Neurotoxicity Study Design: The Essential Element of Location Assessment

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#435

Location of neuronal degeneration

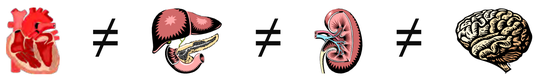
Each of the more than 600 different anatomic populations of the brain has a unique profile with regards to toxicity and each element warrants consideration. Each population of the brain has different cell types, connectivity, and functionality. Our understanding of the brain has been increasing exponentially but we still do not fully understand the comprehensive functions of each population or the interactions of all the populations. In this study, examining the neurotoxic profiles identified for various compounds we find that:

(1) Cells impacted by a neurotoxic compound are rarely widespread and are more often in small and specific regions of the brain.
(2) Neurotoxins often affect just one or perhaps several distinct and possibly distant regions.
(3) Affected regions can be very small, but functionally significant.
(4) The location of effects is unpredictable based on other pathologic and behavioral indicators and even between compounds that share similar chemical structures.

Although our understanding of the brain is perhaps not as complete as with other organs, we do not know of any regions of non-importance. These findings highlight the importance of adopting a well-defined safety assessment approach that examines all elements of the brain. Based on our research, we find that 50-60 uniformly-spaced coronal sections provide a complete, comprehensive sample of the brain's diverse structures.

The Brain is a "Multi Organ" structure: Each region of the brain has unique vulnerabilities to neurotoxins

Different organs are of course considered independently during routine toxicity assessments

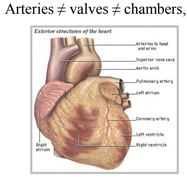


Heart ≠ Liver ≠ Kidney ≠ Brain, etc.

As with other many organs, it is appropriate to independently assess major structures for unique vulnerabilities.

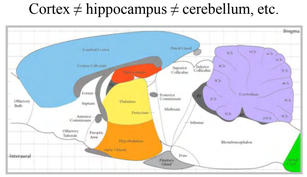
Heart

Arteries ≠ valves ≠ chambers, etc.



Brain

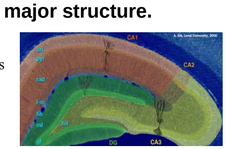
Cortex ≠ hippocampus ≠ cerebellum, etc.



In the brain, it is important to appreciate that neurodegeneration is more likely to occur in a specific subpopulation than an entire major structure.

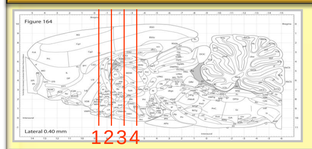
Within a major structure like Hippocampus:
CA1 ≠ CA3 ≠ ventral dentate gyrus ≠ dorsal dentate gyrus

Each major region of the brain is comprised of multiple and independently vulnerable subpopulations.

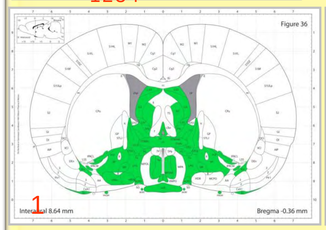


There is no "appendix of the brain". Every population/structure warrants consideration and each must be assessed individually.

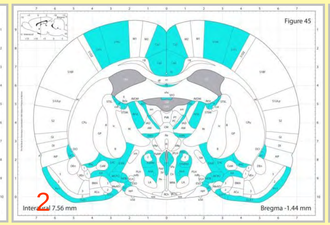
Significantly different populations are present in levels just one mm apart



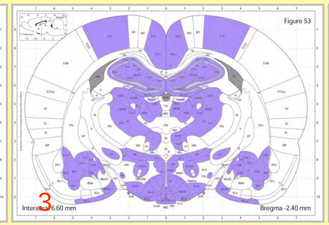
A rat brain is ~21 mm long. This sagittal image of the rat brain shows the location (red lines) of 4 coronal cross sections that are depicted below. The cross sections are only 1mm apart, yet there are quite significant changes in the structures that could possibly be sampled from one section to the next. Shading indicates structures that "disappear" when viewing the next level.



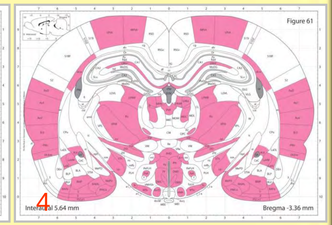
35 structures seen that are not visible 1mm posterior→



←55 structures seen that are not visible 1mm anterior
45 structures seen that are not visible 1mm posterior→

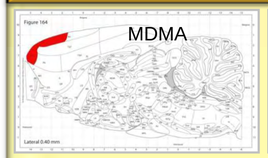


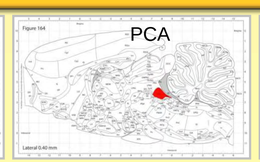
←62 structures seen that are not visible 1mm anterior
33 structures seen that are not visible 1mm posterior→

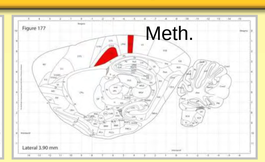


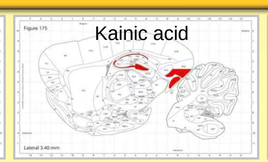
←48 structures seen that are not visible 1mm anterior

