



Allen Reference Atlases

One of the primary goals of the Allen Brain Atlas (ABA) is to create a cellular-resolution, genome-wide map of gene expression in the mouse brain. To complement ABA gene expression data, Allen Reference Atlases (ARAs) have been designed and created by Dr. Hong Wei Dong in the coronal and the sagittal plane. The reference atlases are full-color, high-resolution, web-based digital brain atlases accompanied by a systematic, hierarchically organized taxonomy of mouse brain structures. The ABA and ARA data are obtained, using identical methodology, from 8-week old C57Bl/6J male mouse brain(s) prepared as unfixed, fresh-frozen tissue.

The ARAs were designed to:

- (I) Allow users to directly compare gene expression patterns to neuroanatomical structures in the ABA Application
- (II) Serve as templates for the development of 3D computer graphic models of mouse brain, providing a foundation for the development of informatics based annotation tools
- (III) Provide a standard neuroanatomical ontology for determining structural annotation and aid in the construction of a detailed searchable gene expression database

The coronal ARA consists of 132 coronal sections evenly spaced at 100 μm intervals and annotated to a detail of numerous brain structures. Examples of these images are shown in **Figure 1**. The sagittal ARA consists of 21 representative sagittal sections spaced at 200 μm , annotated for 71 major brain regions identified at the top level(s) of the brain structure hierarchal tree (**Appendix 1**). In the sagittal ARA, a number of cell groups are used as landmarks to indicate specific brain levels, i.e. the red nucleus and cranial motor nuclei.

Ontology and Brain Structure Tree Development

ARA ontology is arranged as a hierarchal organization of brain structures, with prioritization levels identified in the brain structure hierarchal tree (**Appendix 1**). ARA nomenclature was adopted from the Swanson (2004) rat brain atlas; however the hierarchical organization of the brain structures provided by Swanson has been modified. Nomenclature was also adopted from Hof *et al.* (2000) mouse brain atlases. The ARAs also referenced two informatics based neuroanatomy resources, the Brain Architecture Management System ([BAMS, http://brancusi.usc.edu/bkms/](http://brancusi.usc.edu/bkms/)) and [BrainInfo \(http://braininfo.rprc.washington.edu/menu/main.html\)](http://braininfo.rprc.washington.edu/menu/main.html).

In the ARAs three basic divisions of the mouse brain are recognized: (i) cerebral hemispheres, i.e. the cerebrum, endbrain, or telencephalon, consisting of the cerebral cortex and cerebral nuclei; (ii) cerebellum, i.e. the pancephalon, as consisting of cerebellar cortex and cerebellar nuclei; and (iii) brainstem, i.e. the cerebrospinal trunk. The regions are subdivided further, using the nomenclature listed in **Appendix 2**.

The definition of cerebral cortex and cerebral nuclei follows Swanson (2004). The organizational scheme of the brainstem, however, is different from that of Swanson (2004); for the ARA the brainstem is delineated following the classical development scheme of the mammalian brain, consisting of the interbrain (diencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). The ARA hindbrain is further subdivided into pons (metencephalon) and medulla (myelencephalon). Within each division of the midbrain, pons, and medulla, brain structures are put into three major categories, sensory related, motor related, and behavioral state related, which are partially compatible with the organization of Swanson (2004).

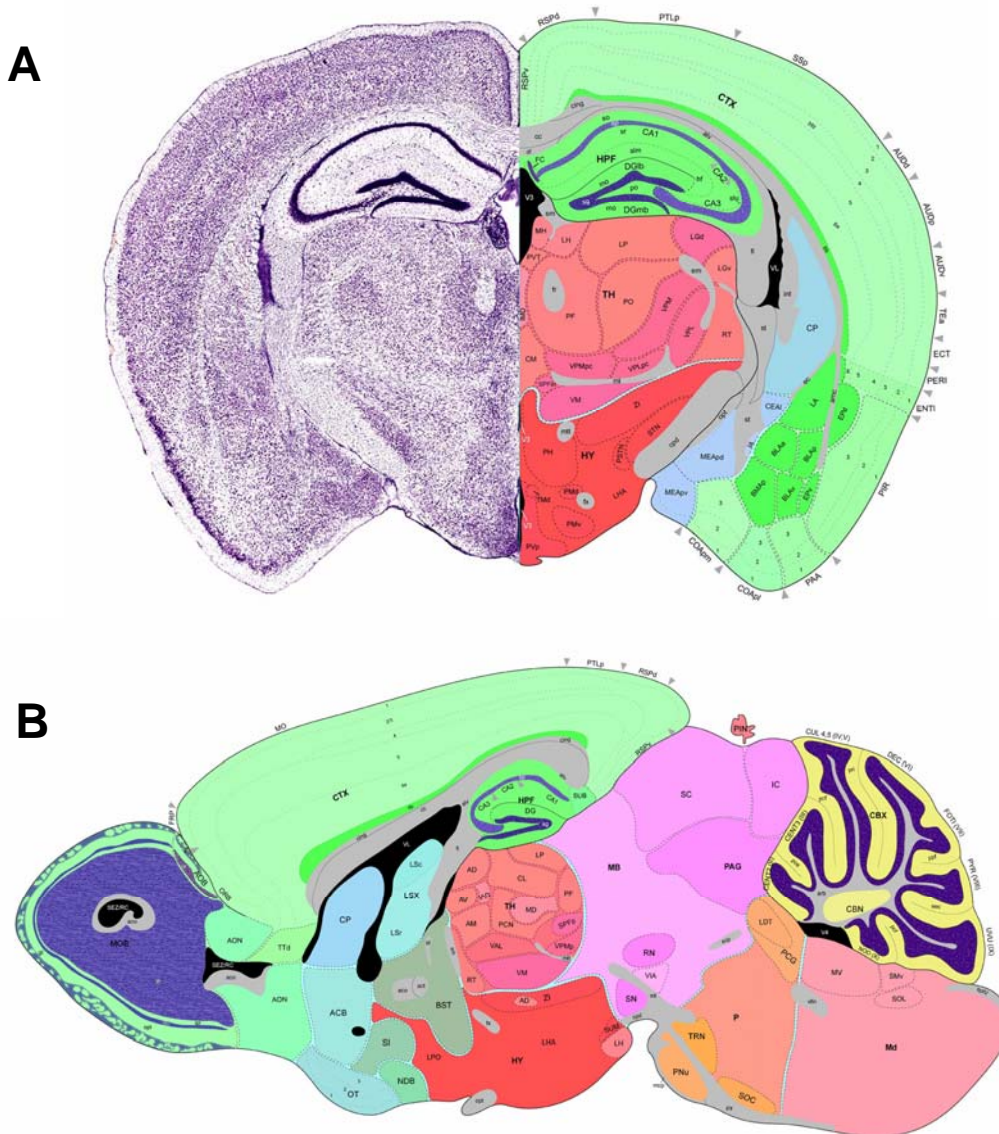


Figure 1 - *Sample images from the coronal (A) and sagittal (B) ARA*

All brain structures annotated in the ARAs are assigned unique colors to visually emphasize their hierarchical positions in the brain. This facilitates the unique definition and segmentation of brain regions. Users can find the hierarchically organized brain structure list (with abbreviations) in the ARA Nomenclature table (**Appendix 2**).

