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1 **Routes of the thalamus through the history of neuroanatomy**

2

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19

20 **Abstract (170 words)**

21

22 The most distant roots of neuroanatomy trace back to antiquity, with the first human
23 dissections, but no document which would identify the thalamus as a brain structure
24 has reached us. Claudius Galenus (Galen) gave to the thalamus the name '*thalamus*
25 *nervorum optitorum*', but later on, other names were used (e.g., *anchae*, or buttocks-
26 like). In 1543, Andreas Vesalius provided the first quality illustrations of the thalamus.
27 During the 19th century, tissue staining techniques and ablative studies contributed to
28 the breakdown of the thalamus into subregions and nuclei. The next step was taken
29 using radiomarkers to identify connections in the absence of lesions. Anterograde
30 and retrograde tracing methods arose in the late 1960s, supporting extension,
31 revision, or confirmation of previously established knowledge. The use of the first
32 viral tracers introduced a new **methodological breakthrough** in the mid-1970s.
33 **Another important step was supported by advances in neuroimaging of the thalamus**
34 **in the 21th century.** The current review follows the history of the thalamus through
35 these technical revolutions from Antiquity to the present day.

36

37

38 **Keywords:**

39 Antiquity; Connectivity tracing; History; Human dissection; Neuroanatomy; Staining;
40 Thalamus

41

42	Content
43	
44	
45	Abstract
46	List of abbreviations
47	
48	1. Introduction
49	2. Greek philosophers and physicians
50	3. Galen
51	4. Albertus Magnus
52	5. De Liuzzi
53	6. Vesalius and Eustachius
54	7. Willis, Vieussens, Vicq d'Azur
55	8. Burdach, Luys and Forel
56	9. Staining-based studies
57	10. Ablation-based studies
58	11. Combination of staining- and ablation-based studies
59	12. Direct tracing methods
60	13. Viruses as tracing tools
61	14. In vitro approaches
62	15. Magnetic resonance imaging
63	16. General conclusionS
64	
65	Acknowlegments
66	Conflict of interest
67	References
68	

69 **List of abbreviations**

70

71 AAV : adeno-associated virus

72 BCE : before common era

73 BDA : biotinylated dextran amines

74 **BOLD : Blood oxygenation level-dependent**

75 CE : common era

76 CNS : central nervous system

77 CA1 : hippocampal region CA1 (*cornu ammonis* 1)

78 ChAT : choline acetyltransferase

79 CTB : Cholera toxin subunit B

80 DA : dopamine (dopaminergic)

81 **DBS : deep brain stimulation**

82 **DTI : diffusion tensor imaging**

83 **DWI : diffusion weighed MRI**

84 **EEG : electroencephalography**

85 EGFP : enhanced green fluorescent protein

86 FITC : Fluorescein isothiocyanate

87 GABA : gamma aminobutyric acid

88 GFP : green fluorescent protein

89 HRP : horseradish peroxydase

90 HSV : herpes simplex virus

91 LIPv : ventral lateral intraparietal area

92 MD : mediodorsal

93 MIP : medial intraparietal area

94 mPFC : medial prefrontal cortex

95 **MRI : magnetic resonance imaging**

96 NPY : neuropeptide Y

97 PHA-I: Phaseolus vulgaris) lectin

98 PNS : peripheral nervous system

99 PRV : pseudorabies virus

100 PV : parvalbumin

101 Re : reuniens nucleus

102 RNA : ribonucleic acid

- 103 TH : tyrosine hydroxylase
104 TK : thymidine kinase
105 TRITC : Tetramethylrhodamine-isothiocyanate
106 VLo : ventralis lateralis pars oralis
107 VMpo : posterior part of the ventral medial (thalamic) nucleus
108 VPLo : ventralis posterior lateralis pars oralis
109 VPm : ventral posteromedial
110 VSV : vesicular stomatitis virus
111 WGA: wheat germ agglutinin
112 ZI : zona incerta
113

114 “Scientific practice is above all a story-telling practice. (...) Biology as a way of
115 knowing the world is kin to Romantic literature, with its discourse about organic form
116 and function. Biology is the fiction appropriate to objects called organisms; biology
117 fashions the facts “discovered” about organic beings.”

118 Donna Haraway (1985)

119 (*Primate Visions: Gender, Race and*
120 *Nature in the World of Modern Science*)

121

122 1. Introduction

123 Perhaps the oldest known document to mention a link between a region of the central
124 nervous system and a behavioral function is the about 36-century old Edwin Smith
125 papyrus (e.g., [Kamp et al., 2012](#)), which could be a copy of an even older document.
126 Edwin Smith was an American antiquity dealer who acquired this papyrus in 1862
127 and subsequently tried to translate it, but he never published his translation. Smith
128 kept the hieroglyphic script until he passed away in 1906. After his death, his
129 daughter gave the papyrus to the New York Historical Society, and the American
130 Egyptologist James Henry Breasted (1865-1935) published his own translation of it in
131 1930 (e.g., [van Middendorp et al., 2010](#)). Therein, words like ‘brain’ (‘skull offal’ is a
132 closer translation; [Standring, 2016](#)), ‘meninges’ or ‘spinal cord’ are used. The
133 document describes 48 cases of more or less dramatic wounds or trauma, each of
134 them according to a standardized presentation principle: heading, description of
135 symptoms, diagnosis, and treatment (or prognosis pointing to hopelessness).
136 Twenty-seven of these reported cases concern head injuries, of which 22
137 descriptions are in line with traumatic brain injuries ([Kamp et al., 2012](#)). Among them,
138 case 20, and perhaps case 22, might be the first descriptions of aphasia ([Minagar et](#)
139 [al., 2003](#)). Remarkably, this papyrus is probably the oldest document providing
140 neuropsychological (as one would say today) arguments in favor of localization of
141 functions in the brain. When open and penetrating fractures of the skull were noticed,
142 where the head had been hit obviously correlated with the type of functions lost or
143 altered. No trace, however, of evidence for neuroanatomical knowledge pointing to
144 the brain as an ensemble of structural entities delimited by specific and clearly-
145 identified landmarks.

146

147 Regarding brain organization, various proposals and types of (sometimes fanciful)
148 anatomo-functional conceptions arose later on. Some of them relied on empirical
149 evidence. For instance, Christian philosopher Nemesius (350?-420? CE), bishop of
150 Emesa (now Homs in Syria), defended a view according to which the different higher
151 functions of an organism originated in the ventricles. In his view, the senses and
152 imagination had a rostral ventricular location, memory a posterior one, and
153 intellectual thought seated in between the two others. Nemesius grounded his
154 anatomo-functional proposal on clinical support (van der Eijk, 2008). Indeed, he had
155 observed that frontal injuries impaired senses, not the other functions, middle injuries
156 affected thought but preserved sensation and memory, and posterior injuries altered
157 only memory. This ventricular doctrine, however, disregarded the cerebral substance,
158 or brain parenchyma, as the crucial support of an organism's functions, and therefore
159 remained far away from the first relevant foundations of brain mapping.

160

161 In such a view, there was no place for cerebral tissue, no place for neuroanatomy
162 other than ventricular, hence no place for the thalamus. **However, it is not impossible**
163 **that these anatomofunctional considerations influenced later localisationist thoughts**
164 **about functional organization of brain tissue.** The current review, which is partly
165 historical, will not discuss in detail the different conceptions of the anatomical and
166 anatomo-functional organization of the thalamus over the ages. Rather, it will focus
167 on showing how the evolution of conceptions of, and knowledge about anatomy of
168 the brain and of the thalamus depended on the investigation possibilities opened up
169 by ideological, initially, and then technical progress.

170

171 **2. Greek philosophers and physicians**

172 Describing the brain and identifying the thalamus as a structural subdivision is not
173 conceivable without accepting dissection as a fundamental, unmissable principle of
174 investigation of an organism's and organ's external and internal organization. Human
175 dissection has an old, sometimes chaotic history that could have roots in
176 mummification, an embalming art used in ancient Egypt to prepare the dead for
177 afterlife. For mummification of the body, the brain was removed with hooks
178 introduced into the skull through the nostrils, while the stomach and intestines were
179 dissolved in intra-abdominally injected cedar oil, or were removed together with the
180 lungs and heart after an incision of the flank; these organs were separately placed

181 into a jar (Turliuc et al., 2015). Turliuc et al. (2015) think that this kind of practice
182 might have led Egyptian priests to overcome their inhibitions with regard to the
183 dissection of human cadavers. At this time, however, the process of dismantling a
184 corpse did not go beyond the field of funeral tradition. Alcmeon of Crotone (exact
185 dates of birth and death are unknown), a Greek philosopher of science and medical
186 theory born in Crotone (now a city in Calabria, Italy) near the end of the 6th century
187 BCE, is considered one of the earliest supporters of anatomical dissections for
188 scientific purposes. Whether he dissected human bodies or only other animals is still
189 under discussion, but his work and insightful analyses led him to consider the brain
190 as the seat of intelligence, as did Hippocrates (460?-377 BCE) and others later on.
191 This opinion stands in clear contradiction with Aristotle's (385-323 BCE) influent
192 cardio-centric hypothesis (e.g., Crivellato and Ribatti, 2007). For Aristotle, the heart,
193 and not the brain, was in fact the seat of intelligence, motion, and sensation, and
194 other organs such as the brain or lungs had a role in cooling the heart and mitigating
195 the passions arising therefrom (for more details, see e.g., Engelhardt, 2018).
196 Surprisingly, this debate between cardio-centrism and encephalo-centrism lasted
197 until the 16th century (Crivellato and Ribatti, 2007).

198
199 Alcmeon of Crotone is credited with a first study of the paths of the optic nerve as
200 well as a theory of senses in which he established a functional connection between
201 sensing and the brain: organs of senses (eyes, ears) are connected to the brain by
202 the way of ducts (*porois* in Greek). He also distinguished understanding/thinking and
203 sense perception as different processes.

204
205 Dissection, whether of human cadavers or of less evolved animals, and thus
206 anatomical knowledge, probably traces back to even earlier than ancient Greece.
207 The documents which could have attested to it, however, did not reach our time,
208 presumably because they disappeared during the destruction of the library of
209 Alexandria (which extended over about seven centuries) or for other reasons. The
210 same may have occurred with the contributions of Herophilus of Chalcedon (about
211 325?-255? BCE; Chalcedon is now a district of Istanbul), and Erasistratus of Ceos
212 (310?-250? BCE; Ceos is now Kea, an island in Greece). Both belonged to the
213 prestigious school of Greek medicine in Alexandria. Herophilus and Erasistratus
214 performed human dissections, and presumably even human vivisections (Strkalj and

215 Chorn, 2008). The first two kings of the Ptolemaic dynasty authorized these
 216 dissections and vivisections. It is a matter of fact that most writings and documents of
 217 Herophilus were lost or have been destroyed, and the information available today is
 218 at least second hand. Nevertheless, it is known that Herophilus identified, described,
 219 and named numerous anatomical structures of the nervous system. For instance, he
 220 distinguished motor from sensory nerves, described seven pairs of cranial nerves,
 221 differentiated the cerebrum (encephalon), cerebellum (parencephalon), and
 222 meninges, discriminated the four ventricles, and described the different layers of the
 223 eye. His dissection/vivisection subjects were criminals provided by Ptolemy I and II,
 224 and one thinks that Herophilus dissected up to about six hundred people over about
 225 40 years of practice (Acar et al., 2005). No trace, however, exists of a reference to a
 226 subdivision of the brain that could correspond to the thalamus (Strkalj and Chorn,
 227 2008).

228

229 3. Galen

230 Galen (131-210 CE), or Claudius Galenus, was a philosopher and physician of the
 231 Roman Empire. During Galen's lifetime, dissections of the human body were no
 232 longer acceptable. Following Aristotle's proposal, who defended the idea of referring
 233 to the parts of other animals that appeared comparable to those of humans, Galen
 234 focused on bovid brains, and also on brains from North African baboons (Acar et al.,
 235 2005), which he described in *De Anatomicis Administrationibus*. He was the one who
 236 used the word 'thalamus' for the first time. This word derives from the Greek word
 237 'θάλαμος', or 'thamos' (Gailloud et al., 2003). In fact the name Galen gave to this
 238 structure was '*thalamus nervorum opticorum*'. 'Thalamus' means inner chamber or
 239 anteroom in a given semantic context and, in another one, bridal bed or bridal
 240 chamber (Gailloud et al., 2003; Jones, 1985; Serra et al., 2019). According to
 241 Gailloud et al. (2003) and Jones (1985), 'thamos' may originate in the even older
 242 word 'thalam', which designates the first of the three chambers of an Egyptian temple
 243 (see Figure 1.2. in Jones, 1985). Interestingly, according to Jones (1985) who cites
 244 Morrison and Williams (1968), the rower sitting on the lowest bench of a Greek battle
 245 galley (i.e., a trireme) was called a *thalamite*, and the oar-hole corresponding to this
 246 place was the *thalamia*, which could be another origin of the word. The rower was
 247 called a *thalamian*. Why '*opticorum*' (which can be translated by 'optical') then?
 248 *Thalamus opticorum* means optical bed, a designation that may sound logical if one

249 considers that, at this time, dissections were started from the ventral side of the brain
250 and what was visible in the region encompassing the thalamus was the optic nerve
251 tracing to an oval mass located near ventricles, on which it seemed to be extending
252 like on a bed. It is possible that Galen saw in this organization a resemblance with
253 the aforementioned *thalamia* from which the oar was emerging. Therefore, for Galen
254 and for those who subsequently relayed his teaching for more than fifteen centuries,
255 the thalamus was a structure not dissociable from visual functions. It is not
256 exceptional that brain structures were named according to their resemblance with
257 objects (e.g., pulvinar or habenula; [Turliuc et al., 2016](#)), animals (e.g., the
258 hippocampus), or body parts (e.g., mammillary bodies).

259
260 It may sound weird that two parenchymal structures like the left and right thalamus
261 received a name designating a room, and thus an empty volume. The explanation for
262 this might be related to both what Galen was actually observing (see below) and his
263 *pneuma* (vital spirit) hypothesis of physiology, which was inspired by the nerve
264 physiology of Herophilus and may have driven his observations. Herophilus, and later
265 Galen, considered that all the *pneuma* or "breath" in the body came from the outside
266 air having entered the body. The inspired air underwent changes in the lungs, then in
267 the left ventricle of the heart, from where the vital *pneuma* went to the ventricles of
268 the brain by the arteries. It is there, in the brain, that the vital *pneuma* (*pneuma*
269 *zoticon*) in turn became elaborated and refined into the psychic *pneuma* (*pneuma*
270 *psykhicon*). From there, the psychic *pneuma* was able to flow into sensory organs
271 and muscles, making sensory experiences and body movements possible. Therefore,
272 it would not be surprising that what Galen defined as the thalamus in fact
273 corresponded not to the two oval diencephalic structures made of gray matter that
274 the noun thalamus nowadays refers to, but in fact to a rather middle inner part of the
275 left and right lateral ventricles that border the thalamus dorsally. **Hence, Galen's**
276 **thalamus was a storeroom for the vital spirit.** Burdach (see below) was even
277 convinced that Galen had never seen the thalamus, as he stated in 1822 ([Serra et](#)
278 [al., 2019](#)).

279
280 Because they were taught to physicians over centuries, Galen's views and theories
281 remained extremely influential during almost fifteen centuries. Human cadaveric
282 dissection was prohibited almost throughout this long period, in part because of the

283 growing influence of religious authorities, who advocated the superiority of the soul
284 over the body, and for whom dissection of human cadavers was therefore considered
285 useless and blasphemous (Ghosh, 2015). To this, one must add that the bulk of the
286 population also viewed dissections of human bodies in a negative light, as already
287 had been the case at the time Herophilus and Erasistratus practiced anatomical
288 explorations in Alexandria (Ghosh, 2015).

289

290 **4. Albertus Magnus**

291 The ventricular conception of brain function, which had roots in the 3rd century BCE
292 (see below), was another obstacle. It was still very relevant in the 13th century, as
293 shown by Albert Magnus' proposals. Albertus Magnus (1193-1280) was a German
294 bishop, theologian, astrologer, and philosopher. He described the ventricular system
295 as the seat of human faculties and distinguished three compartments: anterior for
296 presumably what we name the lateral ventricles, middle for the third ventricle, and
297 posterior for the fourth ventricle. The ventricles contained so-called 'sensory vapor'
298 (Stratton, 1931), a point of view inspired by Erasistratus of Geos' explanation of the
299 function of the *pneuma psychicon* which, from the lateral ventricles, controlled
300 structure-function relationships and set the body in motion by getting through the
301 motor nerves to the muscles (Duque-Parra et al., 2017). For Magnus, common sense
302 and basic imagination were located in the anterior ventricle. Imaginative, cogitative
303 and formative powers, and evaluation capacities were located in the middle ventricle.
304 Finally, mnemonic power operated from the posterior ventricle (Figure 1).

305

306 This ventricular doctrine was also clearly influenced by Avicenna's (980-1037)
307 conceptions. He considered the following faculties to be arranged along the rostro-
308 caudal axis of the ventricular system according to the following enumeration order:
309 five senses, representation, sensitive imagination, evaluation, retention, and recall.
310 Despite his anatomo-functional elaboration, Albertus Magnus did not seem to pay
311 much attention to the cerebral and cerebellar substance. It was also the case of
312 Gregor Reisch's encyclopedic compilation (1504), in which a drawing shows lines
313 illustrating the convergence to the most anterior ventricle of sensory information
314 arising from the eyes, nose, tongue, and ears (Figure 2). Such views are reminiscent
315 of the views of Nemesius we mentioned in our introduction. They gave little chance
316 that a tissue-made structure of the brain came under focus, be it the thalamus or any

317 other parenchymal region. It is interesting to note that the ventricular theory was
318 preserved for more than fifteen centuries before Leonardo da Vinci provided the first
319 realistic drawing of the ventricular system. He performed it from a wax cast of an ox
320 brain (Duque-Parra et al., 2017).

321

322 *****

323 Insert Figures 1 & 2 about here

324 *****

325

326 5. De Liuzzi

327 During the 12th century, a period corresponding to the setting up of the first European
328 universities (e.g., Paris in 1150, Bologna 8 years later, and Oxford another 9 years
329 later), the Church ceased to forbid human cadaveric dissections in the domain of
330 anatomical science. The emperor Frederick II even made it mandatory in 1231 (1238
331 in Sinha, 2015) for all people who were to practice medicine or surgery (Ghosh,
332 2015). **The imperial decision was that one dissection be performed every fifth year**
333 **(Sinha, 2015)**. In 1299, however, Pope Boniface VIII issued a Bull that prohibited the
334 boiling and dismembering of human cadavers, a practice that **had** enabled the
335 dissemination of different pieces of a same corpse for e.g., reasons of prestige
336 (pieces of a same person in different burials), ease of transport (**much used by the**
337 **crusaders**), respect of the premortem will to rest in different places, or whatever. This
338 Bull, however, had little effects on dissection of humans motivated by scientific
339 reasons, perhaps more in Italy than elsewhere.