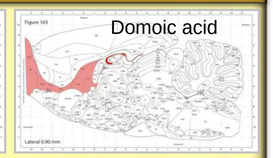
Neurodegeneration is most often confined to specific, small populations for any given toxin









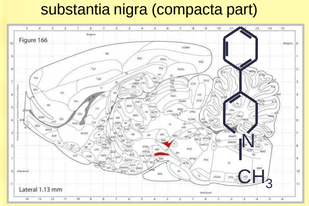


The shading is a depiction of the area in which of these known, prolific neurotoxins causes neurodegeneration. It is normal for neurotoxins to target very specific and discrete populations in the brain. Appreciation of the damage caused can only be accomplished by sampling sections containing the appropriate population susceptible to the particular neurotoxin. Prior to a full evaluation,

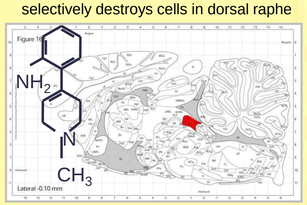
Similar compounds can effect very different populations

The location at which neurodegeneration may occur is UNPREDICTABLE. All populations must be considered for each compound tested. This example highlights the difference in location (and effect) between two similarly structured neurotoxins:

MPTP:
destroys cells in the VTA and substantia nigra (compacta part)



2'-NH_2-MPTP:
selectively destroys cells in dorsal raphe



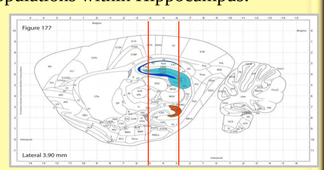
MPTP damages the dopaminergic system while 2'-NH_2-MPTP damages the serotonergic system

The neurotoxic profile of a compound is often a poor predictor of potential neurotoxicity of compounds of similar class, structure or mechanism. All populations must be assessed.

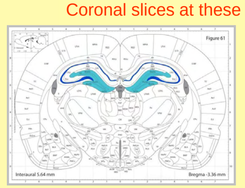
Within the same "Major" structure, specific neurotoxins effect different populations

Assessing a major division of the brain requires sampling from individual populations **within** that region. This example depicts the effects of 3 compounds that impact different populations within Hippocampus:

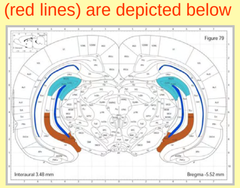
Domoic acid destroys cells in the **pyramidal layer of hippocampus**
PCP destroys cells in **dorsal dentate gyrus**
Alcohol destroys cells in **ventral dentate formation**



Coronal slices at these levels (red lines) are depicted below



In a commonly used view of hippocampus, ventral structures cannot be seen. Alcohol effects would not be observable.



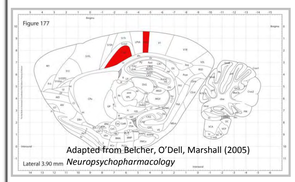
A more posterior section allows ventral structures to be seen

Case Study: Neurodegeneration can only be observed in locations that are evaluated

Two separate studies evaluated D-amphetamine for neurodegeneration. Neurodegeneration was missed in the first study since only expected areas of damage were evaluated

Study #1:

A limited area of cell death was witnessed

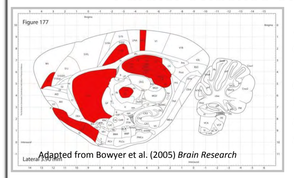


In this study, researchers anticipated, looked for and found that D-amphetamine destroys cells in parietal cortex and somatosensory barrel field cortex.

While the positive findings were correct, the conclusion was incomplete.

Study #2:

Further evidence of cell death was observed



Another group of researchers looked elsewhere and confirmed that D-amphetamine destroys cells in parietal cortex and somatosensory barrel field cortex **as well as** the frontal cortex, piriform cortex, hippocampus, caudate putamen, VPL of thalamus, and (not shown): *tenia tecta, septum and other thalamic nuclei*

Lesson: Neurotoxicity can occur in unexpected locations. Look everywhere-not just where expected.

Recommended Approach to Sampling

The assessment of each population within the brain is a foundation principle in neurotoxicity assessments. A well-designed approach is required to achieve this requirement in an efficient manner. In rats, sampling every 350µ-450µ is adequate and translates to ~50-60 evenly spaced coronal sections. Conveniently, for any species, sampling the same number of levels provides comparable representation.

Suggested Sampling Rates by Species

	Brain Length (mm)	Interval (mm)
Mouse	12	0.20
Rat	21	0.35
Monkey	65	1.08
Dog	75	1.25

Yields ~60 evenly spaced coronal sections

A sampling rate of 50-60 levels achieves adequate sampling for routine safety assessments.

The most commonly observed study design pitfalls are:

- Looking only where damage is expected
- Failing to assess all populations

For more information about neurotoxicity study design principles, a detailed presentation may be found @ http://www.nsalabs.com/Presentations/neurotox_study_design.zip

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