Histology and Photomicrographs

A comparison of general methodologies used for the ARAs and two other published mouse brain atlases (Paxinos and Franklin, 2003; Hof et al., 2000) are shown in **Appendix 3**. Unfixed, fresh-frozen C57Bl/6J



mouse brains were sectioned at 25 μm thickness for the ARAs using the same methods described for the ABA *in situ* hybridization process. The Nissl staining protocol used was modified from that of Paxinos and Watson (1998) and is described in **Appendix 4**. Nissl-stained sections were scanned at high resolution (10X, 0.95 $\mu\text{m}/\text{pixel}$) using a Leica DC 500 CCD camera mounted onto a Leica DM 6000 microscope.

Annotation of 2D Nissl Sections

Digital Nissl stained images were imported into Adobe Photoshop and contrast adjustments were made to the drawing. Annotated maps based on these images were then drawn using Adobe Illustrator CS.

Displaying the Allen Reference Atlases

The ARA is displayed through the [ABA Application \(http://www.brain-map.org/\)](http://www.brain-map.org/). In order to facilitate the use of the ARAs images of the annotated reference sections are presented side-by-side with images of *in situ* hybridization (ISH) gene expression data. The image viewer is designed to present the reference atlas image that most closely matches the plane of section of the ISH data being viewed. The coronal ARA is displayed with half of the brain presented as a Nissl image, mirrored by the corresponding annotated drawing. The sagittal ARA is displayed to allow users to view the annotated map and Nissl images separately. Users have the option to toggle between the Nissl images and corresponding annotation. The Application provides a virtual microscope for the *in-situ* hybridization data and the ARAs by allowing magnification, “zoomification”, of the atlas images.

References

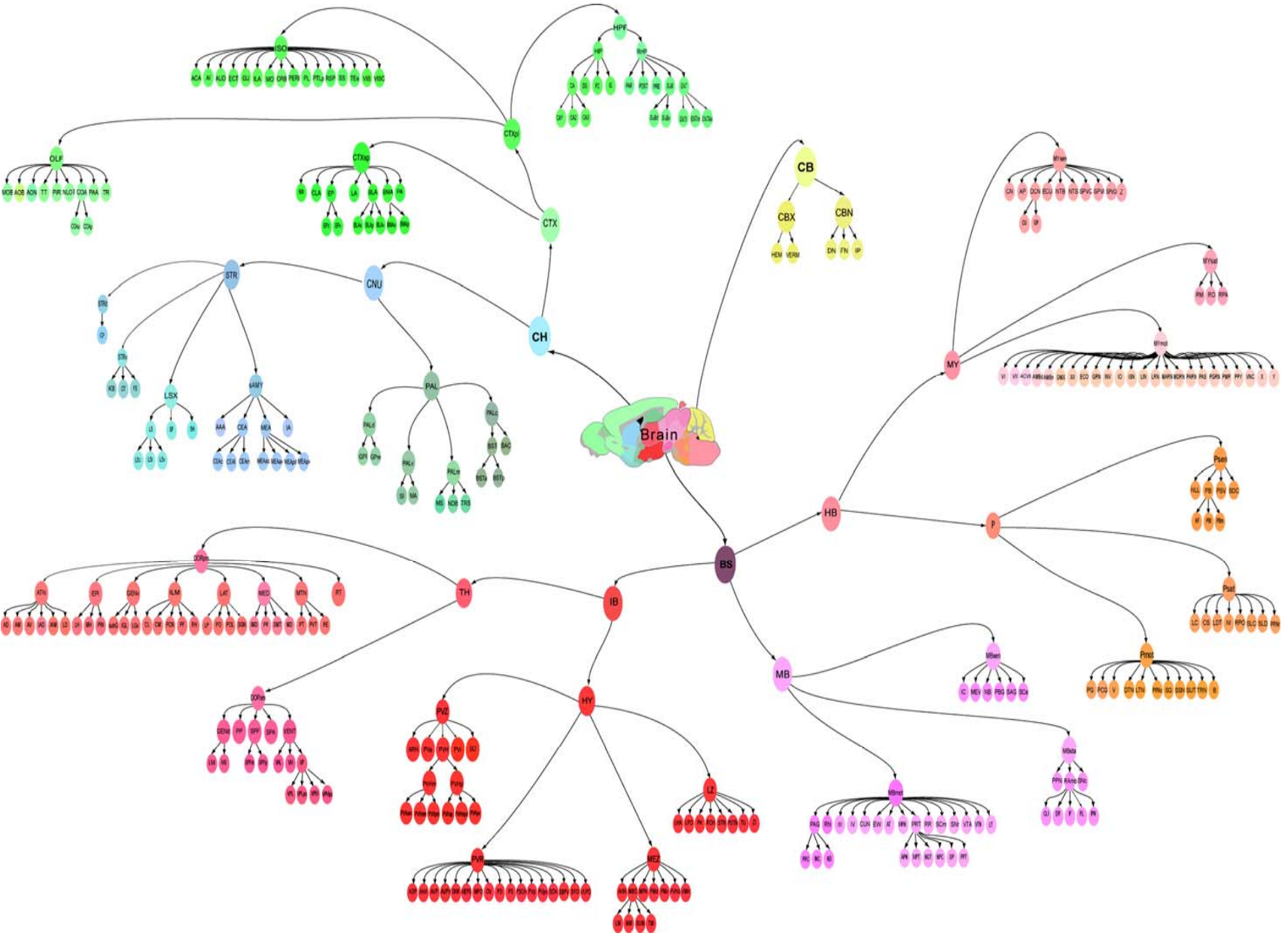
Hof, P.R. and Young, W.G. (2000) Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129 Sv Mouse Brains. Elsevier, Amsterdam.

Paxinos, G. and Franklin, K.B.J. (2004) The Mouse Brain in Stereotaxic Coordinates: Compact Second Edition. Elsevier Academic Press, San Diego, CA.

Paxinos, G. and Watson, C. (1998) The Rat Brain in Stereotaxic Coordinates (Fourth Edition). Academic Press, San Diego, CA.

Swanson, L. (2004) Brain Maps: Structure of the Rat Brain (Third Edition). Elsevier Academic Press, San Diego, CA.

