Safety Pharmacology Society Webinar:
Safety Pharmacology of the Nervous System

Will Redfern, PhD
Drug Safety & Metabolism
AstraZeneca UK
Basic cell – neuron(e)

All cells have a resting membrane potential. Excitable cells (e.g. neurones and muscle cells) can depolarise to produce an ‘action potential’ in response to an appropriate stimulus. In neurones the action potentials are propagated from the cell body along the axon to the axon terminals.

Effector neurones terminate on either muscle cells (smooth, skeletal or cardiac) or secretory cells (endocrine or exocrine). Sensory neurones terminate on dendrites of neurones located in the central nervous system. Neurones within the central nervous system terminate on dendrites of other neurones in the central nervous system.
Neurones don’t exist in isolation, and are supported by glial cells*, which provide both structural and biochemical/nutritional support. Neurones communicate with each other largely via specialised junctions called **synapses**.

‘Neurotoxicity’ can involve any of these elements, but most ‘safety pharmacology’ effects on neurones involve an interaction at the synapse…

*Glial cells are non-neuronal, and include astrocytes (functions include mopping-up neurotransmitters and ions released during neurotransmission), microglia (macrophages which respond to brain injury), and oligodendrocytes (form the myelin sheath of myelinated neurones).
Neurotransmitters can be either excitatory or inhibitory with respect to the downstream neurone, depending on the neurotransmitter and the post-synaptic receptor subtypes.

The **synapse** is the predominant site for acute (i.e., safety pharmacology) effects.

Axon terminals (terminal boutons) release neurotransmitter(s) at the synapse on arrival of an action potential.
Macro-organisation of the nervous system...

**NERVOUS SYSTEM**

- **CNS**
  - Brain
    - Forebrain
    - Midbrain
    - Hindbrain
  - Spinal Cord
- **PNS**
  - Autonomic
    - Sympathetic
    - Parasympathetic
  - Somatic
The nervous system can be viewed either in its full complexity...

...or in the following simple way...
Some key features of the **autonomic nervous system**…

Parasympathetic: ‘Rest and digest’

Sympathetic: ‘Fight or flight’
There are numerous different neurotransmitter pathways within the brain. Here are just 4, each with its own distribution, but with some overlap…
### Stage of first detection of commonly-observed nervous system adverse effects (AEs)

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<th>In vitro</th>
<th>Preclinical tox/SP</th>
<th>Phase I</th>
<th>Phase II-III</th>
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<td>Cognitive dysfunction</td>
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<td>Auditory dysfunction</td>
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<td>Suicidality</td>
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- ■ Reasonable ability to detect/predict the AE (but may or may not be available/performe)
- ■ AE detected/detectable

'In vitro' includes primary pharmacology and secondary pharmacology profiling, cell-based and tissue-based assays, and larval zebrafish assays.

Note that there are animal models of each AE in the above table, with variable knowledge of their translation to humans, and differing complexity and applicability. But, resources being finite, pharma companies tend to focus on what is required by regulatory authorities!
Approaches to studying adverse effects on the nervous system preclinically

**IN VITRO**

- Neuronal cultures
- In vitro electrophysiology (ion channels; neurones; slices)

**IN VIVO**

- Behavioural/neurological
- Neurophysiological recordings (e.g. EEG; ERG; EMG; BAER; nerve conduction velocity)
- Neurochemical (e.g. in vivo microdialysis; biomarkers)
- Neuroimaging (e.g. MRI; MRS; PET; SPECT)

**POST MORTEM**

- Neurohistopathology

Core battery studies
Motor activity, behavioural changes, coordination, sensory/motor reflex responses and body temperature should be evaluated. For example a Functional Observation Battery (FOB), modified Irwin’s or other appropriate test can be used.

Follow-up studies
Behavioural pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory and/or electrophysiology examinations, etc.
CNS Safety Pharmacology Core Battery Studies

- The principal core battery test should assess multiple behavioural, neurological and autonomic parameters in the same animals.
- The collective name for such tests is ‘neuro-behavioural assessment’ tests\(^1\).
- The 2 main tests used are either the **Irwin test\(^2\)** or the **Functional Observational Battery (FOB\(^3\))**. Both are systematic evaluations of nervous system function.