340

341 In Bologna, Mondino de Liuzzi (1270-1326) introduced public dissections of executed
342 criminals as a support to anatomical studies and lectures. De Liuzzi broke with the
343 tradition that required the eminent teacher to read a descriptive text – Galen’s work
344 was often used for this purpose – while a barber (the sector) performed the gestures
345 of the dissection, and a third person (the ostensor) pointed to the organs named by
346 the lecturer (Standing, 2016). In fact, de Liuzzi performed some dissections on his
347 own, taking on all roles, as in 1315 in Bologna when he publicly dissected what could
348 have been the cadaver of an executed woman. Surprisingly, he let Galen’s mistakes
349 **(due to transposition or extrapolation from animal anatomy)** uncorrected and only
350 made a few additions to Galen’s work. These additions encompassed the description

351 of structures located underneath the lateral ventricles and bordering the third
 352 ventricle, which “are of the same substance as the brain”, and which he called
 353 ‘*anchae*’ (a latin word that, placed in its medical context, could mean ‘shaped like
 354 buttocks’; see [Gailloud et al., 2003](#)). There is no evidence, however, that de Liuzzi’s
 355 ‘*anchae*’ actually designated the thalamus. Indeed, what he named so could have
 356 been parts of the caudate nuclei instead ([Swanson, 2015](#)). Whatever may have
 357 been, it seems that until 1610, when Jean Riolan (1580?-1657) resumed the
 358 expression ‘*thalamus nervorum opti-corum*’, the word thalamus was abandoned in
 359 favor of other names, including de Liuzzi’s *anchae*, but also *monticulus* (little
 360 mountain), *nates* (buttocks), and even *testes* ([Serra et al., 2019](#)).

361

362 **6. Vesalius and Eustachius**

363 With the birth of printing (around 1470), anatomical engravings underwent a real
 364 boom and were more than exceptionally the work of artists who acquired
 365 unquenchable fame during their lifetime or after their death. **Beside Dürer, Donatello,**
 366 **Michelangelo, and Raphael, one can also mention** Leonardo da Vinci ([Sinha, 2015](#);
 367 [Swanson, 2000](#)). He illegally practiced overnight candle-lit dissections of human
 368 cadavers in the crypt of Santa Maria Nuova hospital, of which he left incredibly
 369 remarkable drawings (e.g., [Perloff, 2013](#)). **Sinha (2015) mentions that da Vinci made**
 370 **750 drawings and might have written more than 100 anatomical books. As was**
 371 **already the case at the end of the 13th century, a main problem for these dissections**
 372 **was the shortage of usable corpses, which led to illegal conducts such as grave**
 373 **openings for cadaver stealing.** What could have been the first printed anatomical
 374 illustration of the brain structure is the representation published by Magnus Hundt
 375 (1449-1519) in 1501 in his *Antropologium de Hominis Dignitate, Natura et*
 376 *Proprietatibus, de Elementis, Partibus et Membris Humani Corporis*. Showing the
 377 head with the organs of senses and their connections, the illustration emphasized the
 378 ventricular cells, thereby attesting to Galen’s still very significant influence.

379

380 In 1519, Lorenz Fries (1490?-1550) published *Spiegel der Artzney* (literally: medicine
 381 mirror) showing six modern-like representations of brain dissections arranged around
 382 the drawing of an open human trunk ([Swanson, 2000](#)). On these drawings, however,
 383 nothing visible that could correspond to the thalamus! In 1543 appeared the *De*
 384 *Humani Corporis Fabrica*, a masterpiece of anatomy by Andreas Vesalius (1514-

385 1564). Vesalius was reputed for his public dissections of human cadavers,
386 sometimes with 500 observers around him it is said (Scatliff and Johnston, 2014),
387 including artists sketching what was presented and commented by the anatomist.
388 The book encompassed 273 woodcut illustrations of so far unmatched quality and
389 precision, among which 25 were of brains (Scatliff and Johnston, 2014). Incredibly, at
390 the beginning of the 1930s, typograph Willy Wiegand found 227 of Vesalius' original
391 woodblocks from the *Fabrica*, and of a summary thereof published a few days earlier
392 and named *Epitome*. In 1932, American physician Samuel Lambert, who thought that
393 this material had perhaps not disappeared, asked Wiegand to investigate. The blocks
394 were discovered in an attic at the University of München. The pictures corresponding to
395 the originally in Basel(Switzerland)-printed woodcuts were republished in 1934
396 (Lambert, 1934) but, unfortunately, all these blocks were destroyed in 1944 during
397 the bombing of München by the allies (Scatliff and Johnston, 2014).

398
399 It is in Vesalius' remarkable anatomical monograph that one can find the first
400 illustrations of the human thalamus, one as a posterior view covering the brainstem
401 and the thalami, and one as a horizontal view of a section through a brain coiled in
402 an open skull (Figure 3). The beauty of the design is impressive. While Vesalius'
403 illustrations were printed from woodcuts, Bartolomeo Eustachius (1513?-1574),
404 another founder of anatomy, used copper plate engravings, which, thanks to the
405 better resolution they offered, permitted the inclusion of even more details. The
406 copper-engraving technique had been much developed by German painter and
407 printmaker Albrecht Dürer (14712-1528). In 1552, Eustachius prepared 47 drawings
408 on copper plates, but only 8 of them showing skeletons and muscles were published
409 during his life, in *Opuscula Anatomica*, in 1564. The 39 other plates disappeared and
410 reappeared almost 150 years after Eustachius' death, in 1722 (Adanir and Bahsi,
411 2019). On one of those plates, Eustachius had represented for the first time the
412 interthalamic commissure (a problematic denomination, as this structure is devoid of
413 crossing axons). Therefore, **intrathalamic** adhesion is a better name (Gailloud et al.,
414 2003) to designate **this** thalamic structure, which apparently not all human subjects
415 possess (Borghei et al., 2020; 2021; Damle et al., 2017).

416
417 *****

418 Insert Figure 3 about here

419 *****

420

421 7. Willis, Vieussens, Vicq d'Azur

422 Before Thomas Willis (1621-1675), there was no monograph which focused
423 exclusively on the nervous system. So far, monographs dealing with anatomy
424 covered the whole body, the brain being only part of their topics. *De Cerebri Anatome*
425 (1664) by Willis, and *Nevrographia Universalis* (1884) by Raymond Vieussens
426 (1641?-1715) were the first monographs to deal exclusively with the anatomy of the
427 nervous system (Meyer, 1971). Willis produced remarkable drawings of the thalamus,
428 which he named *thalamus opticus*. He also insisted on the fact that there was no
429 cavitation in this structure, despite being otherwise rather seduced by Galen's
430 *pneuma* hypothesis at a purely functional level. His drawing placed the thalamus
431 underneath the striated bodies, for the first time illustrated at the top of the medulla
432 oblongata (i.e., the brainstem). The outstanding quality of Willis' observations and
433 analyses (e.g., the conception that voluntary movements originate in the cortex,
434 involuntary ones in the cerebellum, that the cortex is the substrate of cognition, etc.
435 Arraez-Albar et al., 2015) most probably owe a lot to the revision of the university
436 statutes at Oxford in 1636. Among other changes, this revision introduced dissection
437 as mandatory in medical studies and allowed, for the purpose of dissection, the
438 anatomy reader to be given the corpse of any person executed in a radius of 21
439 miles around Oxford. Before this, teaching of anatomy largely relied on Aristotle's,
440 Hippocrate's, and Galen's outdated contributions (Arraez-Albar et al., 2015).

441

442 A famous anecdote of that time might be worth a short relating. Anne Greene, a
443 maid, was wrongly accused of having murdered her, in fact, miscarried baby. On
444 December 14, 1650, she was hung and, after her apparent death, taken off the
445 gallows and placed in a coffin that was brought to the private home of Dr William
446 Petty, the reader in anatomy. When Petty and Willis opened the coffin, they were
447 dumbfounded to hear a breath and a noise in Ms Greene's throat. The idea of
448 dissecting her was given up and they both, with the help of colleagues, tried to
449 reanimate her, which they actually managed to do. Over days and weeks, Anne
450 Greene returned to normal life and died 15 years later after having married. From her
451 and her husband's nuptial bed, three children were born in the meantime (e.g.,
452 Hughes, 1982).

453
454 **Beside coining neuroanatomical terms (Molnar, 2004)**, Willis also strongly relied on
455 careful observations of clinical cases to which he tried to relate his anatomical
456 observations (Finger, 1994); **he is well known for correlating symptoms and brain**
457 **morphology in his patients (Molnar, 2004)**. Lacking a traditional medical education,
458 he had also much interest in experimental sciences (Arraez-Albar et al., 2015). **For**
459 **more details on Willis, see Molnar (2004)**. According to Gailloud et al. (2003),
460 Raymond Vieussens, who was the first to introduce the distinction between white and
461 gray matter, placed the thalamus on the back of the caudate nucleus and
462 documented the connection of the optic nerve with the posterior part of the thalamus.
463 He also named the *corpus album subrotundum*, which corresponds to the part of the
464 anteroventral nucleus protruding into the third ventricle. Félix Vicq d'Azur (1748-
465 1794), who was the first neuroanatomist to systematically dissect the human brain in
466 the coronal plan, provided a first accurate description of the mamillo-thalamic tract,
467 along with a precise description of this bundle's course through the thalamus. Vicq
468 d'Azur is also known for advocating the adoption of a single nomenclature to
469 designate the same anatomical parts in all species (Parent, 2007).

470

471 **8. Burdach, Luys and Forel**

472 In his *Vom Baue und Leben des Gehirns* published in 1822 (see illustration in Figure
473 4), Karl Friedrich Burdach (1776-1847) introduced the word pulvinar (meaning pillow
474 or cushion) to denominate the largest nucleus of the thalamus. He also identified the
475 *lamina medullaris interna* and *externa*, which he used as delimitations to subdivide
476 the thalamus into superior, internal, and external regions. Thus, the thalamus
477 encompassed four regions if one adds the pulvinar to the aforementioned three.
478 Thereby, Burdach did not only open the possibility of addressing the question of a
479 link between architectonic thalamic subdivisions and possible functional
480 specializations, assuming the two can actually be related to each other, but also
481 paved the way towards a modern nomenclature of the thalamic architecture (Meyer,
482 1970). Jean Bernard Luys (1828-1897), in his *Recherches sur le Système Nerveux*
483 *Cérébrospinal: sa Structure, ses Fonctions et ses Maladies (1865)*, identified 4
484 thalamic subregions he called centers because of their functional implications, all
485 related to senses: the anterior center (*centre antérieur*; olfactory), the middle center
486 (*centre moyen*; optic), the median center (*centre médian*; somaesthetic), and the

487 posterior center (*centre postérieur*, acoustic). According to Jones (1985), Luys saw in
488 the thalamus a kind of relay between spinal cord-associated functions and high level
489 activities of the brain. In his 1881 book, Luys wrote: “*From a physiological standpoint,*
490 *the optic thalami are intermediary regions interposed between the purely reflex*
491 *phenomena of the spinal cord and the activities of psychical life...*”. Luys also named
492 ‘Luys body’ what we now call the subthalamic nucleus (Pearce, 2001). Finally, in
493 1877, the year he became *Privatdozent* in München (Germany), Auguste-Henri Forel
494 (1848-1931) introduced the distinction of four different thalamic regions. He named
495 them anterior, posterior, ventral, and lateral, to which he added a fifth center he
496 called medial (Gailloud et al., 2003). This nomenclature subsequently inspired
497 denominations when it came up that these regions had to be split into smaller
498 subregions based on staining and connectivity studies (see below). The thalamus
499 was the topic of Forel’s PhD thesis defended in 1872 (Osiro et al., 2012).

500

501 From antiquity to the turn of the 19th century, knowledge about the thalamus
502 progressed very slowly, partly because of technical limitations, partly because of
503 ideological pressures and beliefs. What can be retained from this period, however, is
504 the impressive progress in the artistic quality of the drawings made over the last three
505 centuries. They all related to essentially a macroscopic description of external and
506 much less frequently internal aspects of the thalamus. Details about the partition of
507 the human thalamus and the inner organization of the nuclei were still lacking, but the
508 latest studies in primates enabled to propose a subdivision of the thalamus into a
509 more or less important number of major subregions based on anatomical landmarks.
510 Knowledge about the connectivity of thalamic subdivisions with other brain regions
511 remained entirely to be established, being waiting for highly reliable staining
512 techniques. But things would quickly change, partly because of technical progress
513 regarding tracing methods, partly because more and more animal studies were
514 undertaken, although animals were also a source of confusion, rats, mice, cats and
515 inframammals having thalamic maps differing, in several aspects, from those of
516 humans (e.g., Butler, 2008). What was still missing, was a precise idea about the
517 functions the thalamus and even more about what its subdivisions were contributing
518 to. Obviously, as advocated by Meynert (1872), considering functional outputs rather
519 than anatomical cues was also one possible criterion to setup a nuclear
520 nomenclature of the thalamus.

521

522 *****

523 Insert Figure 4 about here

524 *****

525

526 **9. Staining-based studies**

527 During the second half of the 19th century and in addition to the popularization of the
528 microscope as a revolutionary tool to explore what so far was invisible to the naked
529 eye, two major technical breakthroughs contributed to further progress in the
530 knowledge of the thalamus, and more generally of brain anatomy. The first one
531 concerned tissue staining techniques, and the second one the use of so-called
532 ablative studies in a large variety of animal models. These two approaches will
533 converge and combine, permitting further gain in neuroanatomical accuracy. It might
534 be worth noting that Joseph Gerlach (1820-1896) introduced the first stain for
535 nervous tissue in 1858. It was carmine, a red powder obtained by grinding the dried
536 body and eggs of the female cochineal (*Dactylopius coccus*), a South American and
537 Mexican cactus parasite (Swanson, 2000). Gerlach's carmine-based staining was
538 useful to stain cell bodies and therein more specifically their nucleus.

539

540 Other methods were developed later on, and to name just two of the determinant
541 ones, let us start with Franz Nissl (1860-1919) in 1884, at a time he was a medical
542 student. Nissl's method was based on alcohol fixation of brain tissue, which was
543 subsequently sectioned and stained with magenta red, methylene blue, and toluidine
544 blue, exactly in this sequence (da Mota Gomes, 2019). Although this method did not
545 permit to visualize the morphology of neurons (the stain binds to nucleic acids and
546 therefore mainly stains the cell body), it proved extremely useful in characterizing the
547 cytoarchitecture peculiarities of different brain structures, including the thalamus. For
548 morphological details, one had to use another method developed by Camillo Golgi
549 (1843-1926) in 1873, the famous black reaction (*la reazione nera*) or silver
550 impregnation method. The reason why this method stains only a small proportion (ca.
551 3%) of neurons is still unknown (Bentivoglio et al., 2019). The staining principle of
552 Golgi's method required that tissues be hardened in potassium bichromate to be
553 soaked in a silver nitrate solution. This technique enabled the perfect visualization of

554 some neurons in their entirety (i.e., axon, dendrites, dendritic spines, and soma) on a
555 well-contrasted background, i.e., devoid of staining residues.

556

557 When Nissl died in 1919, he was studying the connectivity between the thalamus and
558 the cortex. Thirty years earlier, hence in 1889, he had given a talk about the
559 organization of the thalamic nuclei in the rabbit during the meeting of the
560 *Naturforscherversammlung* (meeting of naturalists) in Heidelberg (Germany). Based
561 on histological arguments, Nissl distinguished 16 nuclei as such: anterior dorsal,
562 anterior ventral, anterior medial, medial middle, posterior medial, anterior lateral,
563 posterior lateral, magnocellularis, medial ventral, lateral ventral, dorsal ventral,
564 posterior, midline, and ventral, dorsal and lateral *Gitterkerne* (lattice nuclei), the latter
565 three corresponding to the reticular nucleus (Andô, 1937). For reasons unbeknown to
566 us, Nissl did not publish the paper corresponding to the talk he gave in Heidelberg
567 before 1913. At this time, his nomenclature encompassed 18 subregions, some of
568 which had already been considered entities in 1889: anterior dorsal, anterior ventral,
569 anterior dorsomedial, anterior ventromedial, middle medial, posterior medial, anterior
570 lateral, posterior lateral, magnocellularis, posterior dorsal, posterior ventral, central,
571 par-ependymal, prebigeminal, the ventral anterior and ventral posterior groups of
572 nuclei, dorsal and ventral *Gitterkerne* (Andô, 1937).

573

574 Meanwhile, Münzer and Wiener (1902) distinguished 14 nuclei, Winkler and Potter
575 (1911) an identical number, d'Hollander (1913) 17, and Miura (1933) 23, which he
576 grouped in 5 *ensembles*. Andô (1937) later proposed a breakdown of the thalamus
577 into 6 *ensembles* encompassing 26 nuclei. These discrepant breakdown proposals,
578 which are just a few selected examples, clearly show that observation of the same
579 brain structure using comparable or constantly improving staining methods in the
580 same (or different) species did not lead to a consensual view on the cytoarchitectonic
581 organization of the thalamus. Such divergencies, essential as they are in the
582 evolution of knowledge, reveal that beyond the brain preparations examined, the
583 species studied, the instruments used, the eye of the observer was most probably
584 just as important as technical tools.

585

586 Le Gros Clark also conducted staining-based (and ablation-based, see hereafter)
587 studies of the thalamus. In an important review article (Le Gros Clark, 1932) on the

588 structures and connections of the thalamus, he emphasized how important it is to
589 have a clear nomenclature in order to be able to apply the appropriate experimental
590 methodology for progressing on anatomical knowledge about this structure. And as
591 the thalamus is a complex structure in high order mammals, he pleaded for
592 comparative anatomy, defending the idea that it was necessary to start from the
593 thalamic organization in lower vertebrates in order to follow its phylogenesis up to
594 primates. Indeed, the thalamus evolved from a structure being mainly concerned by
595 sensory impulses from brain stem, spinal cord and hypothalamus in cyclostomes, to
596 a structure on which evolution superimposed an upper level connected with
597 association areas of the cortex, reaching a culmination in primates (for more details,
598 see e.g., Butler, 2008). Le Gros Clark was also the one to propose that, beyond
599 cellular groupings, subregions of the thalamus be delimited by additional
600 consideration of fiber connections, pointing to the fact that differences in
601 cytoarchitecture do not necessarily parallel functional differentiations. This is perhaps
602 more the case for intralaminar nuclei than for others.

603

604 **10. Ablation-based studies**

605 Beside these histology-based approaches, an ablation-based strategy aiming to
606 characterize the organization of the thalamus according to its connectivity emerged.
607 One of the first scientists to use such a strategy was Bartolomeo Panizza (1785-
608 1867). He damaged the visual pathway in newborn animals (e.g., rabbits, dogs) at
609 some place (e.g., a unilateral ablation of the eye bulb) in order to, later on in adults,
610 post-mortem trace the lesion-induced degeneration indicated by the shrunken
611 regions (Figure 5). Sometimes he also used animals with congenital or accidental
612 lesions of e.g., an eye bulb. This strategy allowed Panizza to realize that following
613 postnatal unilateral enucleation, parts of the thalamus (and of the posterior cerebral
614 cortex) had substantially shrunken. When the thalamic parts he had found to have
615 degenerated were then destroyed in the intact adult brain, the animals showed
616 blindness contralateral to the lesion (Colombo et al., 2002). Observations of this kind
617 provided additional functional support to the implication of thalamic regions in visual
618 functions. They also contributed to extend knowledge about thalamic connectivity.

619

620 Other ablative studies were performed to study the connections between the
621 thalamus and cortical regions, the principle being then to damage circumscribed

622 regions of the cortical mantle and observe the resulting retrograde degeneration in
623 the thalamus. When thalamic degeneration was observed it was interpreted as
624 evidence for existing connections between the damaged cortical and the
625 degenerated thalamic zones. It soon emerged that after lesions restricted to
626 particular cortical regions only delimited neuronal clusters of the thalamus underwent
627 degeneration, and the location of these clusters depended on which of the cortical
628 regions had been damaged. Bernhard von Gudden (1824-1886) was one of the first
629 scientists to use this ablation method to describe the organization of the thalamus
630 from a connectivity and possibly functional point of view. As the animals with lesions
631 had to survive post-surgically for a while, sometimes several months, they could be
632 observed and behavioral consequences of their lesions could be evaluated. Von
633 Gudden also invented a microtome, this instrument enabled the preparation of clean
634 human brain sections, 55 μm in thickness after Forel's improvements. Von Gudden is
635 best known for his studies showing a partial decussation of the optic paths, on which
636 he spent about 30 years of his researcher life (Sarikcioglu, 2007). As a renowned
637 psychiatrist, von Gudden was assigned to follow the health of King Ludwig II of
638 Bavaria, who was suffering mental illness diagnosed as paranoia. On June 13th,
639 1886, they both had an after-dinner walk by the Starnberg Lake, in whose waters
640 they were found dead half an hour before midnight. What exactly happened remains
641 mysterious, but it is well possible that King Ludwig II, who was very corpulent, killed
642 von Guden before himself dying of cold water shock.