Appendix 1 - ARA Brain Structure Tree



Appendix 2 - ARA Nomenclature

1. Cereberum [cerebral hemisphere, endbrain, telecephalon] (CH)
 - 1.1. Cerebral cortex (CTX)
 - 1.1.1. Cortical plate (CTXpl)
 - 1.1.1.1. Isocortex (ISO)*
 - Frontal pole, cerebral cortex (FRP)
 - Somatomotor areas (MO)
 - Primary motor area (MOp)
 - Secondary motor area (MOs)
 - Somatosensory areas (SS)
 - Primary somatosensory area (SSp)
 - Primary somatosensory area, nose (SS-n)
 - Primary somatosensory area, barrel field (SSp-bfd)
 - Primary somatosensory area, lower limb (SSp-l)
 - Primary somatosensory area, mouth (SSp-m)
 - Primary somatosensory area, upper limb (SSp-ul)
 - Primary somatosensory area, trunk (SS-tr)
 - Supplemental somatosensory area (SSs)
 - Infralimbic area (ILA)
 - Gustatory areas (GU)
 - Visceral area (VISC)
 - Auditory areas (AUD)
 - Dorsal auditory area (AUDd)
 - Primary auditory area (AUDp)
 - Posterior auditory area (AUDpo)
 - Ventral auditory area (AUDv)
 - Visual areas (VIS)
 - Anterolateral visual area (VISal)
 - Anteromedial visual area (VISam)
 - Lateral visual area (VISl)
 - Primary visual area (VISp)
 - Posterolateral visual area (VISpl)
 - posteromedial visual area (VISpm)
 - Rostrolateral visual area (VISrl)
 - Anterior cingulate area (ACA)
 - Anterior cingulate area, dorsal part (ACAd)
 - Anterior cingulate area, ventral part (ACAv)
 - Prelimbic area (PL)
 - Orbital area (ORB)
 - Orbital area, lateral part (ORBl)
 - Orbital area, medial part (ORBm)
 - Orbital area, ventral part (ORBv)
 - Orbital area, ventrolateral part (ORBvl)
 - Agranular insular area (AI)
 - Agranular insular area, dorsal part (AId)
 - Agranular insular area, posterior part (AIp)
 - Agranular insular area, ventral part (AIv)
 - Retrosplenial area (RSP)
 - Retrosplenial area, lateral agranular part (RSPag)
 - Retrosplenial area, dorsal part (RSPd)
 - Retrosplenial area, ventral part (RSPv)
 - Posterior parietal association areas (PTLp)
 - Temporal association areas (TEa)
 - Perirhinal area (PERl)
 - Ectorhinal area (ECT)

* The six-layered scheme of the isocortex has been recognized almost 100 years (Brodmann, 1909; Zilles and Wree, 1995). These layers are named from superficial to deep: 1, molecular layer (ISO1); 2, superficial supragranular pyramidal layer (ISO2); 3, deep supragranular pyramidal layer (ISO3); 4, granular layer; 5, infragranular pyramidal layer; 6, polymorph layer. Per Swanson (2004), layer 6 is subdivided into two layers, 6a & 6b. The 6b is assigned separately from other cortical layers into the cortical subplate, while other cortical layers remain in the cortical plate (Alvarez-Bolado and Swanson, 1996).

1.1.1.2. Olfactory areas (OLF)

- Main olfactory bulb (MOB)
 - Main olfactory bulb, glomerular layer (MOBgl)
 - Main olfactory bulb, granule layer (MOBgr)
 - Main olfactory bulb, inner plexiform layer (MOBipl)
 - Main olfactory bulb, mitral layer (MOBmi)
 - Main olfactory bulb, outer plexiform layer (MOBopl)
- Accessory olfactory bulb (AOB)
 - Accessory olfactory bulb, glomerular layer (AOBgl)
 - Accessory olfactory bulb, granular layer (AOBgr)
 - Accessory olfactory bulb, mitral layer (AOBmi)
- Anterior olfactory nucleus (AON)
 - Anterior olfactory nucleus, dorsal part (AONd)
 - Anterior olfactory nucleus, external part (AONE)
 - Anterior olfactory nucleus, lateral part (AONI)
 - Anterior olfactory nucleus, medial part (AONm)
 - Anterior olfactory nucleus, posteroventral part (AONpv)
- Taenia tecta (TT)
 - Taenia tecta, dorsal part (TTd)
 - Taenia tecta, ventral part (TTv)
- Dorsal peduncular area (DP)
- Piriform area (PIR)
 - Piriform area, molecular layer (PIR1)
 - Piriform area, pyramidal layer (PIR2)
 - Piriform area, polymorph layer (PIR3)
- Nucleus of the lateral olfactory tract (NLOT)
 - Nucleus of the lateral olfactory tract, molecular layer (NLOT1)
 - Nucleus of the lateral olfactory tract, pyramidal layer (NLOT2)
- Cortical amygdalar area (COA)
 - Cortical amygdalar area, anterior part (COAa)
 - Cortical amygdalar area, posterior part (COAp)
 - Cortical amygdalar area, posterior part, lateral zone (COApl)
 - Cortical amygdalar area, posterior part, medial zone (COApm)
- Piriform-amygdalar area (PAA)
- Postpiriform transition area (TR)

1.1.1.3. Hippocampal formation (HPF)

- Hippocampal region (HIP)
 - Ammon's Horn (CA)
 - Field CA1 (CA1)
 - Field CA1, stratum lacunosum-moleculare (CA1slm)
 - Field CA1, stratum oriens (CA1so)
 - Field CA1, pyramidal layer (CA1sp)
 - Field CA1, stratum radiatum (CA1sr)
 - Field CA2 (CA2)
 - Field CA2, stratum lacunosum-moleculare (CA2slm)
 - Field CA2, stratum oriens (CA2so)
 - Field CA2, pyramidal layer (CA2sp)
 - Field CA2, stratum radiatum (CA2sr)
 - Field CA3 (CA3)
 - Field CA3, stratum lacunosum-moleculare (CA3slm)
 - Field CA3, stratum lucidum (CA3slu)
 - Field CA3, stratum oriens (CA3so)
 - Field CA3, pyramidal layer (CA3sp)
 - Field CA3, stratum radiatum (CA3sr)
 - Dentate gyrus (DG)
 - Dentate gyrus crest (DGcr)
 - Dentate gyrus crest, molecular layer (DGcr-mo)
 - Dentate gyrus crest, polymorph layer (DGcr-po)
 - Dentate gyrus crest, granule cell layer (DGcr-sg)

