoints. Results showed significant effects of mexiletine that indicate reduced excitability of the muscle cells, as would be expected from a sodium channel blocker.[5]

We have also performed MVRC in a first-in-human study, to investigate the effects of a CI-C1 blocker on MVRC.

Technical specifications

- Digitimer DS5 electrical stimulator
- Digitimer D440-2 EMG amplifier
- Quest Scientific Hum Bug Noise Eliminator
- Daylight heat lamp D/E31150/9 with a 250 watt infrared light bulb, E27, 220-240V / 50 Hz (General Electric)
- QTRAC software (UCL Institute of Neurology, London, UK) including MVRC protocol

[5] Ruijs, T Q., Koopmans, I. W., de Kam, et al. Muscle velocity recovery cycles as pharmacodynamic biomarker: Effects of mexiletine in a randomized double-blind placebo-controlled cross-over study. *Clinical and Translational Science*. 2022; 10.1111/cts.13418.

Why choose CHDR?

The Centre for Human Drug Research specialises in early-phase clinical drug research. CHDR's overall mission is to improve the drug development process by collecting as much information as possible regarding the candidate drug in the early phases of development. This information helps sponsors make informed decisions regarding the course of clinical development for their product.

Why choose CHDR?

Research at CHDR covers a wide range of fields, including the central nervous system (CNS) and pain, the cardiovascular system, haemostasis, immunology, and dermatology. In addition, CHDR is at the forefront in developing novel biomarkers and methods for measuring drug-related effects in all of these research areas.

Pharmacology matters

Whether studying a new cognitive-enhancing drug, a next-generation painkiller, or a new monoclonal antibody designed to treat rheumatoid arthritis, the goal is to determine how the compound's effects correlate with both the dose and blood concentration at any given moment. In addition, understanding which biological systems are activated is an essential first step towards quantifying this relationship. At CHDR, our focus on pharmacology is reflected clearly in what we call question-based drug development.

Question-based drug development

CHDR actively uses question-based drug development - or QBD - as a more rational approach to drug development compared to conventional approaches. QBD can be best described as a series of questions that are addressed throughout the process. These questions often seem simple enough, but failing to answer even one question - or even addressing the questions in the wrong order - can have dire consequences. Thus, using this approach can potentially save companies millions of dollars by helping predict a catastrophic issue early in the development process, before the more expensive latter stages (for example, large-scale clinical trials or the marketing phase).

From a general perspective, the most important questions are:

1. Does the biologically active compound and/or active metabolite(s) reach the intended site of action?
2. Does the compound cause its intended pharmacological and/or functional effect(s)?
3. Does the compound cause any unintended pharmacological and/or functional effect(s)?
4. Does the compound have a beneficial effect on the disease and/or clinical pathophysiology?
5. What is the compound's therapeutic window?
6. How does any variability with respect to the drug response in the target population affect the product's development?

Contact

To learn about CHDR's full range of services, contact us today.

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