643
644 Before von Gudden, Luigi Rolando (1773-1831) had performed thalamic lesions in a
645 variety of animals (lambs, pigs, dogs, hamsters, chickens, ducks, turtles), and he
646 noticed that motor incoordination emerged in mammals, blindness in birds, and
647 cognitive decline in reptiles (Serra et al., 2019). On his side, François Magendie
648 (1783-1855), who also used lesion approaches in frogs and birds, found functional
649 impairments that went beyond the visual sphere as they encroached onto motor
650 functions. In general, however, the ablative methods lacked – sometimes seriously –
651 precision. For instance, when used in young animals or after very long survival times,
652 as sometimes in brain-damaged humans, degeneration could extend both
653 retrogradely and anterogradely over a few synapses. Therefore, although pointing
654 towards interesting directions, the von Gudden method did not permit an accurate
655 description of the organization and functions of the thalamus. As a result, where a

656 consensual agreement would have been welcome, the room for controversy and
657 dispute remained large. In particular, Marie Jean Pierre Flourens (1794-1867) was
658 inexhaustible in matters of sharp criticism of the ablative method, which he
659 nevertheless systematized by introducing a principle of rigorous delimitation of the
660 damaged region and a requirement of reproducibility of the observations made (Serra
661 et al., 2019). At this time, however, the thalamus was clearly more than just a
662 question of visual functions. It is noteworthy that, despite this skepticism, until the
663 1940s, the retrograde cell degeneration method was frequently used (Jones, 1985).

664

665 *****

666 Insert Figure 5 about here

667 *****

668

669 **11. Combination of staining- and ablation-based studies**

670 A true progress in connectivity tracing occurred when Vittorio Marchi (1851-1908)
671 went to Camillo Golgi's laboratory as a specialization fellow, and there developed a
672 novel staining method that enabled unambiguous visualization of degenerating
673 nervous fibers (Talamonti et al., 2013). Quickly this method became very popular in
674 the field of neuroanatomical studies. It consists in placing tissues in a mixture of
675 osmium tetroxide and potassium chlorate used as an oxidizing agent. So exposed,
676 degenerating myelin becomes black; the material stained is a cholesterol ester or a
677 polysaccharide (Strich, 1968). As with the aforementioned ablation method, a lesion
678 was essential for successful tracing with the Marchi fluid, but this technique now
679 allowed perfect localization of the pathways that underwent Wallerian degeneration
680 triggered by the lesion. A major drawback, however, was that it only reveals
681 degenerating myelin sheaths, and thus was restricted to tracks of myelinated fibers
682 (Figure 5). Furthermore, it was reputed capricious, variable and generating artefacts
683 (Prickett and Stevens, 1939).

684

685 Nevertheless, the technique led to the identification of deep thalamic connections
686 such as the ansa peduncularis of the thalamus, the auditory pathway, the
687 cerebellothalamic afferences, the optic tract, the spinothalamic tract, and the
688 trigeminothalamic tract (Serra et al., 2019). It clearly aided agreements in many
689 discussions concerning the intrathalamic localization of endings of afferent pathways

690 (Jones, 1985). To get a more precise idea of how studies relying on Marchi's staining
691 were constructed and how the connections of thalamic nuclei could be visualized
692 under a microscope, the reader might see e.g., Le Gros Clarke and Boggon (1933a;
693 1933b). Le Gros Clarke made his mark on the history of the thalamus with an
694 impressive review paper he published in 1932, and in which he summarized a large
695 part of what had been done so far (Jones, 1985). While Marchi's method has been
696 decisive in the study of corticothalamic connections, this is not true the other way
697 round, i.e., in the thalamocortical direction (Jones, 1985). It was not before 1882 that
698 myelinated fiber tracts could be nicely stained in the absence of lesions, when Carl
699 Weigert (1845-1904) introduced his technique based on the use of potassium
700 dichromate and hematoxylin to stain the myelin sheaths of axons. Conversely to
701 reduced silver methods which stained axons themselves (e.g., Ramon Y Cajal,
702 1891), and thus led to a jumbled staining in which detail was difficult to follow,
703 Weigert's methods produced a nice staining with weak background.

704

705 Further progress in fiber tracing appeared with the revolutionary contribution of Walle
706 Nauta (1916-1994), his colleague Loyd Ryan, and the former's doctoral student Paul
707 Gygax, near the middle of the 20th century. Already as a student, Nauta had been
708 attracted by the hypothalamus, a structure in which most fiber tracts are
709 unmyelinated. This characteristic unfortunately precluded the use of Marchi's method
710 to study fiber tracks. In Nauta's view, an ideal stain would, therefore, highlight the
711 axoplasm affected by the degenerative process, but without interfering with the other
712 cells of the hypothalamic parenchyma. At the time Nauta tried to develop a technique
713 meeting these specifications, he was a lecturer in Zürich, where he spent four years
714 (1947-1951) in the laboratory headed by Gian Töndury, a renowned anatomist also
715 interested in embryology. Nauta's method derived from the silver impregnation
716 method of Max Bielschowsky (1908). Bielschowsky's method had two major
717 drawbacks: a high staining background and an unstable reproducibility. A third
718 drawback was its time-consuming implementation, as, before additional
719 improvements brought later on by other users, it took about 49 hours before the
720 tissue was observable under a light microscope.

721

722 What Nauta tried to set up was a technique marking degeneration of amyelinic fibers
723 in association with a reduced background from the co-staining of intact fibers. It is

724 beyond the scope of this article to present the many attempts Nauta made, and how
725 he tried to meet this challenge, but success finally relied on perseverant empiricism
726 and some serendipity (Jones, 2006). The method (Nauta and Gyax, 1954) was
727 subsequently used in many places throughout the world to discover or reinvestigate
728 all major connections in the central nervous system (Jones, 2006), including those of
729 the thalamus. The principal gain of this method was an – at the time –, unachieved
730 resolution quality, which made it remain the principal method for investigating
731 neuroanatomy until the 1970s. For instance, in 1960, Nauta published a paper on the
732 neuroanatomical organization of the spinothalamic track. Minderhoud (1971) used
733 Nauta's method in order to visualize the efferent connections of the reticular nucleus
734 in the rat, and Jones (1967) described cortical and thalamic connections of the
735 somatosensory cortex. Johnson (1961) used it to study the connections between the
736 thalamus and the striatum in the cat. Other descriptions concerned the cerebellar
737 projections to the thalamus in the monkey (Kusama et al., 1971). These are just a
738 few examples. Thanks to Nauta, the visualization of degenerating fibers had much
739 improved, but the problem concerning the control of the localization and extent, and
740 thus of the exact delimitations of the lesions and the latter's reproducibility remained
741 unchanged. The progress nevertheless came at a point where neuroanatomy, which
742 for a long time had been content with being barely a little more than structural, began
743 to question itself more and more firmly in terms of functional analyses, because
744 investigation tools then allowed acceptable tracking of connectivity.

745

746 Almost at the same time as intensive connectivity studies were performed with the
747 Nauta technique, the introduction of glutaraldehyde (a molecule first produced in
748 1951) by Karnovsky (1965) and the availability of pure paraformaldehyde allowed
749 improved brain fixation, compatible with reliable electron microscopy observations.
750 These new approaches were considered a true revolution in the investigation of the
751 nervous system anatomy. Indeed, electron microscopy gave access to the
752 recognition of axons, dendrites, dendritic spines, synaptic structures, organelles...
753 (e.g., Peters et al., 1976). Amongst major advances was the finding that the majority
754 of thalamic fiber terminations in the cerebral cortex were upon dendritic spines,
755 implying that the fibers did not only end on cells other than the not well-understood
756 'stellate cells' of layer IV, but also on pyramidal neurons. This would not have been a
757 surprise for Cajal, who proposed at the end of the 19th century that the pyramidal

758 cells were the major recipients of thalamic afferents. In the 1960-1970s, the German
759 school of cytoarchitectonic saw thalamic afferents ending on vaguely-defined ‘granule
760 cells’ of layer IV. The latter were supposed to relay the afferent impulses to the
761 pyramidal cells (Sholl, 1956; Jones, 2007 for a review). The renewed gain of interest
762 for the Golgi technique that could be used on fixed tissue permitted the
763 (re)examination, with both light and electron microscopy, of the classification of
764 cortical neurons in the primate visual and somatosensory cortex. Thalamocortical
765 projections were also (re)investigated, often in conjunction with terminal degeneration
766 of specific pathways (Fairen et al., 1977; Jones, 1975). In particular, it became
767 possible to identify with precision the cortical neurons in lower layer III and in layer IV,
768 with which thalamic afferents establish synapses (e.g., Peters, 1979).

769

770 For instance, Jones (1975) used a combination of **i)** axonal degeneration (Nauta
771 technique, see above), **ii)** autoradiography ($[^3\text{H}]$ proline, see below), both targeting
772 the ventrobasal and/or the ventrolateral thalamus, and **iii)** rapid Golgi coloration and
773 light microscopy in order to precisely study the terminations of thalamic afferents in
774 the sensorimotor regions of the primate cerebral cortex. He showed, for the first time
775 in somatic cortical areas, a great proportion of thalamic terminals in a part of layer III,
776 in addition to the expected dense projection in layer IV, previously evidenced using
777 degeneration techniques. He also showed that this part of layer III encompassed
778 large pyramidal cells and corresponded to layer IIIb of Brodmann. A second major
779 finding concerned qualitative and quantitative differences of thalamic terminations in
780 area 3 as compared to areas 1 and 2 of the somatic cortex: terminals were found to
781 be largely denser in area 3 than in the two other ones.

782

783 Another example is by Morest (1975), who studied the synaptic relationships of Golgi
784 type II cells (neurons with a short ramifying axon) in the medial geniculate body of the
785 cat using combined Golgi and silver-degeneration methods, and electron microscopy.
786 He showed that these cells form dendro-dendritic synapses with the principal neuron
787 in terminal aggregates called synaptic nests, and that Golgi type II cells that receive
788 endings from the afferent axons send processes to principal cells, which are also
789 contacted by the same afferent axons. Furthermore, Morest’s analyses pointed to the
790 origin of different types of afferent axonal endings and defined the morphological
791 types of axonal endings and their relationship with the Golgi type II cells. Finally,

792 Morest provided a fine description of the cytological varieties of synapses according
793 to their origins and their relationships to the surface of the Golgi type II cells. To give
794 a last example, [Somogyi \(1978\)](#) also used combined degeneration, Golgi staining
795 and both light and electron microscopy methods to study thalamocortical
796 connections. After damage to a lateral geniculate body, he visualized thalamocortical
797 projections synapsing on two neurons located in layer IV, presumably a spiny
798 interneuron and a small pyramidal one, which established connections with the
799 dendrites of two cortical stellate neurons. Thus, he was able to describe three links in
800 a thalamo-cortical-intracortical neuronal chain.

801

802 **12. Direct tracing methods**

803 The aforementioned problem related to the fact that lesions were mandatory would
804 be solved with a tracing technique that does not require its combination to a lesion
805 approach, and thus a method enabling direct visualization of fibers connecting two
806 brain structures. In the early 1890s, as mentioned above, silver impregnation
807 techniques (e.g., [Ramon y Cajal, 1891](#)) **enabled the staining of neurons**, but they
808 stained all axons, which **negated** any precise investigation **in the jungle of axons**. It is
809 also noteworthy that a gain in precision of the lesions was achieved by the use of
810 stereotaxic apparatuses that were adapted from primates to other animal species
811 (e.g., [Blomstedt et al., 2007](#)), but even with an inframillimetric assistance to the 3D
812 positioning of lesion devices in the brain, there remained between-subject variability.

813

814 In the late 1960s and early 1970s, methods relying on the axonal transport of injected
815 molecules to either axonal processes (anterograde tracers; Figure 6, top) or cell
816 bodies (retrograde tracers; Figure 6, middle) began to develop. [Kristensson and
817 Olsson \(1971a, b\)](#) found that the intrinsic cellular transport mechanisms can be used
818 to spread stains within neurons and label the origin, the course and termination of
819 axons. This discovery, combined with immunohistochemistry, genetics, and
820 neurophysiology, **signaled** the beginning of a new era in neuroanatomy ([Lanciego
821 and Wouterlood, 2011 for a review, Figure 6, bottom](#)), including for the thalamus. The
822 first tracers (anterograde) were radiolabeled amino acids ($[^3\text{H}]$ leucine or $[^3\text{H}]$ proline).
823 They were injected into the cerebral tissue, whence they were incorporated in
824 polypeptides, transported to axons and terminals, and subsequently identified by
825 autoradiography ([Droz and Leblond, 1962, 1963; Grafstein, 1967](#)). They were used

826 systematically by Cowan, Hendrickson and collaborators (e.g. Cowan et al., 1972,
827 see also Woolsey, 2016). The Cowan et al. review article summarized publications
828 using autoradiography alone or in combination with degeneration methods and
829 became a **standard** reference.

830

831 **For example**, the retino-thalamic pathway in the monkey and chick was labelled
832 without any lesion after injection of **a** radioactive tracer in the eye vitreous
833 (Hendrickson et al., 1970; Hendrickson, 1972; Schonbach and Cuénod, 1971;
834 Schonbach et al., 1971). **Likewise**, the cortico-thalamic pathways were defined after
835 radioactive tracer injection in the mouse somatosensory or motor cortex (Price and
836 Woolsey, 1971). In the macaque, using [³H]proline, Nelson and Kaas (1981)
837 described the somatotopic pattern of the connections between the ventroposterior
838 nucleus of the thalamus and areas of the somatic cortex. In dogs, using a
839 combination of [³H]leucine or [³H]proline, Person et al. (1986) traced the contralateral
840 projections of the fastigial nucleus to – among other targets – the thalamus, including
841 the paraventricular complex and the medial dorsal nucleus, as well as the central
842 medial, paracentral, parafascicular central lateral, ventral medial, and ventral lateral
843 nuclei. **The eye-specific segregation of the retinal input in the dorsal lateral geniculate
844 nucleus was identified with [³H]proline combined to local eye lesions or eye removal
845 (e.g., Guillery et al., 1980). Notice that ocular dominance columns were identified by
846 Wiesel et al. (1974) with the same technic, which was also used to study the
847 development of these columns (e.g., Ruthazer et al., 1999).**

848

849 Such autoradiographic techniques provided a very sensitive method for tracing fine-
850 fibered systems and for visualizing pathway terminals. Furthermore, these methods
851 did not suffer from retrograde transport of labeled materials or from the fiber *en*
852 *passage* problem (Figure 7) observed with the previous degeneration-based
853 techniques. This approach supplanted the Nauta techniques (Jones, 2007 for a
854 review). Tracers such as [³H]leucine were also used in functional imaging studies.
855 For instance, Pohle and Matthies (1974) injected [³H]leucine intraperitoneally to
856 identify the structures implicated in the consolidation of a brightness discrimination
857 task; an increased radioactivity was found in the hippocampus and cortex, not in the
858 lateral thalamus, disqualifying the latter from a possible contribution to consolidation
859 of that type of memory. Although the autoradiographic method had a huge impact on

860 the field of neuroanatomy, radioactive tracers were not used for very long. Different
861 reasons included: **i)** the long duration of the experiments, **ii)** the nature of the labeling
862 (indirect, i.e., silver grains outside of the labeled neurons vs direct, i.e.,
863 immunohistochemistry labeling inside the neurons), and **iii)** the restrictions and
864 measures associated with the short- and long-term management of radioactive
865 isotopes ([Lanciego and Wouterlood, 2011](#)).

866
867 In the 1970s-1980s, the use of retrograde tracers arose. Macromolecules injected
868 around a set of axon terminals were transported back to the cell bodies of origin.
869 These tracers are compatible with light or electronic microscopy using histological or
870 immunohistological processing, fluorophores, or conjugation of a fluorophore with an
871 enzymatically active probe ([Saleeba et al., 2019 for a review](#)). The first – mainly –
872 retrograde tracer, namely the glycoprotein and enzyme horseradish peroxidase
873 (HRP), started to be used in the early 1970s. It could be visualized by a simple
874 histochemical reaction. The first reports with HRP labeling concerned the peripheral
875 nervous system ([Kristensson et al., 1971a,b,c](#)). These authors introduced HRP in the
876 gastrocnemius muscle of rats and after a few days detected HRP activity in spinal
877 motoneurons. A similar experiment with HRP injected into the tongue of rats showed
878 labeled motoneurons in the hypoglossal nucleus. [LaVail and LaVail \(1972\)](#)
879 demonstrated retrograde transport of HRP in the CNS itself after intraocular injection
880 in young chicks; 23 to 30 hrs later, they visualized peroxidase in cell bodies of the
881 isthmo-optic nucleus.

882
883 The HRP method was also applied to the study of thalamic connections. For
884 instance, in the cat, [Nakano et al. \(1980\)](#) traced projections from the cerebellum to
885 the ventral nuclei of the thalamus. Still in the cat, [Somogyi \(1978\)](#) described fibers
886 innervating the three anterior nuclei of the thalamus from the hippocampal region,
887 septum, cingulate cortex, and mammillary nuclei. In the rat, [Herkenham \(1978\)](#)
888 provided the first evidence of direct thalamo-hippocampal connections using HRP
889 injection in the reuniens nucleus and anterograde fiber tracing by autoradiography.
890 Indeed, efferents from the reuniens nucleus were shown to innervate the entorhinal
891 and parahippocampal cortices, and Ammon's horn. Entorhinal afferents were
892 localized to layers I and III, while hippocampal ones were restricted to the stratum
893 lacunosum moleculare of the CA1 field and the corresponding stratum in the ventral

894 subiculum. [Hayes and Rustioni \(1979\)](#) developed a method based on the combined
895 use of enzymatically active HRP and inactive radioactive [³H]apo-HRP for exploration
896 of axon collaterals in the somatosensory systems of adult cats. They showed the
897 topography of double labeled and single labeled neurons in the ventralis
898 posterolateralis thalamus (VPL) after ipsilateral HRP injection in the somatosensory
899 cortex I and [³H]apo-HRP in the somatosensory cortex II , with 10% of cells
900 combining both labels. Finally, [Hayes and Rustioni \(1979\)](#), using the same approach,
901 showed that the projections from the rostral cuneate nucleus to the VPL and
902 cerebellar cortex arise from 2 separate cell populations.

903

904 *****

905 Insert Figures 6 & 7 about here

906 *****

907

908 Although HRP suffered from the “fiber *en passage*” problem (Figure 7), as did the
909 Nauta technique, its far more limited technical demands as compared to
910 autoradiography made it the next ‘*revolutionary*’ method for connection tracing in the
911 nervous system. Some refinement of the HRP technique came from its possible
912 combination with the lectin wheat germ agglutinin (WGA), which improved both
913 uptake and transport within neurons, increasing the sensitivity by up to 40 times
914 ([Gonatas et al., 1979](#); [Köbbert et al., 2000](#)). WGA binds to N-acetylglucosamine and
915 the plasma membrane-bound sugar sialic acid, and it is rapidly and actively
916 transported in both anterograde and retrograde directions providing more extensive
917 labeling of the neuron as compared to HRP alone ([Levy et al., 2017](#)). For instance,
918 WGA-HRP was used to study the olfactory representation in the rat thalamus. [Price
919 and Slotnick \(1983\)](#) injected the tracer into the mediodorsal thalamus and showed
920 labeled cells in the polymorphic cell zone deep to the olfactory tubercle, the ventral
921 endopiriform nucleus deep to the piriform one, and in a similar position deep to the
922 periamygdaloid and lateral entorhinal cortices. After an injection into the submedius
923 nucleus, the number of labelled cells was smaller in similar areas, except deep to the
924 lateral part of the piriform cortex. These results were confirmed using the anterograde
925 tracer [³H]leucine injected between the anterior piriform cortex and the olfactory
926 tubercle.