- Dentate gyrus lateral blade (DGlb)
 - Dentate gyrus lateral blade, molecular layer (DGlb-mo)
 - Dentate gyrus lateral blade, polymorph layer (DGlb-po)
 - Dentate gyrus lateral blade, granule cell layer (DGlb-sg)
- Dentate gyrus medial blade (DGmb)
 - Dentate gyrus medial blade, molecular layer (DGmb-mo)
 - Dentate gyrus medial blade, polymorph layer (DGmb-po)
 - Dentate gyrus medial blade, granule cell layer (DGmb-sg)
- Dentate gyrus, granule cell layer (DG-sg)
- Fasciola cinerea (FC)
- Induseum griseum (IG)
- Retrohippocampal region (RHP)
- Entorhinal area (ENT)
 - Entorhinal area, lateral part (ENTl)
 - Entorhinal area, medial part, dorsal zone (ENTm)
 - Entorhinal area, medial part, ventral zone (ENTmv)
- Parasubiculum (PAR)
- Postsubiculum (POST)
- Presubiculum (PRE)
- Subiculum (SUB)
 - Subiculum, dorsal part (SUBd)
 - Subiculum, dorsal part, molecular layer (SUBd-m)
 - Subiculum, dorsal part, pyramidal layer (SUBd-sp)
 - Subiculum, dorsal part, stratum radiatum (SUBd-sr)
 - Subiculum, ventral part (SUBv)
 - Subiculum, ventral part, molecular layer (SUBv-m)
 - Subiculum, ventral part, pyramidal layer (SUBv-sp)
 - Subiculum, ventral part, stratum radiatum (SUBv-sr)
- 1.1.2. Cortical Subplate (CTXsp)
 - 1.1.2.1. Layer 6b, isocortex (6b)
 - 1.1.2.2. Claustrum (CLA)
 - 1.1.2.3. Endopiriform nucleus (EP)
 - Dorsal part (EPd)
 - Ventral part (EPv)
 - 1.1.2.4. Lateral amygdalar nucleus (LA)
 - 1.1.2.5. Basolateral amygdalar nucleus (BLA)
 - Anterior part (BLAa)
 - Posterior part (BLAp)
 - Ventral part (BLAv)
 - 1.1.2.6. Basomedial amygdalar nucleus (BMA)
 - Anterior part (BMAa)
 - Posterior part (BMAp)
 - 1.1.2.7. Posterior amygdalar nucleus (PA)
- 1.2. Cerebral nuclei [corpus striatum, basal ganglia] (CNU)
 - 1.2.1. Striatum (STR)
 - 1.2.1.1. Striatum dorsal region (STRd)—Caudoputamen (CP)
 - 1.2.1.2. Striatum ventral region (STRv)
 - Nucleus accumbens (ACB)
 - Fundus of striatum (FS)
 - Olfactory tubercle (OT)
 - Islands of Calleja (isl)
 - Major island of Calleja (ism)
 - Olfactory tubercle, molecular layer (OT1)
 - Olfactory tubercle, pyramidal layer (OT2)
 - Olfactory tubercle, polymorph layer (OT3)
 - 1.2.1.3. Striatum medial region (STRm)—Lateral septal complex (LSX)
 - Lateral septal nucleus (LS)
 - Caudal (caudodorsal) part (LSc)
 - Rostral (rostroventral) part (LSr)

- Ventral part (LSv)
- Septofimbrial nucleus (SF)
- Septohippocampal nucleus (SH)
- 1.2.1.4. Striatum caudal region (STRc)—Striatum like amygdalar nuclei (sAMY)
 - Anterior amygdalar area (AAA)
 - Bed nucleus of the accessory olfactory tract (BA)
 - Central amygdalar nucleus (CEA)
 - capsular part (CEAc)
 - lateral part (CEAl)
 - medial part (CEAm)
 - Intercalated amygdalar nucleus (IA)
 - Medial amygdalar nucleus (MEA)
 - Anterodorsal part (MEAad)
 - Anteroventral part (MEAav)
 - Posterodorsal part (MEApd)
 - sublayer a (MEApd-a)
 - sublayer b (MEApd-b)
 - sublayer c (MEApd-c)
 - Posteroventral part (MEApv)
- 1.2.2. Pallidum (PAL)
 - 1.2.2.1. Pallidum dorsal region (PALd)—Globus Pallidus (GP)
 - External segment (GPe)
 - Internal segment (GPi)
 - 1.2.2.2. Pallidum ventral region (PALv)
 - Substantia innominata (SI)
 - Magnocellular nucleus (MA)
 - 1.2.2.3. Pallidum medial region (PALm)
 - Medial septal complex (MSC)
 - Medial septal nucleus (MS)
 - Diagonal band nucleus (NDB)
 - Triangular nucleus of septum (TRS)
 - 1.2.2.4. Pallidum caudal region (PALc)
 - Bed nuclei of the stria terminalis (BST)
 - Anterior division (BSTa)
 - anterolateral area (BSTal)
 - anteromedial area (BSTam)
 - dorsomedial nucleus (BSTdm)
 - fusiform nucleus (BSTfu)
 - juxtacapsular nucleus (BSTju)
 - magnocellular nucleus (BSTmg)
 - oval nucleus (BSTov)
 - rhomboid nucleus (BSTrh)
 - ventral nucleus (BSTv)
 - Posterior division (BSTp)
 - dorsal nucleus (BSTd)
 - principal nucleus (BSTpr)
 - interfascicular nucleus (BSTif)
 - transverse nucleus (BSTtr)
 - strial extension (BSTse)
 - Bed nucleus of the anterior commissure (BAC)
- 2. Cerebellum [Parencephalon] (CB)
 - 2.1. Cerebellar cortex (CBX)
 - 2.1.1. Vermal regions (VERM)
 - Lingula (l) (LING)
 - Central lobule (CENT)
 - lobule II (CENT2)
 - lobule III (CENT3)
 - Culmen (CUL)

- Lobules IV, V (CUL4,5)
- Declive (VI) (DEC)
- Folium-tuber vermis (VII) (FOTU)
- Pyramus (VIII) (PYR)
- Uvula (IX) (UVU)
- Nodulus (X) (NOD)
- 2.1.2. Hemispheric regions (HEM)
 - Simple lobule (SIM)
 - Ansiform lobule (AN)
 - crus 1 (ANcr1)
 - crus 2 (ANcr2)
 - Paramedian lobule (PRM)
 - Copula pyramidis (COPY)
 - Paraflocculus (PFL)
 - Flocculus (FL)
- 2.2. Cerebellar nuclei (CBN)
 - 2.1.1. Fastigial nucleus (FN)
 - 2.1.2. Interposed nucleus (IP)
 - 2.1.3. Dentate nucleus (DN)
- 3. Brainstem [Cerebrospinal trunk] (BS)
 - 3.1. Interbrain [diencephalons] (IB)
 - 3.1.1. Thalamus (TH)
 - 3.1.1.1. Sensory-Motor cortex related (DORsm)
 - 3.1.1.1.1. Ventral group of dorsal thalamus (VENT)
 - ventral anterior-lateral complex of the thalamus (VAL)
 - ventral medial nucleus of the thalamus (VM)
 - ventral posterior complex of the thalamus (VP)
 - ventral posterolateral nucleus of the thalamus, principal part (VPL)
 - ventral posterolateral nucleus of the thalamus, parvocellular part (VPLpc)
 - ventral posteromedial nucleus of the thalamus, principal part (VPM)
 - ventral posteromedial nucleus of the thalamus, parvocellular part (VPMpc)
 - 3.1.1.1.2. Subparafascicular nucleus (SPF)
 - magnocellular part (SPFm)
 - parvocellular part (SPFp)
 - 3.1.1.1.3. Subparafascicular area (SPA)
 - 3.1.1.1.3. Peripeduncular nucleus (PP)
 - 3.1.1.1.4. Geniculate group of dorsal thalamus (GENd)
 - medial geniculate complex (MG)
 - dorsal part (MGd)
 - ventral part (MGv)
 - medial part (MGm)
 - lateral geniculate complex, dorsal part (LGd)
 - 3.1.1.2. Polymodal association cortex related (DORpm)
 - 3.1.1.2.1. Lateral group of the dorsal thalamus (LAT)
 - lateral posterior nucleus of the thalamus (LP)
 - posterior complex of the thalamus (PO)
 - posterior limiting nucleus of the thalamus (POL)
 - suprageniculate nucleus (SGN)
 - 3.1.1.2.2. Anterior group of the dorsal thalamus (ATN)
 - anteroventral nucleus of the thalamus (AV)
 - anteromedial nucleus of the thalamus (AM)
 - dorsal part (AMd)
 - ventral part (AMv)
 - anterodorsal nucleus of the thalamus (AD)
 - interanteromedial nucleus of the thalamus (IAM)
 - interanterodorsal nucleus of the thalamus (IAD)
 - lateral dorsal nucleus of the thalamus (LD)
 - 3.1.1.2.3. Medial group of the dorsal thalamus (MED)

