Outcome Assessment of Non-Matrix-Assisted Trimming Utilizing Anatomic Landmarks for Reliable Canine Brain Sampling in Nonclinical General Toxicity Studies

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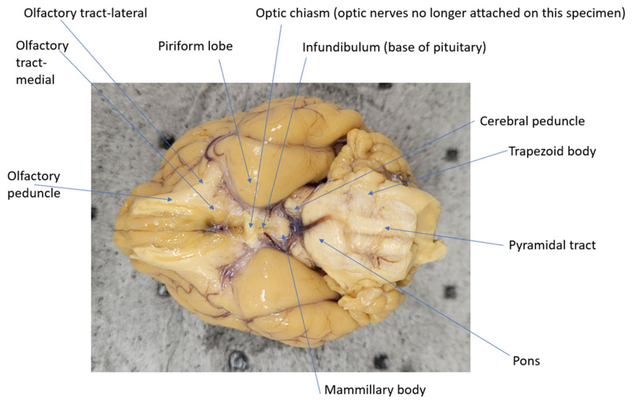

Figure 1. External landmarks can be utilized in a free-hand approach to sample required neuroanatomic structures for evaluation in nonclinical general toxicity studies

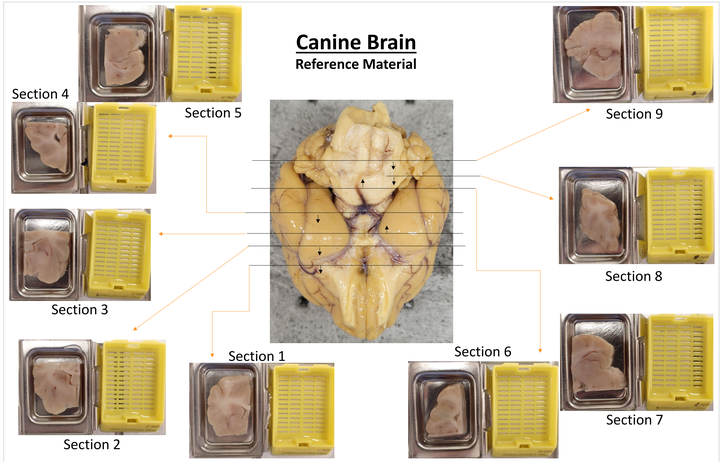

Figure 2. Seven sections are collected as indicated (horizontal black lines, arrow side face down) and further sectioned into nine cassettes as indicated.