927

928 Cholera toxin subunit B (CTB) was introduced in 1977 (Stoeckel et al., 1977) and
929 started to be used for retrograde tracing, either alone or combined to HRP, the HRP-
930 CTB conjugate showing better staining quality than free HRP (Trojanowski et al.,
931 1981, 1982). For example, Krout et al. (2002) provided a very fine description of all
932 the projections from the brainstem to the midline and intralaminar thalamic nuclei.
933 CTB was injected in each of the 6 nuclei constituting the intralaminar thalamus, the 6
934 nuclei of the midline thalamus, as well as in the anteroventral parvocellular part of the
935 ventral posterior and caudal ventral medial nuclei. It was found that each of these
936 thalamic nuclei receives input from a selective set of brainstem nuclei. Previous data
937 showed that the efferent projections from these thalamic nuclei to the cerebral cortex,
938 striatum and amygdala are highly specific (Berendse and Groenewegen, 1990, 1991;
939 Moga et al., 1995; Turner and Herkenham, 1991). Krout et al.'s findings contributed
940 to change the concept of the midline and intralaminar thalamic nuclei as forming a
941 *non specific* relay center that globally activates the cerebral cortex in response to
942 signals from the brainstem reticular formation.

943

944 During the 1980s, more sensitive inorganic fluorescent retrograde tracers such as
945 FluoroGold™, 'the gold standard' tracer in rodents, were developed (Lanciego and
946 Wouterlood, 2011). Fast Blue, Diamidino Yellow, True Blue, and the carbocyanines
947 Dil and DiO (Figure 8) are other such tracers (e.g., Bentivoglio et al., 1980; Kuypers
948 et al., 1980; Puigdellivol-Sanchez et al., 1998). Importantly, these fluorescent dyes
949 can be combined in the same experiment for multiple retrograde labeling, allowing
950 the demonstration of collateral projections. Typically, a dye fluorescing at one
951 wavelength is injected into one terminal site, and a second dye fluorescing at a
952 different wavelength into a second site, so that both are transported to the same
953 parent cells (Akintunde and Buxton, 1992; Jones, 2007). The carbocyanine dye
954 tracing method allowed the visualization of the topography of the thalamocortical
955 connectivity and interactions with subplate (Molnar and Blakemore, 1995), as well as
956 of its development in human post-mortem specimens (e.g., Molnar et al., 1998). The
957 subplate is a zone containing neurons supporting several crucial steps of cortical
958 development and which largely disappears thereafter.

959

960

961 *****

962 Insert Figure 8 about here

963 *****

964

965 Hoover and Vertes (2012) injected fluorogold into the mPFC and fluororuby into the
966 hippocampus, or *vice versa*, and found that 3-9% of the neurons of the nucleus
967 reuniens had collaterals in both regions. At the same period, the vision of a traditional
968 anatomy essentially focused on connectivity and cytoarchitecture in a one-dimension
969 scale, rapidly changed. Indeed, reports described two- or multi-dimensional
970 procedures associating tracing connectivity with double- or multiple
971 immunohistochemical (functional, e.g. neurotransmission- or neuroactivity-related
972 markers [calcium binding proteins, enzymes, transporters...]) identification of neurons
973 and brains areas (e.g. van der Kooy and Steinbush, 1980; rev Lanciego and
974 Wouterlood, 2020); multi-fluorescence microscopy was used for determining
975 colocalization. Thus, Gerfen and Sawchenko (1985) used the uptake and transport of
976 leucoagglutinating subunits complexes of the red kidney bean (*Phaseolus vulgaris*)
977 lectin (PHA-L, Gerfen and Sawchenko, 1984), one of the earliest and most widely
978 '*conventional anterograde tracer*' with high sensitivity and specificity.

979

980 PHA-L introduction marked the beginning of multi-dimensional anterograde tracing
981 (rev Groenewegen et al., 1990; Lanciego and Wouterlood, 2011). PHA-L, like WGA,
982 binds to membrane bound carbohydrates (N-acetyl D-glucosamine and mannose) to
983 enter cells and is detected via antibodies against PHA-L. Gerfen and Sawchenko
984 (1985), using PHA-L injection in the substantia nigra and tyrosine hydroxylase (TH)
985 immunoreactivity, showed that most nigrostriatal and nigrocortical projections were
986 dopaminergic (Figure 6, bottom) and unexpectedly, some nigrostriatal afferents were
987 non-dopaminergic and morphologically distinct, with a much thicker caliber than the
988 dopaminergic afferents (0.1 vs 0.5 μm diameter). These authors pointed to the
989 importance of the combination of anterograde tracing with immunohistochemical
990 identification of labeled fibers to get new information on the neurochemical tag of the
991 neuroanatomical pathways. Originally most of these approaches used non
992 fluorescent reported markers.

993

994 One of the first studies to use PHA-L for tracing thalamic connections visualized
995 thalamocortical axon terminals in vibrissae area of the mouse somatosensory cortex

996 (Keller et al., 1985). Aarnisalo and Panula (1995) were among the first to introduce
997 fluorescence reporting targeting neuropeptide FF-containing efferent projections from
998 the medial hypothalamus. They combined PHA-L tracing with labeling of a small
999 population of target neurons with neurobiotin (dendrite labeling) and opened the
1000 modern “*fluorescence age*”.

1001

1002 Approximately at the same time as the development of the anterograde PHA-L tracer,
1003 Glover et al. (1986) introduced conjugates of dextran-amines and fluorescein and
1004 rhodamin as neuroanatomical tracers. Two key methodological papers using the
1005 systemic application of biotinylated dextran amines (BDA) in rats and monkeys were
1006 published by Veenman et al. (1992) and Brandt and Apkarian (1992). This tracer,
1007 which can be conjugated to fluorescent dyes or biotinylated, was rapidly adopted and
1008 still remains widely used, due to its high quality of cell processes labeling and
1009 suitability for electron microscopy without prior alteration. Although first considered as
1010 an anterograde tracer, many studies indicate bidirectional travel that is exploited for
1011 conventional tracing (e.g., Sivertsen et al., 2014) or for the delivery of calcium-
1012 sensitive indicators for optical recording of neurons selected by axonal trajectory
1013 (O’Donovan et al., 1993).

1014

1015 One interesting example of its use to study the thalamus is by Craig et al. (1994). In
1016 the macaque monkey, these authors described a thalamic nucleus specific for pain
1017 and temperature sensation. They used: **i)** a combination of retrograde PHA-L-Texas
1018 Red to label the spinothalamic tract and single unit recordings, **ii)** injections at the
1019 trigeminal and cervical levels and at cervical and lumbar levels of FITC- and TRITC-
1020 labelled dextrans, and **iii)** calbindin immunoreactivity (IR) with FITC. They showed
1021 that a distinct nucleus in the posterior thalamus received a dense topographic input
1022 from spinothalamic lamina I neurons, and that this nucleus is constituted of
1023 nociceptive or thermoreceptive-specific neurons. Calbindin IR showed that this
1024 nucleus, named VMpo (posterior part of the ventral medial nucleus) is defined by a
1025 dense calbindin-positive fiber plexus. Applied to human thalamus sections, Craig et
1026 al. (1994) found a nearly identical fiber plexus localized in a nucleus that is
1027 cytoarchitecturally homologous to the lamina I relay nucleus in the macaque
1028 monkey. Two other examples of the same group used the BDA and CTB methods to
1029 study the thalamus connectivity, and especially the cortical afferents of the zona

1030 incerta (ZI) ([Mitrofanis and Mikuletic, 1999](#)), as well as its efferents to the dorsal
1031 thalamic nuclei ([Power et al., 1999](#)). In the first study, the BDA tracer was injected
1032 into various parts of the cortex (frontal, cingulate, parietal, occipital...) or the CTB
1033 tracer into the ZI. The authors showed that the cingulate cortex has the heaviest
1034 projection to ZI, the occipital cortex the weakest, and that all retrogradely-labelled
1035 cells in the neocortex were limited to layer V. The second paper showed that there
1036 are large projections from the ZI to various dorsal thalamic nuclei, mostly the
1037 association and intralaminar ones.

1038
1039 In addition, biotin derivatives, such as BDA, but also biocytin and neurobiotin, are
1040 used to fill neurons as a neutral marker after intracellular recording. Biocytin and
1041 neurobiotin were introduced by [Horikawa and Armstrong \(1988\)](#) and [Kita and
1042 Armstrong \(1991\)](#), respectively. The quite short survival time of these dyes (1-4 days)
1043 is due to a quick metabolism and renders them useful for short-distance
1044 anterograde/retrograde tracing, notably in combination with electrophysiological
1045 recording ([Taverna et al., 2004](#)) and chemical phenotyping ([Toney and Daws, 2006](#)).
1046 [Pinault \(1996\)](#) elegantly described a novel single-cell staining procedure *in vivo*
1047 under electrophysiological control, targeting thalamic cells with biocytin or neurobiotin
1048 via juxtacellular injection. He showed that the reticular cells projected to restricted
1049 regions of a single thalamic nucleus, including anterior thalamic nuclei, and that the
1050 thalamus and reticular complex have reciprocal connections.

1051
1052 Finally, despite their durable popularity, it is to note that for most of the '*conventional*
1053 *tracers*' cited above (whether retrograde or anterograde), there are some well-
1054 described drawbacks. These include: **i)** a direction of axonal transport that is rarely
1055 exclusive, complicating circuit analysis, **ii)** that these tracers are taken up by fibers *en*
1056 *passage*, **iii)** the spread around the injection sites that induces intense and diffuse
1057 labeling (see more details in [Saleeba et al., 2019](#)).

1058

1059 **13. Viruses as tracing tools**

1060 **The mid-1970s** began a new era with the introduction of much more sensitive
1061 techniques than those using '*conventional tracers*', based on the use of viruses as
1062 markers. These techniques exploited the natural capacity of some neurotropic
1063 viruses to be transported along the axons and to travel across neuronal pathways. In

1064 addition, viruses have a unique ability to self-amplify by replicating in recipient
1065 neurons, producing intense transneuronal labeling (Kuypers and Ugolini, 1990;
1066 Ugolini, 2010 for a review). It is out of the scope of this historical review to list all viral
1067 transneuronal tracing methods, the corresponding viruses, their combination, as well
1068 as the great variety of transgenic mice designed to serve some of these
1069 neuroanatomical tracing approaches. For more details, see the reviews by Lanciego
1070 and Wouterlood (2011, 2020), Nassi et al. (2015), Ugolini (2010) and Wouterlood et
1071 al. (2014). Here, we focus on the main viral approaches and how they were used to
1072 reveal/confim/validate aspects of thalamic connectivity.

1073
1074 The origin of viruses has its roots in human medicine with a so-called ‘mysterious
1075 agent’, transmitted via kissing or sexual contact; it causes blisters on the lips, in the
1076 oral region or genitals. Hippocrates and the emperors in Rome seemed to be aware
1077 of this phenomenon, and Shakespeare may have mentioned it in his ‘Romeo and
1078 Juliet’ tragedy (excerpt: *“O'er ladies lips, who straight on kisses dream, which off the
1079 angry Mab with blisters plagues, because their breaths with sweetmeats tainted are”*).
1080 The vector of the disease was identified in the 1920s as Herpes simplex virus (HSV).
1081 Goodpasture and Teague (1923) described the type 1 virus, HSV-1, as an infectious
1082 agent in skin epithelial cells, which migrates to nerve ending and then travels along
1083 sensory nerves to settle in neuronal perikarya inside spinal cord and cranial nerve
1084 ganglia. Hill et al. (1972) confirmed by electron microscopy the presence of HSV
1085 nanoparticles inside axons. Evidence that such viruses might move through neuronal
1086 circuits has been found in quite old literature (e.g., Howe and Bodian, 1942
1087 [poliovirus]; Kristensson, 1996 [Borna disease virus, influenza virus...]; Sabin, 1938
1088 [herpes virus]). These authors established the fundamental principles of the use of
1089 these viruses for analysis of brain circuitry. For example, in mice, Sabin (1938)
1090 examined the different routes of entry into the CNS of a variety of neurotropic viruses
1091 following intranasal infection. He showed that all produced infection, but some
1092 viruses infected multiple neural systems (e.g. pseudorabies virus, PRV) except the
1093 olfactory one, while others (e.g. vesicular stomatis virus, VSV) only infected the
1094 olfactory system. This pointed to some specificity and thus differential applications
1095 according to the virus type.

1096

1097 In the 1970s, with the pioneering work of Krister Kristensson and collaborators,
1098 began studies using a neurotropic virus for pathway tracing in both the CNS and
1099 PNS. [Kristensson et al. \(1974\)](#) provided the first clear example of transneuronal
1100 infection of sensory neural pathway using Herpes Simplex virus type 2, HSV-2,
1101 injected intra-ocularly. A few years later, the same authors used HSV-1 to trace
1102 olfactory sensory pathways after unilateral snout infection ([Kristensson et al., 1982](#);
1103 [Kristensson, 1996](#)). However, it is not until the early 1990s that the use of viruses
1104 became a consistent part of neuroanatomy literature. In the rat, [Ugolini et al. \(1989\)](#)
1105 nicely described HSV-1 labeling of multisynaptic circuits in cortical and brainstem
1106 neurons after HSV-1 injection in peripheral (forelimb, hindlimb) nerves. One example
1107 targeting the thalamo-cortical pathways in the cebus monkey was provided by
1108 [Zemanick et al. \(1991\)](#), who used 2 types of HSV-1 viruses: the HSV-1 (McIntyre-B)
1109 virus as a retrograde tracer, and HSV-1 (H129) as an anterograde tracer. After
1110 injection of HSV-1 (McIntyre-B) in the primary motor cortex, they showed densely
1111 labeled neurons in all the cortical and subcortical regions that project to the arm area
1112 of the primary motor cortex and notably, in two subdivisions of the ventrolateral
1113 thalamus, the ventralis lateralis pars oralis (VLo), and ventralis posterior lateralis pars
1114 oralis (VPLo). In addition, densely labeled neurons were present within specific
1115 portions of the reticular thalamus that projects to the VLo and VPLo. Globally, within
1116 various brain areas, a very different pattern of transport was observed with HSV-
1117 1(H129) injected into the primary motor cortex, but concerning the thalamus, the
1118 ventrolateral part was labeled as with the HSV-1 (McIntyre-B). For the authors it was
1119 unclear whether these thalamic neurons were labeled by retrograde or anterograde
1120 transport of HSV-1(H129).

1121
1122 In the beginning, viruses were used as '*conventional tracers*' with the clear
1123 advantage of improving the retrograde filling of neurons with Golgi-like retrograde
1124 labeling, notably with rabies viruses. In addition, RNA viruses (e.g., rabies) showed a
1125 low cytopathogenicity and there was no uptake through '*fibers en passage*' ([Nassi](#)
1126 [and Callaway, 2006](#); [Ugolini, 1995, 2008](#)). Some viruses are particularly well-adapted
1127 for transynaptic labeling and allowed the visualization of entire functional neuronal
1128 networks (*first order neurons, i.e. the initially infected one, 2nd order, i.e., after the first*
1129 *synapse, and so forth*, [Kuypers and Ugolini, 1990](#)). Since the 1990s, two main
1130 classes of viral transneuronal tracers have been available, the Herpes Simplex virus

1131 type 1 (HSV-1) and pseudorabies (PRV), both derived from alpha-herpes viruses,
1132 and the rhabdovirus (e.g., RV) belonging to rabies virus (Kuypers and Ugolini, 1990).
1133 The introduction of the retrograde transneuronal tracing methodology based on the
1134 use of HSV-1 or PRV viruses was the major step in the development of sensitive
1135 transneuronal tracers (e.g., Middleton and Strick 1994; Ugolini, 1995). An example of
1136 its application to rat thalamus connectivity is described in the study of the visual
1137 pathways labeled with 2 strains of PRV, the PRV-Be (wild type and virulent strain), or
1138 the PRV-bartha (attenuate vaccine strain) after intravitreal injection (Card et al., 1991,
1139 1998). The data showed a marked infection of the geniculate complex of the
1140 thalamus. However, if the 3 sub-areas (dorsal, intermediate, or ventral) of the
1141 geniculate nucleus were labeled with the wild type PRV (PRV-Be), only the
1142 intermediate and ventral geniculate nuclei were infected after PRV-bartha injection.
1143 This pointed to the differential sensitivity of brain cells according to the virus strain. In
1144 addition, advances in the use of virus rapidly led to the introduction of less
1145 aggressively spreading strains of virus. Another example is by McLean et al. (1989)
1146 who injected the HSV-1 in the primary visual cortex of rats and showed intense
1147 labeling (Golgi-like) in several ipsilateral visual relay region, notably the dorsal lateral
1148 geniculate nucleus of the thalamus and back to the retina.

1149
1150 Some improvement of tracing methods was obtained by combining virus
1151 transneuronal tracing with other methodologies such as: **i)** rabies virus and
1152 neurotransmitter or cell markers: these approaches are possible because infected
1153 neurons remain metabolically viable for a long time (unlike the narrow window by
1154 alpha-herpes viruses) (Ugolini, 2010); among the immunomarkers are antibodies
1155 against choline acetyl transferase (ChAT, for motoneurons and autonomic
1156 preganglionic neurons), oxytocin, calbindin, or parvalbumin, and **ii)** mixing rabies
1157 virus with CTB fragment to allow a precise definition of the injection area and
1158 simultaneous identification of first order (CTB) and higher-order neurons (rabies
1159 virus); **see Figure 10 for definition of and distinction between first and higher order**
1160 **nuclei**. To illustrate the latter method, Prevosto et al. (2010) were interested in
1161 cerebellar pathways involved in the control of eye movements and coordination of
1162 arm/eye/head movement. They injected unilaterally both rabies virus and CTB in the
1163 ventral lateral intraparietal area (LIPv) or in the medial intraparietal area (MIP) in 3
1164 monkeys to trace their direct thalamocortical and polysynaptic inputs. They notably

1165 showed that the first order (CTB) inputs from the thalamus to MIP and LIPv were
1166 largely topographically segregated. For example, the pulvinar inputs to MIP derived
1167 mostly from the ipsilateral dorsal and lateral parts as well as from the anterior
1168 pulvinar, while inputs to LIPv originated from the more caudal medial and ventral
1169 portion of the pulvinar complex. The rabies transneuroal disynaptic transfer (at 2.5
1170 days) showed labeling in the ipsilateral reticular thalamus and in some additional
1171 thalamic nuclei. At this delay, rabies virus labeling also occurred in some
1172 contralateral thalamic nuclei that mirrored the distribution of first order ipsilateral
1173 labeling (CTB). At 3 days, the rabies tracer crossed an additional synaptic step, as
1174 shown by the labeling of the contralateral reticular thalamic nucleus.