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- intermediodorsal nucleus of the thalamus (IMD)
- mediodorsal nucleus of the thalamus (MD)
 - central part (MDc)
 - lateral part (MDl)
 - medial part (MDm)
- submedial nucleus of the thalamus (SMT)
- perireuniens nucleus (PR)
- 3.1.1.2.4. Midline group of the dorsal thalamus (MTN)
 - paraventricular thalamic nucleus (PVT)
 - paratenial nucleus (PT)
 - nucleus of reunions (RE)
- 3.1.1.2.5. Intralaminar group of the dorsal thalamus (ILM)
 - rhomboid nucleus (RH)
 - central medial nucleus of the thalamus (CM)
 - paracentral nucleus of the thalamus (PCN)
 - central lateral nucleus of the thalamus (CL)
 - parafascicular nucleus (PF)
- 3.1.1.3. Reticular nucleus of the thalamus (RT)
- 3.1.1.4. Geniculate group, ventral thalamus (GENv)
 - intergeniculate leaflet, lateral geniculate complex (IGL)
 - ventral part of the lateral geniculate complex (LGv)
 - lateral zone (LGvl)
 - medial zone (LGvm)
 - subgeniculate nucleus (subG)
- 3.1.1.5. Epithalamus (EPI)
 - medial habenula (MH)
 - lateral habenula (LH)
 - pineal gland (PIN)
- 3.1.2. Hypothalamus (HY)
 - 3.1.2.1. Periventricular zone (PVZ)—neuroendocrine motor zone (NEM)
 - Supraoptic nucleus (SO)
 - Accessory supraoptic group (ASO)
 - Nucleus circularis (NC)
 - Paraventricular hypothalamic nucleus (PVH)
 - magnocellular division (PVHm)
 - anterior magnocellular part (PVHam)
 - medial magnocellular part (PVHmm)
 - posterior magnocellular part (PVHpm)
 - lateral zone (PVHpml)
 - medial zone (PVHpmm)
 - parvicellular division (PVHp)
 - anterior parvicellular part (PVHap)
 - dorsal zone (PVHmpd)
 - periventricular part (PVHpv)
 - Periventricular hypothalamic nucleus, anterior part (PVa)
 - Periventricular hypothalamic nucleus, intermediate part (PVi)
 - Arcuate hypothalamic nucleus (ARH)
 - 3.1.2.2. Periventricular region (PVR)
 - Anterodorsal preoptic nucleus (ADP)
 - Anterior hypothalamic area (AHA)
 - Anteroventral preoptic nucleus (AVP)
 - Anteroventral periventricular nucleus (AVPV)
 - Dorsomedial nucleus of the hypothalamus (DMH)
 - Dorsomedial nucleus of the hypothalamus, anterior part (DMHa)
 - Dorsomedial nucleus of the hypothalamus, posterior part (DMHp)
 - Dorsomedial nucleus of the hypothalamus, ventral part (DMHv)
 - Median preoptic nucleus (MEPO)
 - Medial preoptic area (MPO)
 - Vascular organ of the lamina terminalis (OV)

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- Posterodorsal preoptic nucleus (PD)
- Parastrial nucleus (PS)
- Suprachiasmatic preoptic nucleus (PSCH)
- Periventricular hypothalamic nucleus, posterior part (PVp)
- Periventricular hypothalamic nucleus, preoptic part (PVpo)
- Subparaventricular zone (SBPV)
- Suprachiasmatic nucleus (SCH)
- Subfornical organ (SFO)
- Ventrolateral preoptic nucleus (VLPO)
- 3.1.2.3. Hypothalamic medial zone (MEZ)—behavioral control column
 - Anterior hypothalamic nucleus (AHN)
 - Anterior hypothalamic nucleus, anterior part (AHNa)
 - Anterior hypothalamic nucleus, central part (AHNc)
 - Anterior hypothalamic nucleus, dorsal part (AHNd)
 - Anterior hypothalamic nucleus, posterior part (AHNp)
 - Mammillary body (MBO)
 - Lateral mammillary nucleus (LM)
 - Medial mammillary nucleus (MM)
 - Medial mammillary nucleus, median part (Mmme)
 - Supramammillary nucleus (SUM)
 - Supramammillary nucleus, lateral part (SUMl)
 - Supramammillary nucleus, medial part (SUMm)
 - Tuberomammillary nucleus (TM)
 - Tuberomammillary nucleus, dorsal part (TMd)
 - Tuberomammillary nucleus, ventral part (TMv)
 - Medial preoptic nucleus (MPN)
 - Medial preoptic nucleus, central part (MPNc)
 - Medial preoptic nucleus, lateral part (MPNl)
 - Medial preoptic nucleus, medial part (MPNm)
 - Posterior hypothalamic nucleus (PH)
 - Dorsal premammillary nucleus (PMd)
 - Ventral premammillary nucleus (PMv)
 - Paraventricular hypothalamic nucleus, descending division (PVHd)
 - dorsal parvicellular part (PVHdp)
 - forniceal part (PVHf)
 - lateral parvicellular part (PVHlp)
 - medial parvicellular part, ventral zone (PVHmpv)
 - Ventromedial hypothalamic nucleus (VMH)
 - anterior part (VMHa)
 - central part (VMHc)
 - dorsomedial part (VMHdm)
 - ventrolateral part (VMHvl)
- 3.1.2.4. Hypothalamic lateral zone (LZ)
 - Lateral hypothalamic area (LHA)
 - Lateral preoptic area (LPO)
 - Preparasubthalamic nucleus (PST)
 - Parasubthalamic nucleus (PSTN)
 - Retrochiasmatic area (RCH)
 - Subthalamic nucleus (STN)
 - Tuberal nucleus (TU)
 - Zona incerta (ZI)
 - Dopaminergic A13 group (A13)
 - Fields of Forel (FF)
- 3.2. Midbrain [mesencephalon] (MB)
 - 3.2.1. Sensory related (MBSen)
 - 3.2.1.1. Superior colliculus, sensory related (SCs)
 - optic layer (SCop)
 - superficial gray layer (SCsg)
 - zonal layer (SCzo)