Global nervous system assessment: The Irwin test/FOB

A multi-parameter assessment of nervous system function in rodents

**AUTONOMIC**
- salivation
- lacrimation
- piloerection
- abnormal urination
- abnormal defaecation
- abnormal respiration
- pupil size
- rectal temperature

**NEUROMUSCULAR**
- posture
- gait
- Straub tail
- body tone
- ptosis
- exophthalmos
- grip strength
- traction response
- tremor
- twitches
- convulsions

**SENSORIMOTOR**
- touch response
- palpebral reflex
- startle reflex
- pinna reflex
- righting reflex

**BEHAVIOURAL**
- arousal
- spontaneous activity level
- vocalisation
- aggressiveness
- sniffing
- grooming
- scratching
- rearing
- stereotypy
- bizarre behaviour
- activity

*Plus:* any miscellaneous observations; body weight gain overnight post-dose
Useful reviews


Why this paper?

1. It’s a review, with 37 references;
2. With respect to nervous system safety pharmacology it covers most of the more obvious in-life in vivo methods (except seizure risk and abuse liability);
3. Although it’s over 10 years old, it is a very good starting point.
Why this paper?

1. It’s a review, with 253 references;
2. With respect to nervous system safety pharmacology it does cover some of the more obvious in-life in vivo methods (except seizure risk and abuse liability);
3. It’s also useful for all other aspects of safety pharmacology.
NB. This is a good source of references on these techniques, some of which (upper table) are also suitable for inclusion in repeat-dose toxicity studies.
Why this paper?

1. It’s a review, with 63 references;
2. It distils all you really need to know about seizure liability of drugs for the exam, including some of the known secondary pharmacology risks, and in vitro and in vivo methodology;
3. It proposes a strategy for detecting, assessing and addressing seizure liability early in Drug Discovery.
Why this paper?

1. It’s a review, with 63 references;
2. It distils all you really need to know about abuse-dependence liability of drugs for the exam, including some of the known secondary pharmacology risks, and methodology;
3. It proposes a strategy for detecting, assessing and addressing abuse-dependence liability.
There are no reviews on ‘Cognition tests for rodents in safety pharmacology studies’!

References for some tests are in Porsolt (2002) and Redfern & Wakefield (2006)

Decision as to which test is most appropriate reflected in the title of the following paper:

**Which Memory Task for My Mouse? A Systematic Review of Spatial Memory Performance in the Tg2576 Alzheimer’s Mouse Model**

*Journal of Alzheimer’s Disease*  
*IOS Press*  
*ISSN: 1387-2877 (Print) 1875-8908 (Online)*  
*Subject: Medicine, Clinical Neurology and Internal Medicine*  
*Issue: Volume 26, Number 1 / 2011*  
*Pages: 105-126*

Simple message...look at the following slide and track down papers yourself!
Avoidance tasks

- Passive Avoidance: Exploits a natural tendency of mice to enter dark environments.
  - Unidirectional: mouse goes from light to dark chamber.

- Active Avoidance: Mouse learns to avoid shock based upon the presentation of a light cue.
  - Unidirectional: mouse is always shocked in the same chamber/location.

- Shuttle Avoidance: Mouse learns to avoid shock based upon the presentation of a light cue which is dependent upon location of the mouse in the apparatus.
  - Bi-directional: mouse learns to monitor for cues in both chambers that predict shock.

Maze tasks

- Y-maze*
  - Maze tasks with two arms extending from a common starting point.
  - Rat learns to find the correct path.

- T-maze*
  - Maze tasks with three arms extending from a common starting point.
  - Rat learns to choose the correct path.

- 8-arm radial maze*
  - Maze tasks with 8 arms extending from a central point.
  - Rat learns to find the correct food source.

- Morris water maze
  - Maze tasks in liquid environment.
  - Rat learns to find a hidden platform.

Operant tasks*

- Rat learns to press correct lever in response to a conditioning cue (e.g., sound; light) – receives food reward.
  - Delayed matching-to-sample
  - Delayed non-matching-to-sample

Object recognition tasks

- Rat learns to recognize objects based on their features.

*Require a partial food restricted diet (so that rats will work for a food reward)
Good luck in the exam in October!