1175
1176 At the end of the 20th century, the development of molecular biology and virology
1177 allowed the construction of viral vectors at will. As a result, combinations of preferred
1178 features from different viruses and assembled into new, unique, and highly specific
1179 vectors (including the ability to switch on gene expression in infected cells), rapidly
1180 developed. One of the first reports used a recombinant virus primarily to act as a
1181 delivery agent to transfect neurons in the CNS with a gene that codes for green
1182 fluorescent proteins (GFP) (Chamberlain et al., 1998). These authors, using an
1183 adeno-associated virus (AAV)-GFP vector injected into the parabrachial nucleus,
1184 showed a robust GFP immunoreactivity in cell bodies, axons and terminals in the
1185 forebrain, brainstem, and spinal cord, attesting of the specific anterograde transport
1186 of GFP.

1187
1188 The utility of neurotropic viruses was greatly improved by controlling what cell types
1189 are initially infected or are permissive for viral infection, or by controlling the number
1190 of synaptic steps that are crossed. De Falco et al. (2001) were originators of the use
1191 of a modified form of PRV-Bartha (Ba2001), unable to replicate unless its genome is
1192 recombined by a cre-recombinase. The strategy is based on the fact that viral
1193 thymidine kinase (TK), required for viral replication, was deleted from the PRV
1194 genome and replaced with a 'floxed stop' sequence followed by coding sequences
1195 for both TK and GFP. This approach also takes advantage of the existence of mouse
1196 lines which express cre-recombinase only in specific cell types. Thus, in Ba2001, cre-
1197 recombinase will excise a 'floxed stop' sequence and allow TK+GFP expression only
1198 in Cre-recombinase expressing cells. So, the GFP-expressing virus replicates only in

1199 neurons that express the Cre recombinase and in neurons that are in synaptic
1200 contact with the originally infected cells. DeFalco et al. (2001) injected the virus into
1201 the arcuate nucleus of mice that express Cre only in NPY neurons (NPY Cre mice).
1202 They showed that 4 days after injection, the virus spread notably to the posterior
1203 hypothalamus and the mediodorsal thalamus, but also to the dentate gyrus, the
1204 piriform cortex, and ventral basal amygdala.

1205

1206 More recently, still with AAV tracing, the projections from cortical layer 6b to thalamic
1207 nuclei could be studied. Hoerder-Suabedissen et al. (2018) used cre-dependent viral
1208 tracing in a mouse line expressing cre-recombinase in a subpopulation of layer 6b
1209 cortical neurons. Unlike neurons from layers 6a and 5, those from layer 6b were
1210 found to project on higher order thalamic nuclei. Similarly, still with cre driver mouse
1211 lines, a hierarchical organization of corticocortical, corticothalamic and
1212 thalamocortical connectivity could be established, leading to the construction of a
1213 feedforward or feedback connection model between these brain regions (Harris et al.,
1214 2019). Crosshierarchical corticothalamic rewiring after loss of visual input (monocular
1215 enucleation) could also be identified and described with such methods (Grant et al.,
1216 2016).

1217

1218 Over the same period, glycoprotein G, a key ingredient in multisynaptic retrograde
1219 transport, allowed the development of a new tool for first-order-neuron-only-
1220 retrograde tracing (rev Callaway, 2008; Callaway and Luo, 2015). Mazarakis et al.
1221 (2001) demonstrated that the rabies-G envelope of the rabies virus is sufficient to
1222 confer retrograde axonal transport properties to a heterologous virus, both *in vivo* and
1223 *in vitro*. They particularly showed, in the rat, strong labeling in several thalamic areas
1224 (i.e., centromedian, submedialis, pericentral, subthalamic) 1 month after intra-striatal
1225 injection of the pseudotyping lentiviral vector (pONY8.0Z) with rabies-G. The
1226 substitution by Wickersham and collaborators of the glycoprotein G gene by a gene
1227 coding for EGFP produced powerful first-in-line-neuron-only retrogradely transported
1228 vectors that, by virtue of expressing EGFP in infected cells, made retrograde labeled
1229 infected neurons directly visible under fluorescence (SAD Δ G-EGFP derived from the
1230 SAD B19 rabies virus; Kim et al., 2016; Wickersham et al., 2007a). In the
1231 Wickersham et al.'s paper, the retrograde virus (RABV Δ G-EGFP) was injected in the
1232 mouse thalamus and many bright EGFP-expressing pyramidal neurons in the

1233 overlying cortex were **apparent**. Finally, [Wickersham et al. \(2007b\)](#) developed a
1234 method in which the initial viral infection is restricted to particular cells. They deleted
1235 the RG gene from the SAD-B19 rabies genome, and replaced it with GFP. Then, they
1236 produced viral particles with a different envelope protein by pseudotyping the RG-
1237 deleted virus with the avian virus envelope protein EnvA; this new virus was called
1238 Env-A-SAD Δ G-GFP. When this virus is injected into the brain of a normal animal, it
1239 does not infect any neuron because the mammalian brain has no receptors for EnvA.
1240 By misexpressing the EnvA receptors, TVA, in particular cells, it was possible to
1241 selectively infect those cells ([Callaway, 2008 for a review](#)).

1242

1243 This system, tested and validated in cultured slices of neonatal rat brain has the
1244 advantage that rabies behaves as a mono-trans-synaptic tracer and permitted the
1245 first unambiguous identification of retrogradely connected cells from an initially
1246 infected cell. [Haberl et al. \(2015\)](#) used a similar approach and injected the
1247 anterograde RABV Δ G (VSV-G^{RtmC}) into the mouse ventral posteromedial (VPm)
1248 thalamic nucleus or the posteromedial (POm) one. This allowed a 3D reconstruction
1249 of 3 excitatory neurons, 1 inhibitory interneuron, and 3 astrocytes within the imaged
1250 volume of the thalamus and cortex.

1251

1252 Monosynaptic restriction of trans-synaptic tracing also occurred in the anterograde
1253 direction with the first demonstration by [Beier et al. \(2011\)](#). These authors
1254 demonstrated that recombinant vesicular stomatitis virus (VSV) vectors can be
1255 endowed with anterograde or retrograde transsynaptic tracing ability by providing the
1256 virus with different glycoproteins (RABV-G or lymphocytic choriomeningitis virus,
1257 LCMV-G). By targeting the visual system with eye injection of the anterograde VSV
1258 (LCMV-G) virus, labeling was restricted to the visual system including primary
1259 retinorecipient areas (lateral geniculate nucleus, superior colliculus, and
1260 suprachiasmatic nucleus), and secondary (V1) visual centers ([Beier et al., 2011](#)).
1261 This paper provided definitive evidence that directionality of a virus is a property of
1262 the G protein, i.e., RABV-G for retrograde and LCMV-G for anterograde properties. In
1263 2013, these authors extended the characterization of the transmission and gene
1264 expression of recombinant VSV either with the rabies virus glycoprotein
1265 (rVSV(RABV-G)) for retrograde labeling, or recombinant VSV-virus glycoprotein
1266 (rVSV(VSVS-G)) for anterograde labeling ([Beier et al., 2011](#)). Using these two

1267 transynaptic virus vectors injected into the motor cortex area 1, they showed that the
1268 thalamic neurons expressed either the retrograde- or the anterograde virus, with no
1269 co-labeling (Beier et al., 2013).

1270

1271 Another example of the contribution of these virus-based neuroanatomical
1272 techniques to the thalamus is by Broms et al. (2017). In mice, these authors very
1273 nicely described microcircuits within the thalamus and habenula using GPR151, a G
1274 protein-coupled receptor whose expression is enriched in specific diencephalic
1275 structures. They traced the afferent connectivity of habenular and thalamic neurons
1276 defined by their GPR151 expression, using a virus/vector combination in a transgenic
1277 Gpr151-cre-mouse. These mice received a mixture of AAV8-Ef1a-FLEX-TVA-
1278 mCherry and AAV8-CA-FLEX-RG unilaterally into the habenula, the paraventricular-
1279 or the lateral posterior thalamic nuclei. After 21 days, the stereotaxic procedure was
1280 repeated for the SADΔG-eGFP(EnvA) vector injection in the same brain areas. The
1281 data showed that thalamic vs habenular GPR151-expressing neurons differed
1282 substantially. As an example, the habenular neurons primarily received inputs from
1283 basal forebrain structures, the bed nucleus of the stria terminalis, the lateral preoptic
1284 area, the entopeduncular nucleus, and the lateral hypothalamic areas. The GPR151
1285 neurons of the paraventricular thalamus were contacted primarily by the medial
1286 hypothalamic areas as well as by neurons from the zona incerta.

1287

1288 Finally, Xu and Südhof (2013) mapped the synaptic projections from the medial
1289 prefrontal cortex (mPFC) and confirmed a specific neural pathway from the mPFC to
1290 the thalamic reuniens nucleus (Re). The neuroanatomical tracing approach used an
1291 anterograde synaptoTag-AAV which co-express red fluorescent mCherry protein and
1292 EGFP-Synaptobrevin 2 (a synaptic vesicle protein). The SynaptoTag-AAV-infected
1293 neurons were filled with diffusible mCherry and selectively localized GFP-
1294 synaptobrevin-2 to efferent synapses formed in the target regions. This allowed a
1295 quantitative assessment of the number of synapses in the target areas by the
1296 SynaptoTag-AAV-infected neurons. Eight weeks after stereotactic injection of the
1297 SynaptoTag virus into the mPFC of adult mice, the authors observed that, within the
1298 thalamus, most projections were targeted to the mediodorsal (MD) and Re nuclei.
1299 Then, to determine if the same mPFC neurons project to both targets, Xu and Südhof
1300 injected a cholera toxin B (CTB)-Alexa-Fluor-488 or -594, the former in the Re and

1301 the latter in the MD. Retrogradely labeled neurons were observed in the 3 major
1302 mPFC subregions, i.e., the prelimbic, infralimbic, and anterior cingulate cortex.
1303 However, most fluorescent mPFC neurons contained preferentially only one
1304 fluorophore indicating that these neurons project to only one of these thalamic
1305 regions. The following experiments consisted in inactivating either the mPFC-Re- or
1306 the mPFC-MD pathway, using double-floxed inverted TetTox AAV in the mPFC and a
1307 WGA-Cre AAV in the Re or MD nucleus. Four weeks after viral injections, fear
1308 conditioning tests were performed and data showed that the mPFC-Re pathway
1309 controls memory specificity (Xu and Südhof, 2013; see also Ferraris et al., current
1310 issue).

1311

1312 One key aspect of the development of neuroanatomical tracing concerns the study of
1313 the brain of transgenic animals in which cells of a particular neurochemical
1314 phenotype express a fluorescent protein, e.g., the green fluorescent protein (GFP)
1315 under Cre-mediated recombination (e.g., GFP-cre fusion gene, Sauer, 1998). Then,
1316 came neurotransmitter-specific transgenic mice with Cre-recombinase expression in
1317 dopaminergic (DA) neurons (Bäckman et al., 2006; Witten et al., 2011; Zhao et al.,
1318 2004), GAD67 neurons (Tamamaki et al., 2003), serotonin (5HT) neurons (Zhuang
1319 et al., 2005), choline acetyltransferase (ChAT) neurons (von Engelhardt et al., 2007),
1320 and parvalbumin(PV)-expressing GABAergic neurons (Tanahira et al., 2009).
1321 However, the drawback of this first generation of mice was that all members of a
1322 particular species of cells light up green simultaneously (as in the brainbow mice,
1323 Weissman et al., 2011). The key vector to achieve trace axonal connectivity in a
1324 small portion of neurotransmitter-specific neurons is an adeno virus (AV) or adeno-
1325 associated virus (AAV). In that case, the role of the virus is to deliver a gene coding
1326 for one of the fluorescent proteins and expression of GFP (or channel rhodopsin) can
1327 be forced in Cre-recombinase mice through focal injection with the viral vector.
1328 Atasoy et al. (2008) developed a Cre-recombinase-dependent viral vector for
1329 targeting channel rhodopsin 2 (ChR2) (e.g., rAAV-FLEX-rev-ChR2mCherry and
1330 rAAV-ChR2EGFP) in order to map long-range circuits. As an example, they injected
1331 a rAAV-FLEX-rev-ChR2mCherry virus in the arcuate nucleus of an agrp-cre;rosa26-
1332 loxSTOPlox-eyfp mice and observed strong axonal labeling in the projections of
1333 AGRP (Agouti-related protein) neurons in the paraventricular hypothalamus as well
1334 as in the paraventricular thalamus. Kuhlman and Huang (2008) also found a solution

1335 by using a transgenic parvalbumin (PV)-cre mouse strain to specifically target
1336 neocortical GABAergic interneurons by switching GFP expression in a small
1337 population of cells. They engineered AAV that express GFP or other markers
1338 (dsRedExpress, Channel rhodopsine 2...) upon Cre/loxP recombinant-mediated
1339 removal of a transcription-translation STOP cassette (Figure 9). They injected an
1340 AAV-lox-STOP-lox (LS1, L) GFP virus into the neocortex of a PV-cre mouse to
1341 specifically label neurons expressing cre-recombinase. They followed the dynamic of
1342 parvalbumin-containing (PV) GABAergic cortical interneurons over 1 week and
1343 showed in young adult mice that inhibitory PV circuits maintained the **potential** for
1344 structural rewiring via boutons on axonal shafts (Kuhlman and Huang, 2008).

1345

1346 Tang et al. (2015) developed a new method allowing the cell type-specific
1347 manipulation with GFP-dependent Cre recombinase (CRE-DOG) to directly induce
1348 Cre/loxP recombination using plasmid electroporation and AAV viral vectors. They
1349 tested their system in the retina and various brain areas using several GFP mouse
1350 lines. As an example, using a Tg(TRHR-GFP) mouse (a line expressing GFP in a
1351 specific retinal ganglion cell subtype), they showed, after retinal injection of the 2
1352 viruses, rAAV-encoding CRE-DOG OPT and rAAV-FLEX-tdTomato(tdT) (the first
1353 virus **allowing he turn on of the 2nd one** in a GFP-dependent manner), a strong tdT
1354 expression in the retinal ganglion cell projection targets, the dorsal lateral geniculate
1355 thalamic nucleus, and the superior colliculus.

1356

1357 *****

1358 Insert Figure 9 about here

1359 *****

1360

1361 Finally, a last paper illustrating the powerful tool of genetic virus-based tracing
1362 methods using Cre driver mouse lines is one by Harris et al. (2019). These authors
1363 described a major expansion of the Allen Mouse brain connectivity Atlas resource
1364 (Oh et al., 2014) involving thousands of experiments in the cortex and thalamus to
1365 map brain-wide connections (cortico-cortical, thalamo-cortical, and cortico-thalamic).
1366 **For this**, they used a cre-dependent AAV vector that express EGFP in the cytoplasm
1367 of cre-expressing infected neurons, the AAV2/1.pCAG.FLEX.EGFP.WPRE. They
1368 also used a Cre-dependent AAV virus expressing synaptophysin-EGFP fusion

1369 protein to specifically label presynaptic terminals, the
1370 AAV2/1.pCAG.FLEX.sypEGFP.WPRE. Injections were made into lines with
1371 regulatable versions of Cre, the tamoxifen- or thimethoprim-inducible Cre line, (CreR)
1372 mice or (dCre) mice, respectively. The huge amount of data collected allowed the
1373 mapping of projections originating from unique cell populations in the same cortical
1374 area and from distinct projection classes in the thalamus. [Harris et al.](#) were then able
1375 to lay the foundation of some anatomical rules of cortical and thalamic connections,
1376 i.e., cell-class specific connections are organized in a shallow hierarchy within the
1377 mouse cortico-thalamic network.

1378
1379 In conclusion, the introduction of viruses as tracing tools in the 1970s allowed
1380 spectacular progress in the field of neuronatomy, particularly thalamus connectivity.
1381 From one-dimensional retrograde tracing with herpes simplex virus 1 ([Kristensson et](#)
1382 [al., 1974](#)), a rapid evolution was noticed with: **i)** virus-based tools being combined
1383 with genetics and molecular biology, and **ii)** the introduction of adeno-associated
1384 viruses (AAV). The latter served as vehicles for altered gene delivery to force
1385 neurons to express green fluorescent proteins (GFP), a major discovery in
1386 neurosciences. The reader interested in more details about virus-based tracing
1387 method is invited to take a look at the recent review by [Lanciego and Wouterlood](#)
1388 [\(2020\)](#).

1389
1390 All of these combined approaches markedly transformed neuronanatomical tracing
1391 techniques into a large multi-dimensional molecular-genetic tracing spectrum: a great
1392 tool, that was also applied, for instance, to research on the organization of thalamic
1393 microcircuits ([Broms et al., 2017](#)), on brain-wide corticothalamic and thalamocortical
1394 connections, and on thalamic cell classes (core, matrix, intralaminar, [Harris et al.,](#)
1395 [2019](#)).

1396
1397 **The progress of tracing techniques, combined to functional studies supported by**
1398 **electrophysiological approaches, led Guillery (e.g., 1995) to make an important**
1399 **distinction between two kinds of thalamic nuclei based on the nature of their inputs,**
1400 **namely first order nuclei and higher order nuclei (Figure 10). First order nuclei (e.g.,**
1401 **lateral and medial geniculate nucleus, ventrolateral and ventrobasal thalamus, and**
1402 **anterior nuclei) receive their sensory inputs from subcortical structures as well as**

1403 cortical afferents originating in layer 6, which have modulatory functions. Higher order
1404 nuclei (e.g., mediodorsal and lateroposterior thalamus, pulvinar), which have
1405 substantially expanded during primate evolution, receive most of their afferents from
1406 pyramidal cells located in layer 5 and 6 of cortical regions and send projections
1407 mainly to cortical regions which are different from those providing afferents. These
1408 nuclei are kind of a thalamic relay for corticocortical communication that often
1409 parallels a direct corticocortical one (see also Wolff et al., 2021, current issue). With
1410 his long date collaborator Murray Sherman (e.g., Sherman, 2019), Guillery also
1411 distinguished two types of glutamatergic pathways, one he called drivers and one he
1412 called modulators (Figure 10). Briefly, driver neurons carry the principal information.
1413 As such, they are the “main conduits of information” (Sherman, 2019) arriving to
1414 thalamic nuclei from where they are passed to the cortex. Conversely, modulator
1415 neurons affect the way the information provided by driving neurons is processed.
1416 Interestingly, if both types of neurons are glutamatergic, they differ in several
1417 structural and functional instances. Indeed, driver neurons act on ionotropic receptors
1418 (modulators on metabotropic ones), have large EPSPs (they are small in
1419 modulators), have thick axons (small ones in modulators), synapse on proximal
1420 dendrites (on terminal ones in modulators), etc (Sherman, 2019). It is noteworthy that
1421 the aforementioned classifications, as well as other ones of pathways (e.g., core vs.
1422 matrix neurons that coexist in defined nuclei) have been questioned recently
1423 (Halassa and Sherman, 2019).

1424

1425 *****

1426 Insert Figure 10 about here

1427 *****

1428

1429 **14. *In vitro* approaches**

1430 Interactions and connections between the thalamus and other structures,
1431 predominantly cortical ones, have also started to be investigated with *in vitro*
1432 approaches near the end of the 1980s. These approaches included organotypic co-
1433 cultures, thalamocortical slice cultures and co-cultured organoids. A cell culture
1434 consists in preparing a usually large amount of tissue from which cells are kept
1435 operational in an isolated environment under conditions enabling their survival. An
1436 organotypic culture is a culture of a tissue or an organ (or pieces thereof) compatible

1437 with structural and functional characteristics closer to *in vivo* conditions than cell
1438 cultures have. Regarding the brain, organotypic co-cultures, or slice cultures or slice
1439 co-cultures are variants of this methodology applied to portions of the brain. These
1440 techniques have the advantage of offering not only possibilities to investigate
1441 connectivity and its development/degeneration/regeneration within or between brain
1442 regions, but also track many functional characteristics therein (e.g., Humpel, 2015).
1443 Regarding the thalamus, the aforementioned approaches have considerably
1444 extended the understanding of the development and specificity of thalamocortical
1445 interactions. Furthermore, slice culture preparations are highly compatible with
1446 functional approaches using localized stimulations and either localized recordings,
1447 including patch clamp recording, or calcium or voltage-sensitive dyes (e.g., Higashi et
1448 al., 2005).