- 3.2.1.2. Inferior colliculus (IC)
 - central nucleus (ICc)
 - dorsal nucleus (ICd)
 - external nucleus (ICe)
- 3.2.1.3. Nucleus of the brachium of the inferior colliculus (NB)
- 3.2.1.4. Nucleus sagulum (SAG)
- 3.2.1.5. Parabigeminal nucleus (PBG)
- 3.2.1.6. Midbrain trigeminal nucleus (MEV)
- 3.2.2. Motor related (MBmot)
 - 3.2.2.1. Substantia nigra, reticular part (SNr)
 - 3.2.2.2. Ventral tegmental area (VTA)
 - 3.2.2.3. Midbrain reticular nucleus, retrorubral area (RR)
 - 3.2.2.4. Midbrain reticular nucleus (MRN)
 - 3.2.2.5. Superior Colliculus, motor related (SCm)
 - deep gray layer (SCdg)
 - deep white layer (SCdw)
 - intermediate white layer (SCiw)
 - intermediate gray layer (SCig)
 - sublayer a (SCig-a)
 - sublayer b (SCig-b)
 - sublayer c (SCig-c)
 - 3.2.2.6. Periaqueductal gray (PAG)
 - Periaqueductal gray, proper (PAG)
 - Precommissural nucleus (PRC)
 - Interstitial nucleus of Cajal (INC)
 - Nucleus of Darkschewitsch (ND)
 - 3.2.2.7. Pretectal region (PRT)
 - Anterior pretectal nucleus (APN)
 - Medial pretectal area (MPT)
 - Nucleus of the optic tract (NOT)
 - Nucleus of the posterior commissure (NPC)
 - Olivary pretectal nucleus (OP)
 - Posterior pretectal nucleus (PPT)
 - 3.2.2.8. Cuneiform nucleus (CUN)
 - 3.2.2.9. Red nucleus (RN)
 - 3.2.2.10. Oculomotor nucleus (III)
 - 3.2.2.11. Edinger-Westphal nucleus (EW)
 - 3.2.2.12. Trochlear nucleus (IV)
 - 3.2.2.13. Ventral tegmental nucleus (VTN)
 - 3.2.2.14. Anterior tegmental nucleus (AT)
 - 3.2.2.15. Medial terminal nucleus of the accessory optic tract (MT)
 - 3.2.2.16. Lateral terminal nucleus of the accessory optic tract (LT)
 - 3.2.2.17. Dorsal terminal nucleus of the accessory optic tract (DT)
- 3.2.3. Behavioral state related (MBsta)
 - 3.2.3.1. Substantia nigra, compact part (SNc)
 - 3.2.3.2. Pedunculopontine nucleus (PPN)
 - 3.2.3.3. Midbrain raphé nuclei (RAmb)
 - Interfascicular nucleus raphé (IF)
 - Interpeduncular nucleus (IPN)
 - Rostral linear nucleus raphé (RL)
 - Central linear nucleus raphé (CLI)
 - Dorsal raphé (DR)
- 3.3. Hindbrain [rhombencephalon] (HB)
 - 3.3.1. Pons [metencephalon] (P)
 - 3.3.1.1. Sensory related (P-sen)
 - Nucleus of the lateral lemniscus (NLL)
 - dorsal part (NLLd)
 - horizontal part (NLLh)
 - ventral part (NLLv)



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- Principal sensory nucleus of the trigeminal (PSV)
- Parabrachial nucleus (PB)
 - Kolliker-Fuse subnucleus (KF)
 - Parabrachial nucleus, lateral division (PBI)
 - central lateral part (PBlc)
 - dorsal lateral part (PBld)
 - external lateral part (PBle)
 - superior lateral part (PBls)
 - ventral lateral part (PBlv)
 - Parabrachial nucleus, medial division (PBm)
 - external medial part (PBme)
 - medial medial part (PBmm)
 - ventral medial part (PBmv)
- Superior olivary complex (SOC)
 - periolivary region (POR)
 - medial part (SOCm)
 - lateral part (SOCl)

3.3.1.2. Motor related (P-mot)

- Accessory abducens nucleus (ACVI)
- Accessory facial motor nucleus (ACVII)
- Barrington's nucleus (B)
- Dorsal tegmental nucleus (DTN)
- Lateral tegmental nucleus (LTN)
- Pontine central gray (PCG)
- Pontine gray (PG)
- Pontine reticular nucleus, caudal part (PRNc)
- Pontine reticular nucleus, ventral part (PRNv)
- Supragenual nucleus (SG)
- Superior salivatory nucleus (SSN)
- Supratrigeminal nucleus (SUT)
- Tegmental reticular nucleus (TRN)
- Motor nucleus of trigeminal (V)
- Abducens nucleus (VI)
- Facial motor nucleus (VII)

3.3.1.3. Behavior state related (P-sat)

- Superior central nucleus raphé (CS)
 - Superior central nucleus raphé, lateral part (CSI)
 - Superior central nucleus raphé, medial part (CSm)
- Locus ceruleus (LC)
- Laterodorsal tegmental nucleus (LDT)
- Nucleus incertus (NI)
- Pontine reticular nucleus (PRNr)
- Nucleus raphé pontis (RPO)
- Subceruleus nucleus (SLC)
- Sublaterodorsal nucleus (SLD)

3.3.2. Medulla [myelencephalon] (MY)

3.3.2.1. Sensory related (MY-sen)

- Area postrema (AP)
- Cochlear nuclei (CN)
 - Granular lamina of the cochlear nuclei (CNlam)
 - Cochlear nucleus, subpeduncular granular region (CNspg)
 - Dorsal cochlear nucleus (DCO)
 - Ventral cochlear nucleus (VCO)
- Dorsal column nuclei (DCN)
 - Cuneate nucleus (CU)
 - Gracile nucleus (GR)
- External cuneate nucleus (ECU)
- Nucleus of the trapezoid body (NTB)



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- Nucleus of the solitary tract (NTS)
 - central part (NTSce)
 - commissural part (NTSco)
 - gelatinous part (NTSge)
 - lateral part (NTSl)
 - medial part (NTSm)
- Spinal nucleus of the trigeminal, caudal part (SPVC)
- Spinal nucleus of the trigeminal, interpolar part (SPVI)
- Spinal nucleus of the trigeminal, oral part (SPVO)
 - caudal dorsomedial part (SPVOcdm)
 - middle dorsomedial part, dorsal zone (SPVomdm)
 - middle dorsomedial part, ventral zone (SPVomdmv)
 - rostral dorsomedial part (SPVOrdM)
 - ventrolateral part (SPVOvl)
- Nucleus z (z)
- 3.3.2.2. Motor related (Md-mot)
 - Nucleus ambiguus (AMB)
 - dorsal division (AMBd)
 - ventral division (AMBv)
 - Dorsal motor nucleus of the vagus nerve (DMX)
 - Efferent cochlear group (ECO)
 - Gigantocellular reticular nucleus (GRN)
 - Infracerebellar nucleus (ICB)
 - Interstitial nucleus of the vestibular nerve (INV)
 - Inferior olivary complex (IO)
 - Intermediate reticular nucleus (IRN)
 - Inferior salivatory nucleus (ISN)
 - Linear nucleus of the medulla (LIN)
 - Lateral reticular nucleus (LRN)
 - Lateral reticular nucleus, magnocellular part (LRNm)
 - Lateral reticular nucleus, parvicellular part (LRNp)
 - Magnocellular reticular nucleus (MARN)
 - Medullary reticular nucleus (MDRN)
 - Medullary reticular nucleus, dorsal part (MDRNd)
 - Medullary reticular nucleus, ventral part (MDRNv)
 - Parvicellular reticular nucleus (PARN)
 - Parasolitary nucleus (PAS)
 - Paragigantocellular reticular nucleus (PGRN)
 - Paragigantocellular reticular nucleus, dorsal part (PGRNd)
 - Paragigantocellular reticular nucleus, lateral part (PGRNI)
 - Perihypoglossal nuclei (PHY)
 - Nucleus intercalates (NIS)
 - Nucleus of Roller (NR)
 - Nucleus prepositus (PRP)
 - Paramedian reticular nucleus (PMR)
 - Parapyramidal nucleus (PPY)
 - Parapyramidal nucleus, deep part (PPYd)
 - Parapyramidal nucleus, superficial part (PPYs)
 - Vestibular nuclei (VNC)
 - Lateral vestibular nucleus (LAV)
 - Medial vestibular nucleus (MV)
 - Spinal vestibular nucleus (SPIV)
 - Superior vestibular nucleus (SUV)
 - Nucleus x (x)
 - Hypoglossal nucleus (XII)
 - Nucleus y (y)
- 3.3.2.3. Behavioral state related (Md-sat)
 - Nucleus raphé magnus (RM)
 - Nucleus raphé pallidus (RPA)
 - Nucleus raphé obscurus (RO)