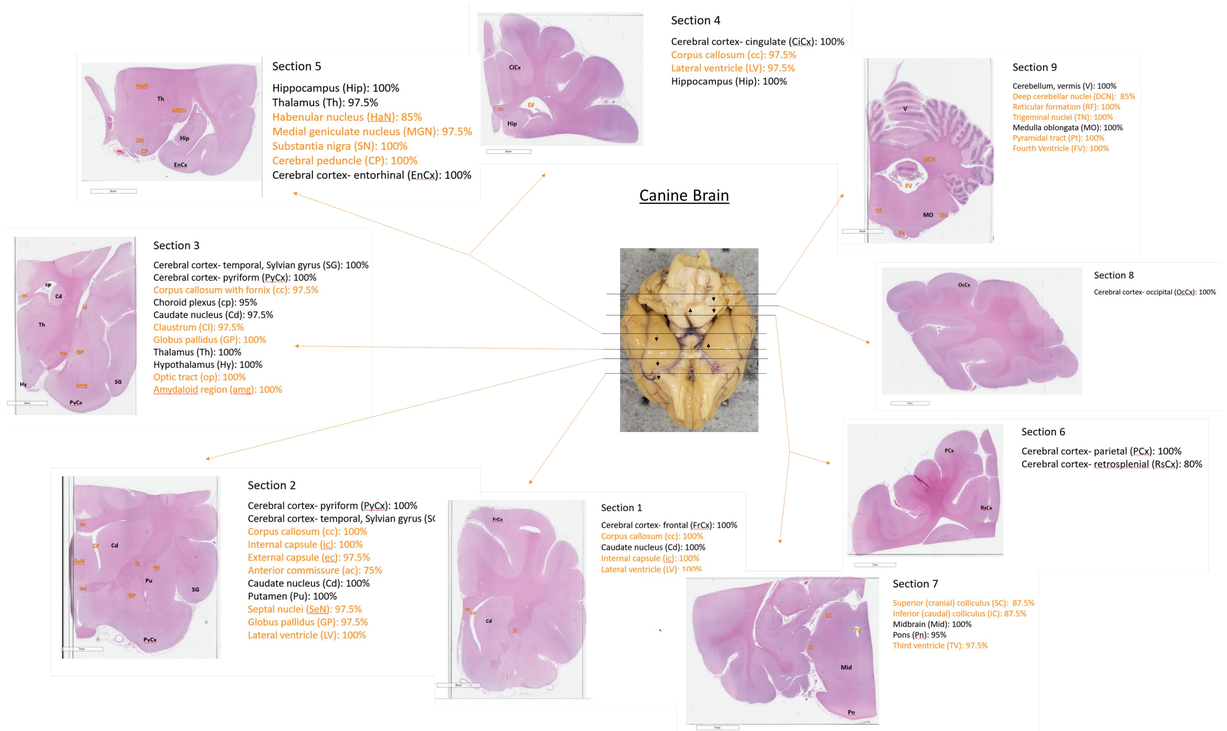

Figure 3. Nine sections were examined to determine the presence or absence of listed structures. Structures in black were considered "core" and structures in orange "supplemental." Capture rate for each core (black) and supplemental (orange) structure is recorded for each slide.

Introduction

Microscopic evaluation of the nervous system is an important component of nonclinical general toxicity studies. The Society of Toxicologic Pathology (STP) has published recommended practices for the sampling of the nervous system in nonclinical general toxicity studies for rodent and non-rodent species, including Beagle dogs.[1] The guidance outlines neural structures to be sampled including caudate/putamen, cerebellum, cerebral cortex, choroid plexus, hippocampus, hypothalamus, medulla oblongata, midbrain, pons, and thalamus. Additionally, technical guides have been published that offer general recommendations with regards to trimming schemes to facilitate sampling. Published sampling guides in dogs have had practical caveats, utilizing specialized matrix-cutting device or requiring extrapolation from trimming of other large animal species such as nonhuman primate brains.[2-6] Utilizing published recommended practices and available guidance, we developed a canine brain trimming scheme that utilized free-hand (non-matrix-assisted) targeting of external anatomic landmarks and could be implemented by trained laboratory staff to consistently capture recommended neuroanatomic structures for evaluation in nonclinical general toxicity studies.

Objective

This study was designed to evaluate the developed canine brain trimming scheme for consistent capture of recommended neuroanatomic structures.

Methods

Coronal sections were acquired from 40 Beagle dog brains, processed routinely according to the targeted free-hand canine brain trimming procedure utilizing readily identifiable external anatomic landmarks (1) olfactory tracts and rostral piriform lobes; (2) optic chiasm; (3) infundibular slit (4) cerebral peduncles and pons; (5) caudal pons, cerebellum; (6) occipital lobe; and (7) cerebellar vermis and parafolliculi (figures 1 and 2). Sections were trimmed, processed in a routine manner, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and processed to slide for pathologist review. Slides were independently evaluated by two pathologists (ML and CH) for the presence of 11 core and 22 supplemental targeted neuroanatomic structures. 11 core structures consisted of cerebral cortex, caudate nucleus, putamen, choroid plexus, thalamus, hypothalamus, hippocampus, midbrain, pons, cerebellum, and medulla oblongata. Supplemental structures included corpus callosum, internal capsule, lateral ventricle, external capsule, anterior commissure, septal nuclei, globus pallidus, claustrum, optic tract, amygdaloid region, habenular nucleus, medial geniculate nucleus, substantia nigra, cerebral peduncle, superior (cranial) colliculus, inferior (caudal) colliculus, third ventricle, deep cerebellar nuclei, reticular formation, trigeminal nuclei, pyramidal tract, and fourth ventricle. Capture rates were calculated for each section and structure.

Results

Consistent evaluation of recommended core structures was achieved when coronal sections or hemisections of the brain were collected using readily identifiable external landmarks. Utilizing major external landmarks, the majority of core structures (caudate, putamen, cerebellum, cerebral cortex, hippocampus, hypothalamus, medulla oblongata, midbrain, and thalamus) were captured in 100% of examined brains in at least one section (figure 3). The pons and choroid plexus were identified in 95% of examined brains. All of the other 22 supplemental structures had ≥75% capture rate. Eleven of those (corpus callosum, internal capsule, lateral ventricle, globus pallidus, optic tract, amygdaloid region, substantia nigra, cerebral peduncle, trigeminal nuclei, pyramidal tract, and fourth ventricle)e were captured 100% of the time in at least one section. The anterior commissure was the least often captured of supplemental structures but was identified in 75% of examined sections.

Conclusions

Consistent capture of recommended neuroanatomic structures was achieved utilizing free-hand targeting of major external landmarks. For the purposes of this evaluation, no additional tissue recuts or reharvesting were attempted, however it is likely that routine rework (recutting or reharvesting) efforts would yield improved capture rates.

This targeted, free-hand trimming procedure required no special equipment and was implemented with standard training processes. A canine brain trimming scheme utilizing free-hand targeting of external anatomic landmarks can be successfully deployed by trained laboratory staff and yield reliable capture of recommended neuroanatomic structures for evaluation in canine nonclinical general toxicity studies.

References

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Acknowledgements

The authors are grateful for the support of pathology and histology staff across Inotiv sites.