1449
1450 To give just a few examples in relation with the thalamus, Higashi et al. (2005)
1451 studied the thalamocortical connectivity in thalamocortical slice preparations from
1452 immediately postnatal rats showing reversed cortical layering. Bolz et al. (1990),
1453 using visual cortex slices co-cultured with thalamic slices observed that the cortico-
1454 thalamic projections developed according to a pattern similar to the one observed in
1455 vivo, cortical axons growing directly to their appropriate thalamic target, most
1456 probably under the influence of a chemotropic attraction. The same is true the other
1457 way around (Bolz et al., 1992; Yamamoto et al., 1989). Agmon and Connors (1991)
1458 have developed a slice preparation containing the ventrobasal nucleus of the
1459 thalamus and the sensorimotor barrel cortex of the mouse. In this slice culture, they
1460 could study the physiology and pharmacology of the thalamocortical synapse, and
1461 map the connections. Lotto et al. (1999), again on thalamic and cortical slice co-
1462 cultures, could observe that axons from the thalamus to the cortex grew to both
1463 superficial and deep cortical layers indifferently, but axons inappropriately located
1464 (i.e., in the superficial layers) were subsequently lost, most probably because of
1465 repressive vs. permissive intracortical, contact-mediated interactions. Finally, still with
1466 thalamic and cortical slice co-cultures, Yamada et al. (2010) labeled individual
1467 thalamocortical axons and reduced firing therein. They found that the reduction of
1468 activity of thalamocortical neurons decreased branching, demonstrating that such
1469 activity also contributes to the correct development of this connectivity.

1470

1471 **15. Magnetic resonance imaging**

1472 Magnetic resonance imaging (MRI) consists in generating images by three-
1473 dimensional detection and computer-assisted reconstruction of the post-excitation (by
1474 application of an electromagnetic field at a given frequency) realignments of the
1475 magnetic moments of spins of particular atoms (e.g., hydrogen). The first images this
1476 technique generated were those of two water-filled capillary tubes (Lauterbur, 1973),
1477 and, for the human body, it was that of a finger in 1976 (Mansfield and Maudsley,
1478 1977; in fact it was one of Maudsley's fingers). Damadian, who had understood that
1479 MRI would become an investigation tool of very high clinical relevance (Damadian,
1480 1971), is the first to have scanned the entire body (Damadian et al., 1977).
1481 Regarding the human brain, Young and Clow obtained the two first MRI images in
1482 1978 (e.g., Hounsfield, 1980).

1483

1484 In 1991, John Belliveau communicated about his dynamic imaging of activation of
1485 visual brain areas in response to visual stimulation. This is how functional MRI made
1486 the first of many subsequent steps (for an exhaustive account of the story and all its
1487 actors, see the special issue of Neuroimage edited by Bandettini, 2012). The
1488 principle of fMRI is relatively simple. When a brain region is active, its blood flow
1489 increases. When oxygenated, hemoglobin is diamagnetic (meaning that there is no
1490 unpaired electron in an orbital of the atom), but once deoxygenated, it becomes
1491 paramagnetic (there are unpaired electrons), the contrast between both being
1492 detectable in a scanner. Thus, by collecting the three-dimensional distribution of
1493 venous blood oxygenation level-dependent (BOLD) magnetic resonance along a time
1494 line, it is possible to image the three-dimensional map of brain activation patterns,
1495 which correlate with local blood flow.

1496

1497 Diffusion weighted MRI (DWI; e.g., Baliyan et al., 2016) is another development of
1498 MRI. It uses the fact that water molecules move in tissues, that their motion is
1499 anisotropic, and that the movement coefficients of these molecules depend on the
1500 nature and composition of the tissues. Detection of these anisotropic movements is
1501 what DWI is doing to construct three-dimensional images of organs and tissues.
1502 Diffusion tensor imaging (DTI) is a particular way to model DWI data sets. It
1503 visualizes axonal organization in nervous system tissues, and more specifically
1504 orientation of fiber tracts. The technique arose in the mid-1990s (e.g., Basser et al.,

1505 1994). It uses the fact that the movement of water molecules is easier along the
1506 axonal fiber tracts than when perpendicular to it. DTI exploits this water-motion
1507 orientation bias as a probe to infer the neuroanatomical paths of fiber ensembles, this
1508 motion undergoing more influence by static anatomy than by physiological factors (Le
1509 Bihan and Johansen-Berg, 2012).

1510

1511 All these technics, which have the huge advantage of being non-invasive and
1512 compatible with small animal explorations (generally with a higher magnetic field than
1513 in humans), contributed to a better knowledge of the anatomy and connectivity of the
1514 thalamus in both humans and other animals. In January 2021, a Pubmed search
1515 crossing the keywords ‘thalamus’ and ‘MRI’ generated a list of > 12,500 hits (and
1516 almost as many when ‘fMRI’ was substituted for ‘MRI’). Suffice to say that it would be
1517 herculean work to summarize them in a few short paragraphs. Therefore, as often
1518 done above, we will only consider a small number of representative examples.

1519

1520 Using DWI, Johansen-Berg et al. (2005) explored the structural connectivity of the
1521 human thalamus with the cortex. They established that the connectivity pattern (white
1522 matter) between thalamus and cortex was an acceptable criterion to segregate
1523 thalamic regions that corresponded to nuclei (gray matter). Before them, Behrens et
1524 al. (2003) had reported on similar observations demonstrating a strong similarity
1525 between the organization of the human thalamic gray matter according to its cortical
1526 connectivity and the different thalamic subregions as shown by histological studies.
1527 Furthermore, when compared to the connectivity map of non-human primates, the
1528 DTI images revealed very similar. Still with DWI and using a whole-brain approach,
1529 Grodd et al. (2020) recently reported on the connectivity of several thalamic nuclei
1530 (e.g., anterodorsal, anteromedial, anteroventral...) with other structures of the limbic
1531 system. These authors could establish high precision maps of this connectivity and
1532 identify some differences with tracer studies. In particular, they distinguished direct
1533 projections of the mediodorsal nucleus to brainstem, and indirect projections of this
1534 nucleus interconnecting temporal and mediofrontal regions. Interestingly, DTI was
1535 also used to follow the timing of the growth of thalamocortical fibers in the human
1536 fetal brain (Krsnik et al., 2017).

1537

1538 The connectivity maps such as the ones mentioned in the previous paragraph were
1539 also explored at a functional level by resting-state fMRI. Resting-state fMRI is a
1540 spatial and temporal signal acquisition approach in which no specific stimulus, no
1541 particular instruction and no cognitive task is used. In other words, recordings occur
1542 while the subject is resting. It relies on the BOLD signal and, by establishing
1543 correlations between the activation patterns, it provides useful information about the
1544 macroscopic organization of systems of neural processing. Using such an approach,
1545 Kim et al. (2013) could establish a detailed map of functional network connectivity
1546 between the basal ganglia, the thalamus, and different regions of the cerebral cortex.
1547 This led to a nice description of the cortico-striato-thalamo-cortical circuits in the
1548 human brain.

1549
1550 Combination of DWI and fMRI enables direct comparison between structural and
1551 functional connectivity patterns. Such combined approaches have also been used to
1552 explore the thalamus. For example, using DWI and fMRI, Zhang et al. (2010) have
1553 focused on the thalamocortical system. They could establish excellent overlapping of
1554 their MRI maps with the maps based on human and non-human primate histology.
1555 Whereas histological, functional and connectional maps were concordant in most
1556 instances, there were also differences, the greatest of them concerning motor and
1557 premotor regions of the cortex. Such differences do not point to the limits of each
1558 technique, whether considered separately or in combination. They rather indicate that
1559 each technique may measure different aspects of connectivity, which can be mutually
1560 enriching and thereby permit a better tuning in the interpretation of relationships
1561 between connectivity and functions. Kumar et al. (2017) also combined DWI and
1562 fMRI to generate a functional map of thalamic parcels. They found these parcels to
1563 correspond to cytoarchitectonally-defined nuclei, of which they characterized the
1564 structural connectivity. Interestingly, for some of these thalamic parcels, there
1565 seemed to be a good correspondence with thalamocortical maps described in mice
1566 according to histological studies.

1567
1568 Anatomical and functional connectivity 'units' can also be modulated functionally with
1569 non-invasive tools, and this is true for thalamic nuclei. For instance, in the study by
1570 Zotev et al. (2018), real-time fMRI was coupled to neurofeedback and
1571 electroencephalographic (EEG) recordings. The principle of neurofeedback is to

1572 provide a subject with information about ongoing brain activity so that self-control of
1573 this activity can occur. Zotev et al. assessed correlations between alpha EEG activity
1574 (in the parieto-occipital region of the brain) and BOLD activity in the mediodorsal and
1575 anterior thalamus. At the same time, subjects were recalling autobiographical
1576 memories or doing control tasks. The results established an enhancement of the
1577 functional connectivity between the mediodorsal thalamus and the inferior precuneus
1578 during the fMRI neurofeedback episodes. In addition, the alpha EEG power was
1579 correlated with the BOLD activity in the mediodorsal thalamus. Finally, the temporal
1580 correlation between the alpha EEG power and the BOLD activity in the mediodorsal
1581 and the anterior thalamus was enhanced during the neurofeedback episodes. Based
1582 on these observations, Zotev et al. suggested that the functional connectivity
1583 between the precuneus and the mediodorsal thalamus contributed to the
1584 enhancement of the correlation between thalamic BOLD and cortical alpha EEG
1585 activity during the neurofeedback procedure.

1586

1587 Another exploration technique may consist in using deep brain stimulation (DBS) in
1588 patients. Usually, DBS is primarily used for a therapeutic purpose. It is invasive, as it
1589 requires a surgical procedure consisting in the intracerebral implantation of a device
1590 delivering pacemaker-like controlled electrical stimulations to specific brain regions.
1591 DBS is a surgical treatment of e.g., Parkinson's disease or some intractable forms of
1592 epilepsy. The effects of DBS can be followed with EEG recordings or fMRI. In
1593 epileptic patients equipped with DBS devices targeting the anterior and medial
1594 thalamus, Zumsteg et al. (2006) observed that the low frequency stimulation of the
1595 anterior and medial thalamus induced rhythmical cortical EEG synchronization in
1596 some patients, indicating an influence of these thalamic nuclei on cortical activity.

1597

1598 Thus, over the last 20 years, nuclear magnetic resonance, quickly called MRI
1599 because people were reluctant to the word 'nuclear', DWI, DTI, fMRI, combined or
1600 not with other approaches (e.g., EEG, neurofeedback, DBS), largely supported the
1601 recent progress of our knowledge of the functional connectivity of thalamic nuclei.

1602

1603 *****

1604 Insert Figure 11 about here

1605 *****

1606

1607 **General conclusions**

1608 In his first chapter of “The Thalamus”, Edward [Jones \(1985\)](#) provided a detailed
1609 account of how knowledge about the organization of the thalamus progressed during
1610 the 19th century and the first two thirds of the 20th century. With the refinement level
1611 of tissue and fiber staining techniques constantly improving, driven by the
1612 omnipresent concern to rid them of their disadvantages, the thalamus could be
1613 subdivided into a set of nuclei characterized by both their cytoarchitectonic
1614 peculiarities and their connection patterns, whether afferent or efferent. This is
1615 exactly what Edward Jones insisted on when he wrote “*The past history of thalamic*
1616 *studies, (...), has been characterized by periods during each of which the work was*
1617 *dominated by a particular methodological approach.*” ([Jones, 1985](#)). Such methods
1618 were, for instance, those developed by von Gudden (retrograde degeneration),
1619 Marchi (stain of myelin in degenerating fiber tracks), or Nissl (with a stain binding to
1620 nucleic acids).

1621

1622 Of course, as is often the case, there was no consensus on the limits of regions or
1623 thalamic nuclei, no more than on where fiber tracks were coursing, starting, ending,
1624 or on the nomenclature. One needs only look at the recent article by [Mai and](#)
1625 [Majtanik \(2019\)](#), for instance, to be convinced that subdividing the thalamus into
1626 coherent nuclei or ensembles of nuclei has generated much debate. Indeed, this
1627 article, which its authors have limited to the human thalamus, lists no less than twelve
1628 proposals for neuroanatomical organization and nomenclature of identifiable thalamic
1629 nuclei on a frontal section passing through the posterior commissure, and all these
1630 proposals arose between 1971 and 2017. This is less than half a century! [Mai and](#)
1631 [Majtanik \(2019\)](#) have also risked a suggestion to achieve better consistency within
1632 the scientific thalamus community. Now let us wait and see what will happen to it.

1633

1634 Providing an exhaustive account of why and how the conceptions about the
1635 macroscopic and microscopic anatomy of the thalamus and of its complex
1636 connectivity has evolved lies beyond the scope of the current contribution, but the
1637 reader may find some relevant information in the first chapter of [Jones’](#) *The*
1638 *Thalamus (1985)*, along with an abundant literature. Instead, using a series of
1639 selected examples in an already plethoric literature, we have, in centuries of

1640 thalamus history, set milestones at times which, according to our own knowledge of
1641 historical and scientific literature, seemed to us to correspond to determinant
1642 advances.

1643

1644 When it comes to the future, the next burst of progress will probably rely on furthering
1645 explorations supported by ‘omics’ approaches. In fact, this type of studies has
1646 already started to develop. In a relatively recent research article, Nagalski et al.
1647 (2016) have used gene expression data from the Allen (mouse) Brain Atlas database
1648 in order to ground a proposal about the molecular anatomy of the thalamus complex
1649 on the combinatorial expression of a series of transcription factor genes. This
1650 combinatorial distinguished nine thalamic nuclei or ensembles of nuclei, each of them
1651 having a particular molecular signature. For instance, the lateral thalamus,
1652 characterized by high *Tcf7/2* and *Lef1* expression, and less pronounced *Gbx2*
1653 expression, encompass the mediodorsal, laterodorsalventral anterior/ventral lateral,
1654 the ventral posteromedial and posterolateral, the submedius, the ventromedial, the
1655 parvicellular part of ventral posterior, the posterior, the dorsolateral and medial
1656 geniculate nuclei or nuclear groups. The reticular thalamic nucleus, which plays a
1657 role in sensory processing, attention and cognition, is characterized by a high
1658 expression of *Six3* and *Esrrg*, and none of the indentifying transcription factors
1659 corresponding to the lateral or other thalamic nuclei.

1660

1661 An even more recently published study focused on this reticular nucleus using a
1662 single-cell transcriptomic approach combined to electrophysiological recordings (Li et
1663 al., 2020). Li et al. established a cellular heterogeneity in this nucleus. Indeed, they
1664 identified four subpopulations of cells: one in which the *Spp1* gene was highly
1665 expressed, one in which it was the *Ecel1* gene, another one which expressed both
1666 genes, and a final one in which neither was expressed. Interestingly, in coronal
1667 sections of the reticular nucleus, *Ecel1* cells formed kind of a shell surrounding a core
1668 of *Spp1* cells. The next step established that *Spp1* neurons projected predominantly
1669 to first order thalamic nuclei, which receive information from ascending pathways,
1670 whereas *Ecel1* neurons projected predominantly to high order nuclei, which gate the
1671 cortical feedback to first order nuclei and monitor/modulate cortico-thalamo-cortical
1672 communication, especially from one cortical area to another (e.g., Guillery, 1995;
1673 Figure 10). *Spp1* neurons, which have a simple, short-extending dendritic

1674 arborization, exhibit greater excitability and a lower action potential threshold than
1675 *Ecel1* neurons. They are of the first type. *Ecel1* neurons, which have a complex
1676 dendritic pattern with longer ramifications in all directions, have a more robust
1677 bursting activity than the *Spp1* ones. Li et al. then examined whether the two types of
1678 neurons had differential contributions to electroencephalographic activity in the
1679 somatosensory cortex. By genetic manipulations, they reduced Ca⁺⁺ currents in one
1680 or the other population and observed that the cortical delta power during non-REM
1681 sleep was reduced by the manipulation in *Spp1* neurons, not by a similar
1682 manipulation of *Ecel1* neurons. When spindle oscillations during non-REM sleep
1683 were analyzed, the reduction of Ca⁺⁺ currents in *Spp1* neurons reduced the
1684 oscillations in number and length, whereas the same modification in *Ecel1* neurons
1685 increased the oscillations in length (not number).

1686

1687 This type of finely tuned approach certainly foreshadows what the functional anatomy
1688 of the thalamus (and of other brain structures) will be in the next years. Technically, it
1689 is now feasible to track the organization of neurons within a thalamic nucleus, their
1690 precise connectivity patterns with e.g., cortical regions, and their functional
1691 characteristics, all of this in close relationship with their transcriptomic peculiarities,
1692 and it is possible to do it at a single-cell resolution level (see e.g., Li et al., 2020).

1693

1694

1695 **Acknowledgments**

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1701

1702 **Conflict of interest**

1703 The authors have no conflict of interest to declare.

1704

1705 **References**

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2597

2598 **Figure captions**

2599

2600 **Figure 1: The cerebral ventricles as illustrated by Albertus Magnus in his**
 2601 ***Philosophia Naturalis* published posthumus in 1506.** This drawing corresponds to
 2602 an anatomo-functional conception in which common sense and basic imagination are
 2603 determined in the most anterior ventricle, creative imagination, phantasy, rational
 2604 thought, and evaluation in the ventricle next behind, and memory and reminiscence
 2605 in the most posterior ventricle. Drawing (Indian ink) is a faithful interpretation by
 2606 Audrey Chateaux according to a reproduction of the original material.

2607

2608 **Figure 2: Ventricular theory of brain functions.** Another illustration of the
 2609 ventricular theory of brain function which points to a connection between the most
 2610 anterior ventricle and four organs of senses (eye, nose, tongue, and ears). The
 2611 drawing is from Gregor Reisch's (ca. 1467-1525), *Margarita philosophica* compilation.
 2612 In fact, this was the first encyclopedic compilation published so far. Drawing (Indian
 2613 ink) is a faithful interpretation by Audrey Chateaux according to a reproduction of the
 2614 original material.

2615

2616 **Figure 3: Thalamus according to Vesalius.** Drawing of a horizontal section through
 2617 the head showing the lateral ventricles, the striatum (S) and the right and left (T)
 2618 thalami. This drawing by Audrey Chateaux (Indian ink) is a faithful interpretation of a
 2619 photograph of a copy by the spanish anatomist Juan Valverde de Amusco ([Lanska](#)
 2620 [and Lanska, 2013](#)) of the original woodcut print of Andrea Vesalius' *Fabrica*.
 2621 Valverde de Amusco's *Historia de la Composición del Cuerpo Humano* was
 2622 published in 1556. It included 42 anatomical illustrations from copperplate
 2623 engravings, of which 38 were copies of Vesalius' work ([Bahsi et al., 2020](#)). Valverde
 2624 de Amusco is considered the greatest Castilian anatomist of this time.