BASIC FIBER SYSTEMS OF THE MOUSE CNS

1. CRANIAL NERVES (cm), SPINAL NERVES (spin), & RELATED terminal nerve (tn)
 - vomeronasal nerve (von)
 - olfactory nerve (In)
 - olfactory nerve layer of main olfactory bulb (onl)
 - lateral olfactory tract, general (lotg)
 - lateral olfactory tract, body (lot)
 - dorsal limb (lotd)
 - accessory olfactory tract (aolt)
 - anterior commissure, olfactory limb (aco)
 - optic nerve (In)
 - accessory optic tract (aot)
 - brachium of the superior colliculus (bsc)
 - superior colliculus commissure (csc)
 - optic chiasm (och)
 - optic tract (opt)
 - tectothalamic pathway (ttp)
 - oculomotor nerve (In)
 - medial longitudinal fascicle (mlf)
 - posterior commissure (pc)
 - trochlear nerve (IVn)
 - trochlear nerve decussation (IVd)
 - abducens nerve (VIIn)
 - trigeminal nerve (Vn)
 - motor root of the trigeminal nerve (moV)
 - sensory root of the trigeminal nerve (sV)
 - midbrain tract of the trigeminal nerve (mtV)
 - spinal tract of the trigeminal nerve (sptV)
 - facial nerve (VIIIn)
 - intermediate nerve (iVIIIn)
 - genu of the facial nerve (gVIIIn)
 - vestibulocochlear nerve (VIIIIn)
 - efferent cocvhleovestibular bundle (cvb)
 - vestibular nerve (vVIIIIn)
 - cochlear nerve (cVIIIIn)
 - trapezoid body (tb)
 - intermediate acoustic stria (ias)
 - dorsal acoustic stria (das)
 - lateral lemniscus (ll)
 - inferior colliculus commissure (cic)
 - brachium of the inferior colliculus
 - glossopharyngeal nerve (IXn)
 - vagus nerve (Xn)
 - solitary tract (ts)
 - accessory spinal nerve (XIIn)
 - hypoglossal nerve (XIIIn)
 - ventral roots (vrt)
 - dorsal roots (drt)
 - cervicothalamic tract (cett)
 - dorsolateral fascicle (dl)
 - dorsal commissure of the spinal cord (dcm)
 - ventral commissure of the spinal cord (vc)
 - fasciculus proprius (fpr)
 - dorsal column (dc)
 - cuneate fascicle (cuf)
 - gracile fascicle (grf)
 - internal arcuate fibers (iaf)

- medial lemniscus (ml)
 - spinothalamic tract (sst)
 - lateral spinothalamic tract (sttl)
 - ventral spinothalamic tract (sttv)
 - spinocervical tract (scrt)
 - spino-olivary pathway (sop)
 - spinoreticular pathway (srp)
 - spinovestibular pathway (svp)
 - spinotectal pathway (stp)
 - spinothalamic pathway (shp)
 - spinotelenchephalic pathway (step)
 - hypothalamohypophysial tract (hht)
- 2. CEREBELLUM RELATED FIBER TRACTS (cbf)
 - cerebellar commissure (cbc)
 - cerebellar peduncles (cbp)
 - superior cerebellar peduncles (scp)
 - superior cerebellar peduncle decussation (dscp)
 - uncinate fascicle (uf)
 - ventral spinocerebellar tract (sctv)
 - middle cerebellar peduncle (mcp)
 - inferior cerebellar peduncle (icp)
 - dorsal spinocerebellar tract (sctd)
 - cuneocerebellar tract (cct)
 - juxtarestiform body (jrb)
 - bulbocerebellar tract (bct)
 - olivocerebellar tract (oct)
 - reticulocerebellar tract (rct)
 - trigemino-cerebellar tract (tct)
 - arbor vitae (arb)
- 3. LATERAL FOREBRAIN BUNDLE SYSTEM (lfbs)
 - corpus callosum (cc)
 - anterior forceps (fa)
 - external capsule (ec)
 - extreme capsule (ee)
 - genu (ccg)
 - posterior forceps (fp)
 - rostrum (ccr)
 - splenium (ccs)
 - corticospinal tract (cst)
 - internal capsule (int)
 - cerebral peduncle (cpd)
 - corticotectal tract (cte)
 - corticorubral tract (crt)
 - corticopontine tract (cpt)
 - corticobulbar tract (cbr)
 - pyramid (py)
 - pyramidal decussation (pyd)
 - corticospinal tract, crossed (cstc)
 - corticospinal tract, uncrossed (cstu)
 - thalamus related (lfbst)
 - external medullary lamina of the thalamus (em)
 - internal medullary lamina of the thalamus (im)
 - middle thalamic commissure (mtc)
 - thalamic peduncles (tp)
- 4. EXTRAPYRAMIDAL FIBER SYSTEMS (epsc)
 - cerebral nuclei related (epsc)
 - pallidothalamic pathway (pap)