2625

2626 **Figure 4: Thalamus according to Burdach.** Drawing of a coronal section passing
 2627 through the human brain at the level of the thalamus. The drawing is a faithful
 2628 interpretation by Audrey Chateaux (Indian ink) according to Burdach (1822) in *Vom*
 2629 *Baue und Leben des Gehirns*. This frontal section is a posterior view. Two original
 2630 annotations are not shown in this drawing. Above, on the left, one could read:
 2631 "Burdach .vom Gehirne" (meaning: "*Burdach. Of the brain*"; on the right: "*IV. Tafel.*"

2632 (meaning: “Board IV”). The visible structures in the middle of the section are: **a**, the
 2633 thalamus; **b**, the medial geniculate nucleus; **c**, the lateral geniculate nucleus; **d**, the
 2634 pineal gland (with the 3rd ventricle above and behind it); **e**, the superior colliculus; **f**,
 2635 the inferior colliculus; **g**, the cerebral peduncle; **h**, the superior cerebellar peduncle.
 2636 On the original board, these indications were not provided. Underneath the section
 2637 shown here, however, was an annotated drawing outlining of the different structures,
 2638 to which either letters or numbers were associated. This picture is the first to mention
 2639 the pulvinar in the literature (as *Polster*; in german, the word means ‘pad’ or ‘pillow’).
 2640 It is indicated by the asterisk on the current drawing.

2641

2642 **Figure 5: Ablation methods.** **A** illustrates the principle of the ablation method to
 2643 identify existing connectivity between brain structures. Regions ‘a’, ‘b’ and ‘c’ project
 2644 to their target regions ‘1’, ‘2’ and ‘3’, respectively. In order to identify the origin of the
 2645 projections to region ‘2’, this region is damaged (top) and, usually, the animal is left
 2646 for a relatively long survival time after which it is killed for neuroanatomical
 2647 investigations. The neuroanatomist will seek the regions in the brain that have
 2648 undergone shrinkage (bottom) due to loss of their target, **which** would then point to a
 2649 connectivity between ‘b’ and ‘2’. **B** illustrates the method developed by Vittorio Marchi
 2650 to visualize degenerating nervous fibers. It consists in placing tissues in a mixture of
 2651 osmium tetroxide and potassium chlorate. So exposed, degenerating myelin
 2652 becomes black. As with the ablation method, a lesion or a fiber track section (top)
 2653 was mandatory for successful tracing, but the staining allowed perfect localization of
 2654 the pathways that had undergone Wallerian degeneration (bottom), **which** the
 2655 ablation method did not. A major drawback of Marchi’s technique was its **restriction** to
 2656 myelinated fibers.

2657

2658 **Figure 6: Principles of anterograde, retrograde and multidimensional**
 2659 **(anterograde+@b) neuroanatomical tracing methods.** **A** Anterograde: a tracer
 2660 undergoing anterograde transport is injected into a nucleus, from where it is
 2661 transported to the axonal terminals. Usually, dendrites, cell bodies (of both projection
 2662 neurons and interneurons) and axons are labelled. The usual tracers are biocytin,
 2663 biotinylated dextran amines (BDA), phaseolus vulgaris leucoagglutinin (PHA-L),
 2664 rhodamine-conjugated dextran amine (RDA), wheat germ agglutinin-horseradish
 2665 peroxidase (WGA-HRP). These tracers do not distinguish the neurochemical identity

2666 of the neurons. **B** Retrograde: a tracer undergoing retrograde transport is injected
 2667 into a nucleus from where it is transported to the cell body and dendrites of the
 2668 projection neurons that innervate this nucleus. The usual tracers are Cholera toxin
 2669 subunit B (CTB), fluoro-gold, pseudorabies virus (PRV), and rabies virus (RV). **C**
 2670 Anterograde + @b: an anterograde (or retrograde; not illustrated) tracing method is
 2671 combined to immunohistochemistry with an antibody that will contribute to precise the
 2672 e.g. neurochemical identity of specific neurons and their terminals within the larger
 2673 population of labelled ones. Thereby the dopaminergic, cholinergic, GABAergic...
 2674 nature of the connections between two structures can be identified. This figure has
 2675 been redrawn according to [Lanciego and Wouterlood \(2020\)](#), figures 2 and 5.

2676

2677 **Figure 7: Illustration of the fiber *en passage* problem with the ablation method**

2678 **(A) or the method of Marchi based on myelin staining of degenerating fibers**

2679 **(B).** **A** In the case of ablation of region 'b' (on top panel), when fibers pass through
 2680 the damaged region, they are also disrupted, which leads to a shrinkage of their
 2681 region of origin ('a' in bottom panel) along with a shrinkage of the region in which
 2682 fibers innervating 'b' originate ('2'). A consequence could be to consider that both
 2683 regions 'a' and '2' innervate 'b'; in fact, the innervation of 'b' by 'a' is a false
 2684 conclusion. **B** In the case of Marchi's method, there is a similar problem. If
 2685 myelinated fibers originating in region 'a' and innervating region '2' pass through
 2686 region 'b', 'b' being a region innervating region '1', and region 'b' is destroyed (top
 2687 panel), these fibers *en passage* will also be disrupted. The consequence of this is
 2688 that Marchi's method will reveal tracks corresponding to fibers *en passage* in region
 2689 'b' and fibers going from region 'b' to region '1' (bottom panel). A consequence is to
 2690 consider that 'b' innervates both region '1' and region '2'. Whereas the conclusion for
 2691 region '1' is correct, the conclusion for region '2' is false.

2692

2693 **Figure 8: Principle of the carbocyanine staining.** Carbocyanine dyes were first
 2694 introduced by Honig and Hume in 1985 (Honig and Hume, 1989). They are lipophilic
 2695 substances undergoing fast axonal transport in both directions (anterograde and
 2696 retrograde). They label the whole neuron (soma, dendrites, axon). Transcellular
 2697 labeling has also been observed (Godement et al., 1987). The dyes (e.g., DiL, DiO,
 2698 DiA, DiAsp) diffuse along the plasma membrane of living tissue and can be even
 2699 used in formalin-fixed tissue (Godement et al., 1987), and this is a huge advantage

2700 in comparison with other staining techniques requiring *in vivo* injections, and thus
2701 stereotaxic surgery. They provide intense fluorescence with little fading (meaning the
2702 staining is persistent) and are particularly well-adapted to post-mortem human brain
2703 tissue. They are compatible with double labeling (e.g., Dil in one structure and DiA in
2704 another one).

2705

2706 **Figure 9: Virus-mediated tracing. A** Principle of neuroanatomical tracing combining
2707 a genetic modification of the animal model in order to get an expression of a reporter
2708 gene in neurons having a specific neurochemical identity. The example illustrated
2709 here is that of a parvalbumin-cre-dependent mouse in a brain structure where a viral
2710 vector is injected, introducing into transfected neurons a stop codon with loxP sites
2711 and the reporter gene (here GFP). With such an approach, only GABAergic neurons
2712 (red circle) of the parvalbumin type will express GFP and appear entirely stained. The
2713 others will not express the reported gene. This figure has been redrawn according to
2714 [Lanciego and Wouterlood \(2020\)](#), figure 10. **B** Principle of retrograde transynaptic
2715 staining with a rabies virus. In the absence of genetic manipulations, rabies are
2716 naturally retrograde, polysynaptic viruses with a very high tropism for neurons. This is
2717 due to their full competence to replicate, including the code of the G protein, which is
2718 a glycoprotein necessary and sufficient to support retrograde transynaptic infection.
2719 When the virus binds to one of its membrane receptors (there are several types), it
2720 undergoes endocytosis-driven internalization and is transported to the soma. Rabies
2721 have the capacity to retrogradely cross a potentially unlimited number of synapses; it
2722 just takes time. **C** Principle of tracing with a (modified) monosynaptic rabies virus.
2723 Monosynaptic rabies virus spread is obtained by a modification of the wild-type virus
2724 such as to delete the gene coding for the G glycoprotein. By this way, the still G-
2725 protein-coated virus infects cells via the G receptor of the afferent neurons. When the
2726 virus is released from an infected cell (the starter cell), it is not taken up by adjacent
2727 nerve terminals. The fact that the virus does not cross more than one synapse is due
2728 to the absence of the code corresponding to the G protein transcription when the
2729 virus is replicating (e.g., Ghanem and Conzelmann, 2016).

2730

2731 **Figure 10: Schematical representation of the connectivity differences between**
2732 **first order thalamic nuclei (dark grey) and higher order nuclei (light grey).** First

2733 order nuclei receive driving afferents from the periphery (peri) and modulating
2734 afferents from the cortex that exert a gating role (layer VI). They send efferents to the
2735 cortex (layer IV). Higher order nuclei also receive afferents from the periphery,
2736 though to a lesser degree than first order nuclei. Their driving afferents come from
2737 the cortex (layer V). They also receive modulating afferents from the cortex (layer VI).
2738 In addition, efferents going to the cortex (layer IV) are implicated in the transthalamic
2739 corticocortical information flow that may parallel the direct corticocortical flow. First
2740 order nuclei are implicated in sensory functions while higher order nuclei participate
2741 in cognitive functions. CTX1, CTX2, and CTX3 represent cortical regions.

2742
2743 **Figure 11: Principle of fMRI (A,B).** When an ensemble of neurons is activated, the
2744 metabolic activity of the region encompassing these neurons increases (compared to
2745 rest, **A**) and induces a correlative increase of blood flow (**B**). This increased blood
2746 flow provides neurons with an additional supply of oxygen and glucose. These
2747 modifications are termed neurovascular coupling. Neurovascular coupling is
2748 supported by chemical signals and astrocytes, what induces a localized dilatation of
2749 blood vessels (**B**). In the activated brain structure or region (stimulation), the
2750 deoxyhemoglobin/oxyhemoglobin ratio undergoes a transient modification (because
2751 of the transfer of oxygen to neural tissue), which is detected by the MRI scanner at a
2752 resolution achieving the mm^3 in space and an order of 1 s in time. **Principle of fiber**
2753 **tract tracing by DTI (C).** In gray matter, diffusion of water molecules is unrestricted,
2754 so that these molecules can diffuse in all possible directions. This diffusion is called
2755 isotropic diffusion. Restricted (anisotropic) diffusion corresponds to a situation in
2756 which water molecules are limited by obstacles such as membranes,
2757 macromolecules or white matter. Indeed, when such an obstacle is a fiber track, the
2758 water molecules tend to diffuse along the track, more rarely perpendicularly to it.
2759 Furthermore, when they diffuse along the track, this diffusion is faster than in case of
2760 a perpendicular one. The track and its orientation can be visualized by detection of
2761 the directionality of the water diffusion process. Such detection make it possible to
2762 create, voxel by voxel, fiber orientation maps. This is called white matter
2763 tractography. On the left part of figure **C**, the short lines in each square indicate the
2764 orientation of a virtual fiber track, here illustrated in a two-dimensional scale, thus for
2765 each virtual pixel (each of the squares delimited by blue lines). In each pixel, the red
2766 line indicates a virtual average orientation of diffusion. Notice that the alignment of

2767 these red lines is influenced by the orientation of the fiber track. For DTI, the average
2768 diffusion vector is computed for each voxel. On the right, the reconstructed image in
2769 the same scale.