- nigrostriatal tract (nst)
- nigrothalamic fibers (ntt)
- pallidotegmental fascicle (ptf)
- striatonigral pathway (snp)
- subthalamic fascicle (stf)
- tectospinal pathway (tsp)
 - direct tectospinal pathway (tspd)
 - doral tegmental decussation (dtd)
 - crossed tectospinal pathway (tspc)
- rubrospinal tract (rust)
 - ventral tegmental decussation (vtd)
 - rubroreticular tract (rrt)
- central tegmental bundle (ctb)
- reticulospinal tract (rst)
 - reticulospinal tract, lateral part (rstl)
 - reticulospinal tract, medial part (rstm)
- vestibulospinal pathway (vsp)
- 5. MEDIAL FOREBRAIN BUNDLE SYSTEM (mfbs)
 - cerebrum related (mfbc)
 - amygdalar capsule (amc)
 - ansa peduncularis (apd)
 - anterior commissure, temporal limb (act)
 - cingulum bundle (cing)
 - fornix system (fxs)
 - alveus (alv)
 - dorsal fornix (df)
 - fimbria (fi)
 - precommissural fornix, general (fxprg)
 - precommissural fornix
 - diagonal band (db)
 - postcommissural fornix (fxpo)
 - medial corticohypothalamic tract (mct)
 - columns of the fornix (fx)
 - hippocampal commissures (hc)
 - dorsal hippocampal commissure (dhc)
 - ventral hippocampal commissure (vhc)
 - perforant path (per)
 - angular path (ab)
 - longitudinal association bundle (lab)
 - stria terminalis (st)
 - hypothalamus related (mfbsbshy)
 - medial forebrain bundle (mfb)
 - ventrolateral hypothalamic tract
 - preoptic commissure (poc)
 - supraoptic commissures (sup)
 - anterior (supa)
 - dorsal (supd)
 - ventral (supv)
 - premamillary commissure (pmx)
 - supramamillary decussation (smd)
 - proprio-hypothalamic pathways (php)
 - dorsal (phpd)
 - lateral (phpl)
 - medial (phpm)
 - ventral (phpv)
 - periventricular bundle of the hypothalamus (pvbh)
 - mammillary related (mfbsma)
 - principal mammillary tract (pm)
 - mammillothalamic tract (mtt)

mammilotegmental tract (mtg)
 mammillary peduncle (mp)
 dorsal thalamus related (mfbst)
 periventricular bundle of the thalamus (pvbt)
 epithalamus related (mfbse)
 stria medullaris (sm)
 fasciculus retroflexus (fr)
 habenular commissure (hbc)
 pineal stalk (PIS)
 midbrain related (mfbsm)
 dorsal longitudinal fascicle (dlf)
 dorsal tegmental tract (dtl)

Ventricular Systems (VS)

1. Lateral ventricle (VL)
 - rhinocele (RC)
 - subependymal zone (SEZ)
 - choroids plexus (chpl)
 - choroids fissure (chfl)
2. Interventricular foramen (IVF)
3. Third ventricle (V3)
4. Cerebral aqueduct (AQ)
5. Fourth ventricle (V4)
 - fourth ventricle proper (V4)
 - lateral recess (V4r)
6. Central canal, spinal cord/medulla (C)

Grooves (grv)

1. Cerebral cortex
 - endorhinal groove (eg)
 - hippocampal fissure (hf)
 - rhinal fissure (rf)
 - rhinal incisure (ri)
2. Cerebellar cortex
 - precentral fissure (pce)
 - preculminate fissure (pcf)
 - primary fissure (pri)
 - posterior superior fissure (psf)
 - prepyramidal fissure (ppf)
 - prepyramidal fissure (pyf)
 - secondary fissure (sec)
 - posterolateral fissure (plf)
 - nodular fissure (nf)
 - simple fissure (sif)
 - intercrural fissure (icf)
 - ansoparamedian fissure (apf)
 - intraparafloccular fissure (ipf)
 - paramedian sulcus (pms)
 - parafloccular sulcus (pfs)

Appendix 3 - Comparison of Mouse Brain Atlas Characteristics and Protocols

	Paxinos & Franklin (2004)	Hof et al. (2000)	Allen Reference Atlas
Strain	C57Bl/6J	C57Bl/6J	C57Bl/6J
Source	Not indicated in the atlas	RCC Ltd, Switzerland	Jackson Lab West Coast, USA
Sex	Male	Male	Male
Age	100 days	110 days	56 days
Body Weight	26-30 g	28.7 g	25.19 +/- 1.59 g
Numbers of brains used for mapping the atlases	1 for forebrain and midbrain, and 1 for medulla	1 entire brain	1 entire brain
Perfusion & fixation	0.1M PBS (pH 7.3) 4% PFA	0.9% saline 4% PFA	Fresh frozen, no fixation
Embedding medium	1.5% gelatin in 0.9% saline	Paraffin	OCT
Thickness of sections	40 µm	5 µm	25 µm
Frequency and numbers of mapped sections	1:4 of total sections (100 coronal levels with 120 -160 µm spacing)	49 of total 1560 coronal sections (49 levels with 130-150 µm spacing)	1:4 of total 528 sections (132 levels with 100 µm spacing)
Nomenclatures	Adopted from Paxinos & Watson (1998) and Paxinos (1995)	Adopted from Swanson (1998-99)	Adopted from Swanson (2004) and Hof et al. (2000)
Nissl staining	Yes	Yes	Yes
Myelin staining	None	Six levels	None
Other stainings	Acetylcholinesterase (AChE)	None	None



Appendix 4 – Tissue Preparation and Nissl Staining Protocol

C57Bl/6J male mice (aged 56 days, with a body weight of 25.19 +/- 1.59g) from the Jackson Laboratory were used for the ARA. All animals were housed and treated in accordance with the NIH Animal Research Committee Guidelines.

The animals were deeply anaesthetized using isoflurane and sacrificed by decapitation. The fresh brains were dissected carefully and rapidly and placed precisely into a grid-line freezing chamber that allows control over the angle and placement of the brains. The chamber was filled with Optimal Cutting Temperature (OCT) mounting medium, and each brain was submerged to the bottom of the chamber, dorsal surface down. The suspended brain was oriented in the chamber under a dissection microscope so that its anterior-posterior axis was parallel to the long axis of the chamber. Its dorsal surface was positioned parallel to the flat skull horizontal plane before the chamber was placed on dry ice to be frozen. The frozen tissue block was then stored at -80°C until sectioning.

The frozen brain block was attached on the chuck of a cryostat with the anterior-posterior axis of the brain perpendicular (for coronal sections) or parallel (for sagittal) to the surface of the chuck. The brains were cut at 25 µm thickness sections on a Leica 3050S cryostat at -15°C. The brain sections were then mounted onto glass slides. Every section through the entire brain was collected with 4 contiguous sections on one glass slide. Sections separated by 100 µm were conveniently located in corresponding positions on two adjacent slides. The coronal sections were collected from the rostral tip of the olfactory bulb through the upper level of the cervical spinal cord. The sagittal sections were collected from the right side to the left side of the entire brain.

The brain sections were desiccated in a 37°C oven for seven days before they were stained with thionin and covered with DPX. The protocol of Nissl staining for fresh brain tissue was modified from Paxinos and Watson (1998). In brief, the slides were placed in 2 washes of xylene for 15 minutes each. The slides were then placed for 3 minutes in each of the following: two washes of 100% EtOH, 90% EtOH, 70% EtOH, 50% EtOH and, finally, water. Next, the slides were stained with 0.25% thionin for 3-5 minutes. The slides were differentiated in fresh water for 3 minutes with 2 washes, and then dehydrated through 50% EtOH, 70% EtOH, 90% EtOH, and 100% EtOH. Finally, the slides were put in xylene and cover-slipped with DPX.