Figure 1

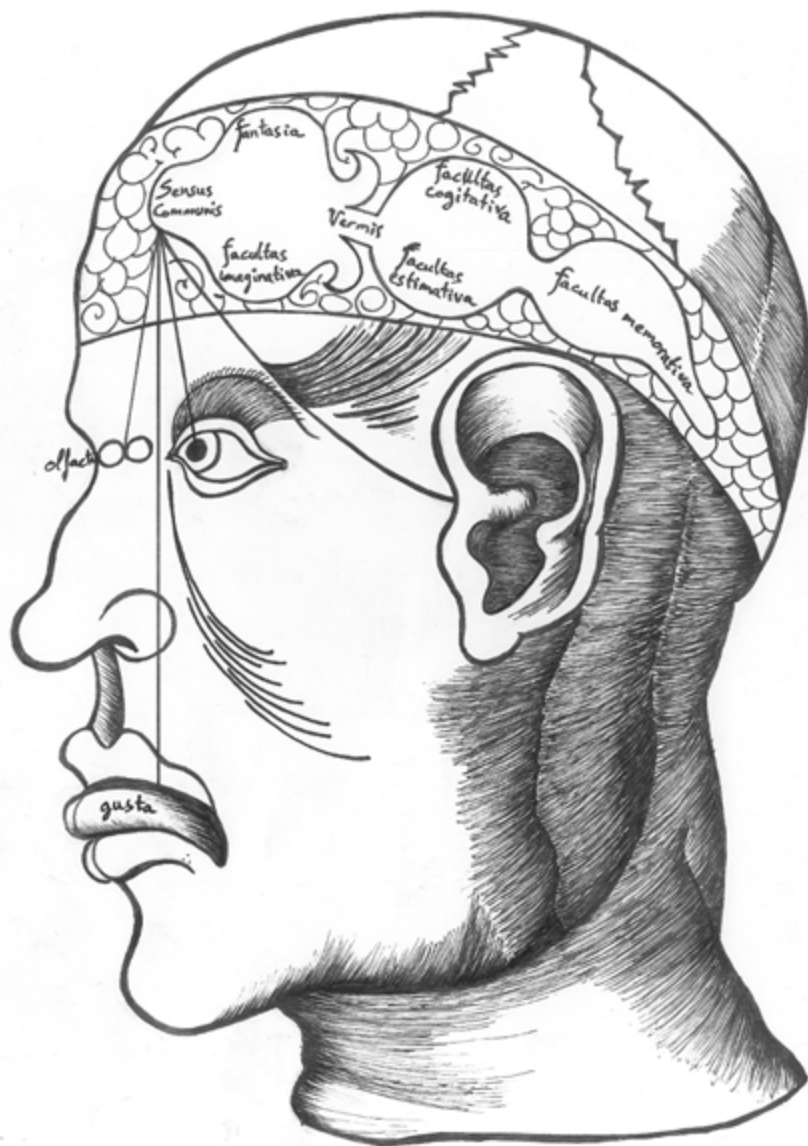


Figure 2

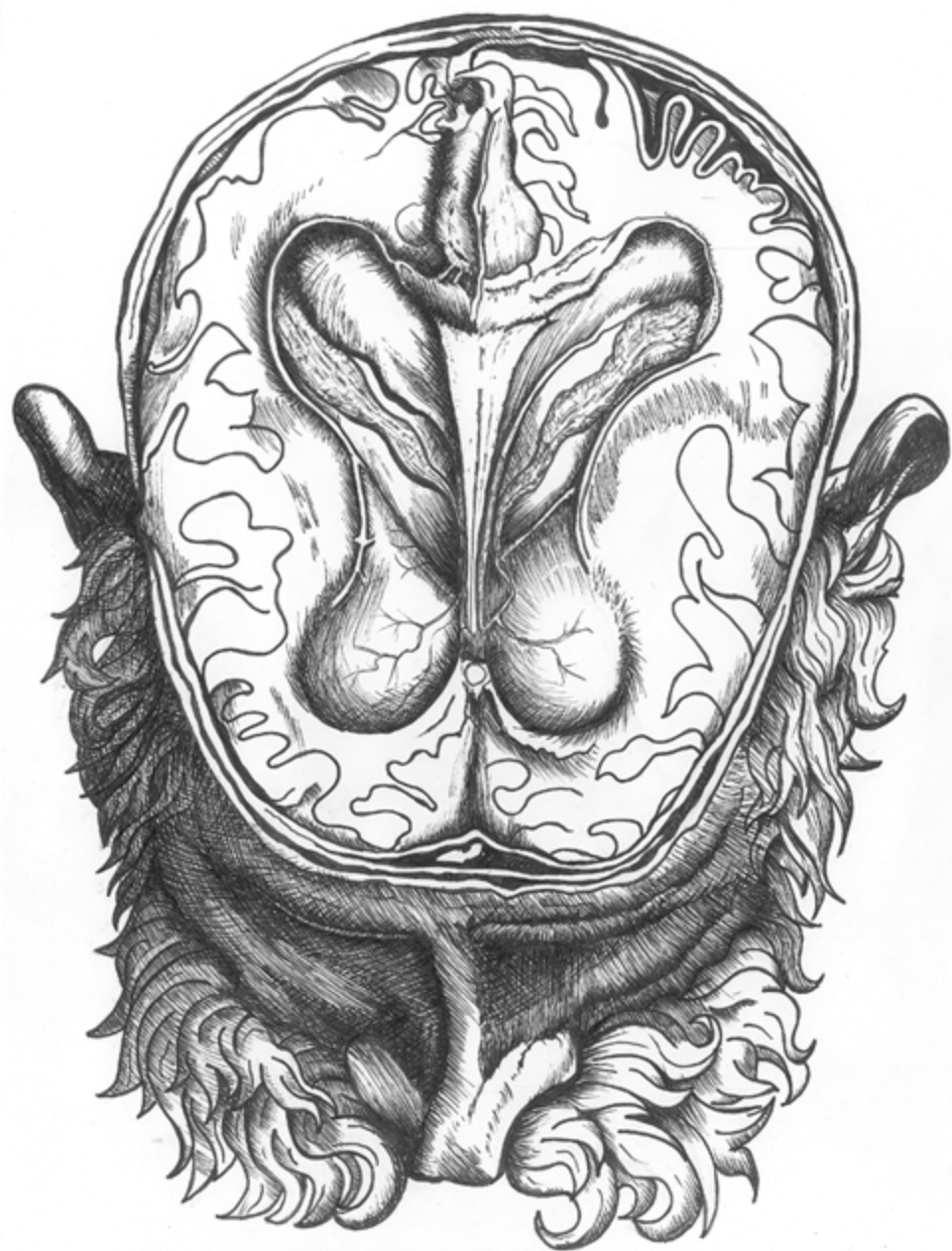


Figure 3

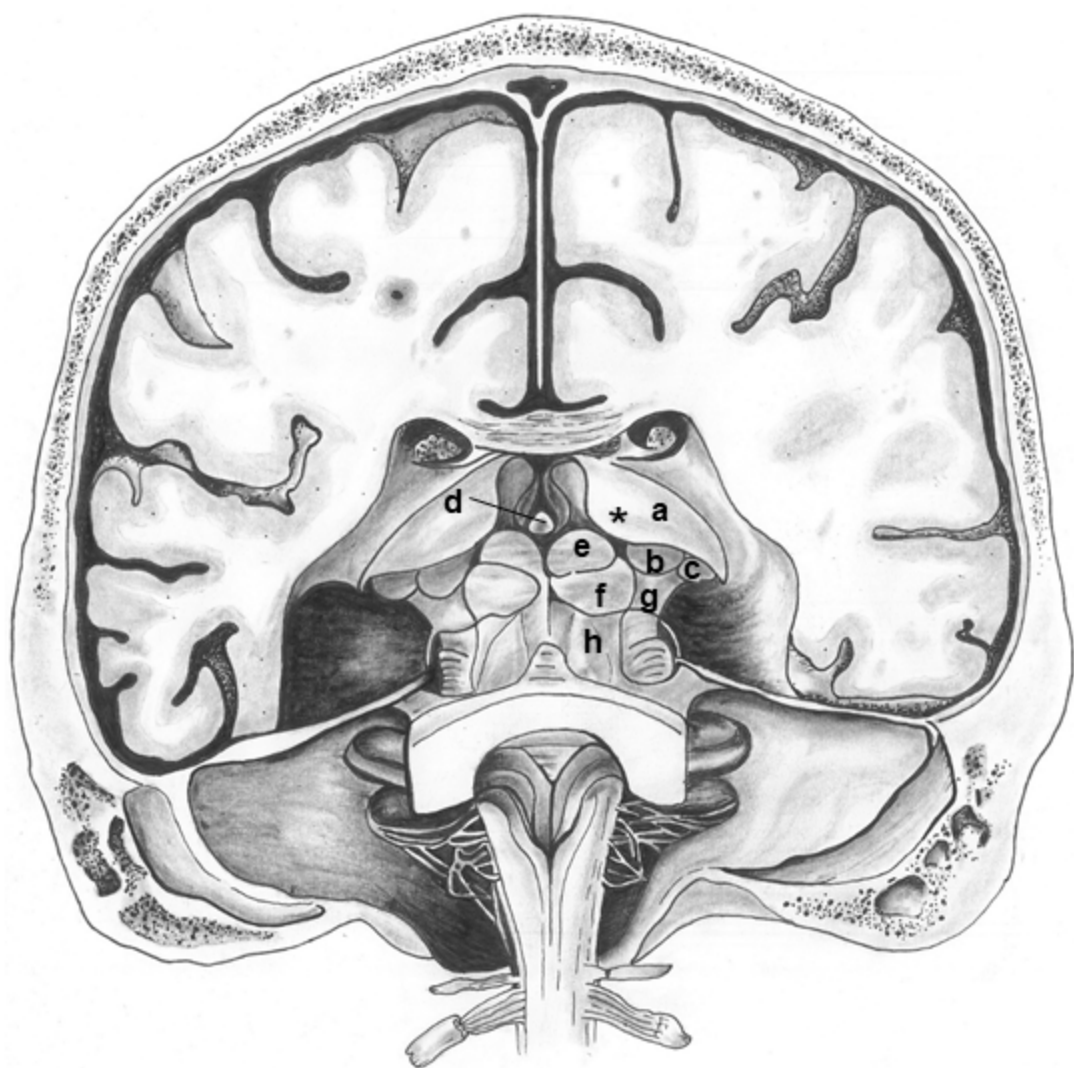


Figure 4

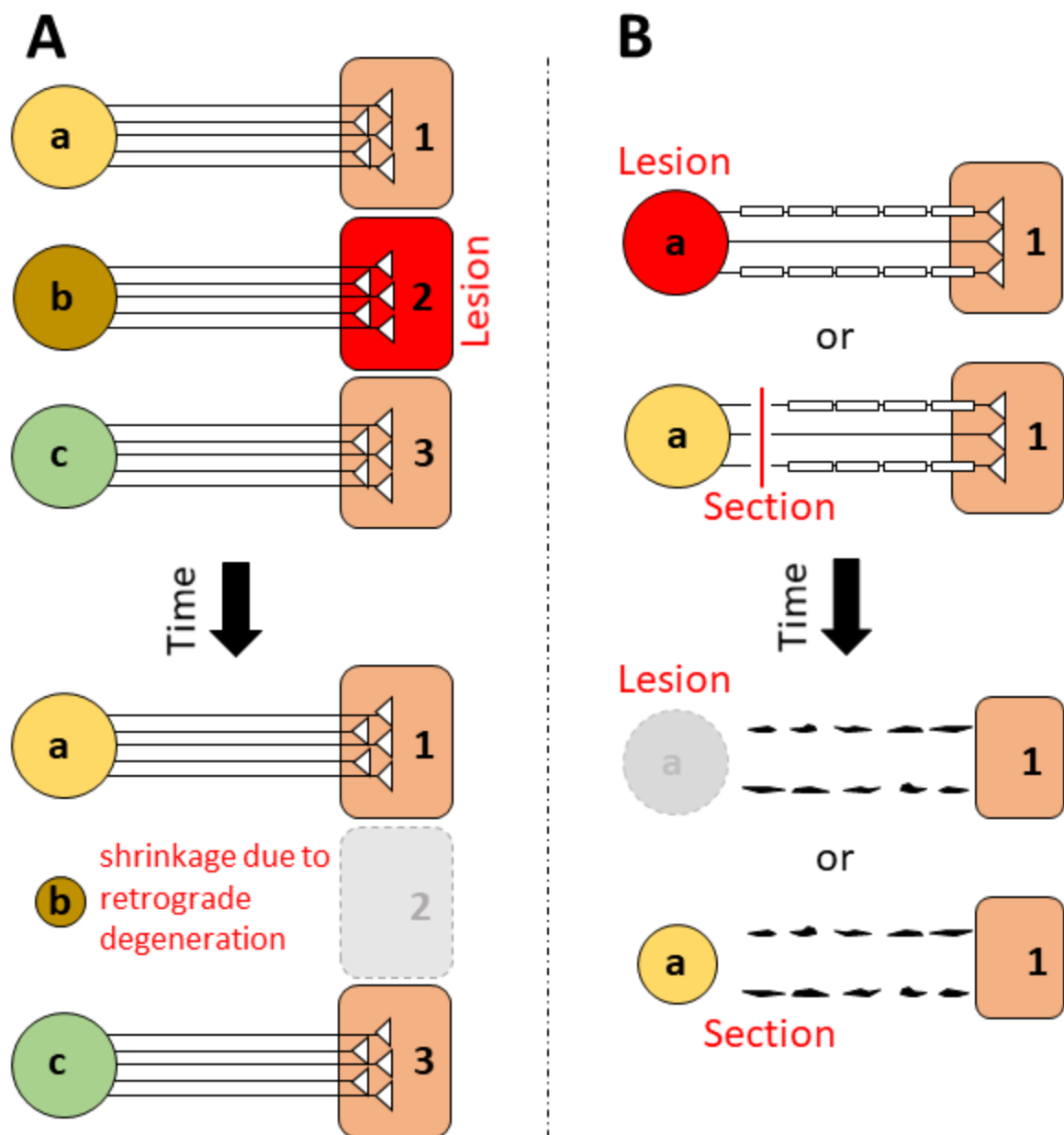
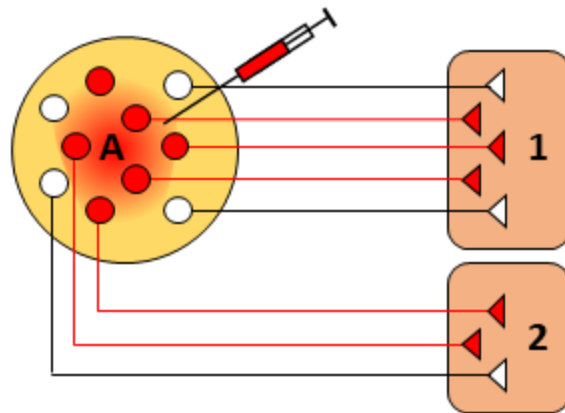
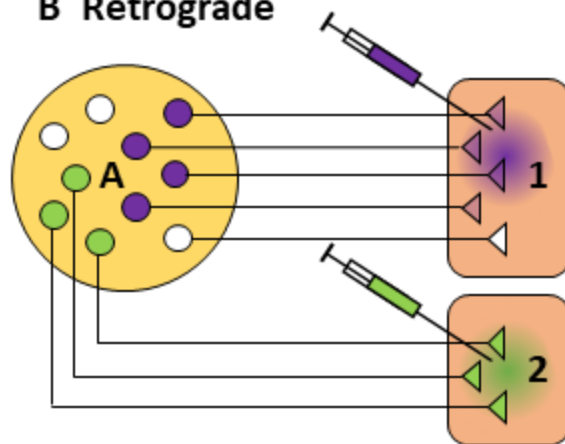


Figure 5

A Anterograde



B Retrograde



C Anterograde + @b

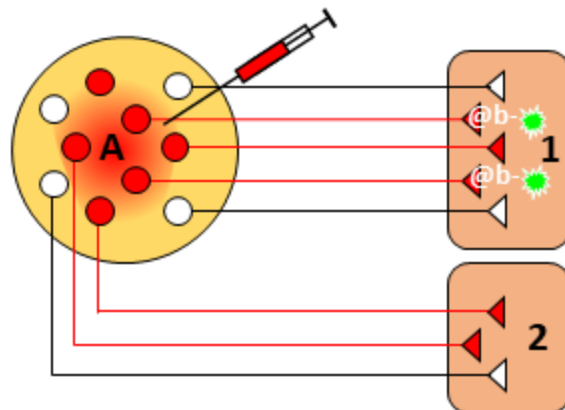


Figure 6

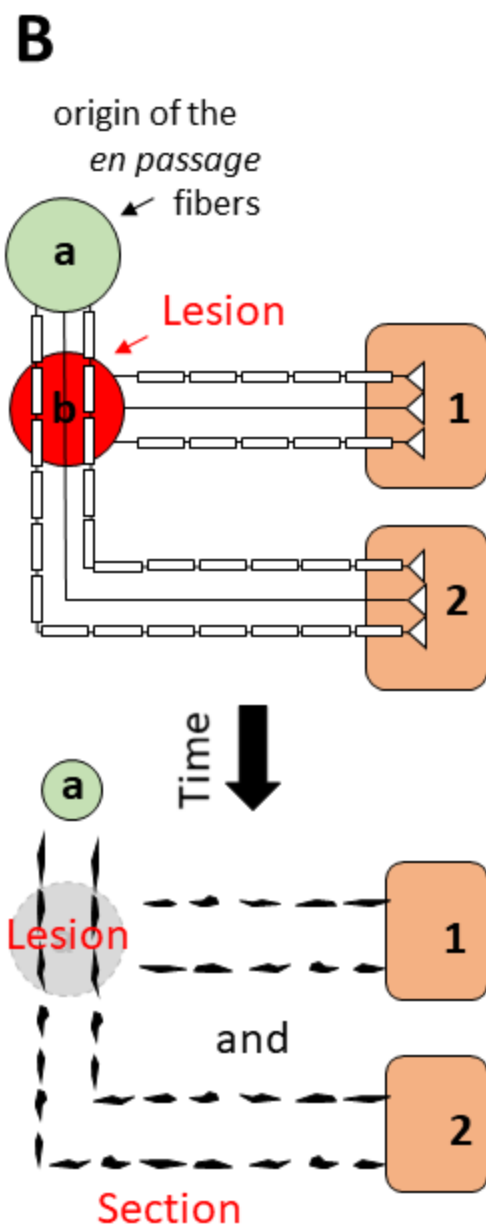
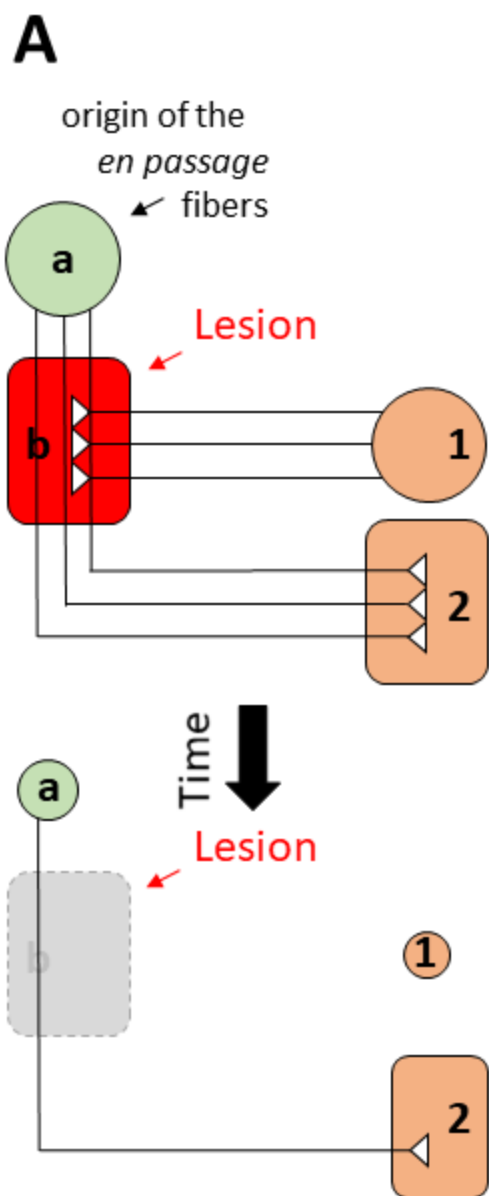


Figure 7

Carbocyanine dyes

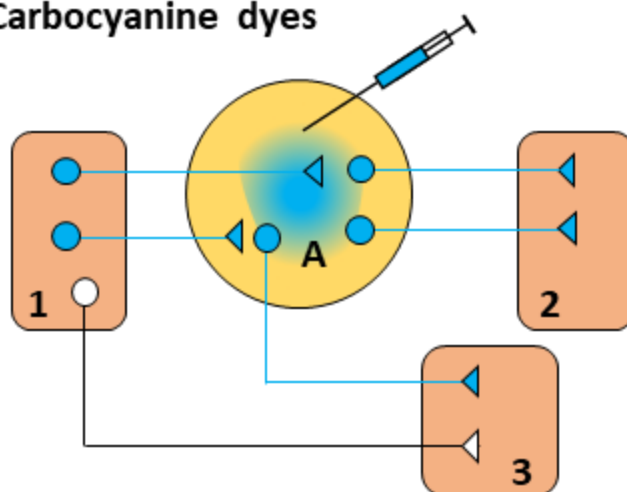
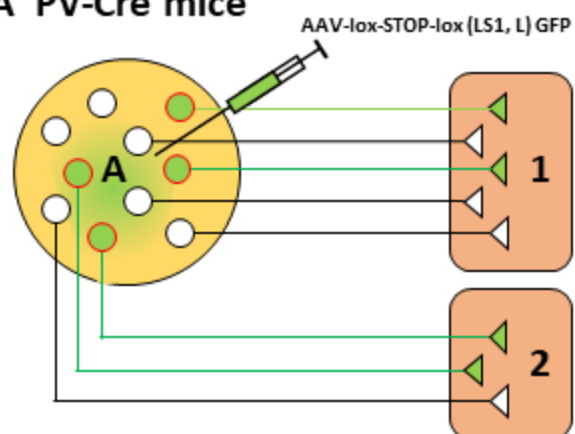
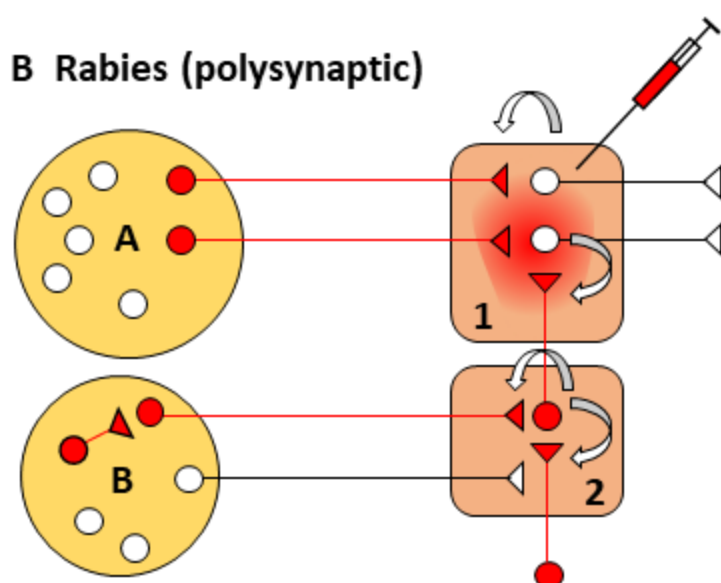


Figure 8

A PV-Cre mice



B Rabies (polysynaptic)



C Modified rabies (monosynaptic)

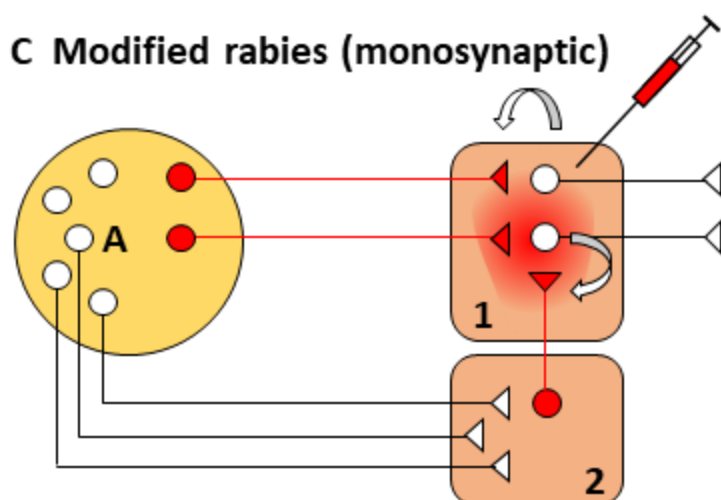


Figure 9

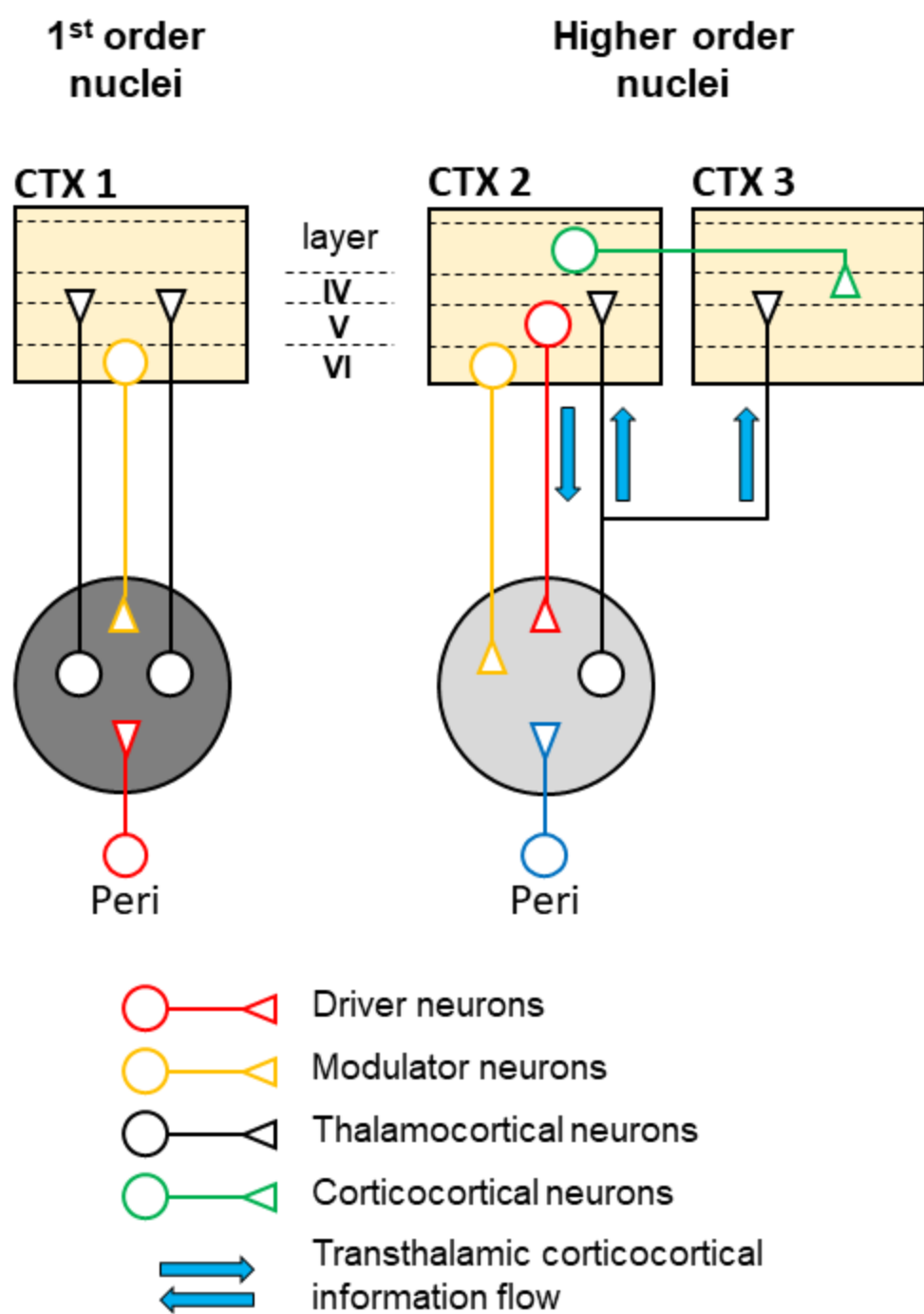
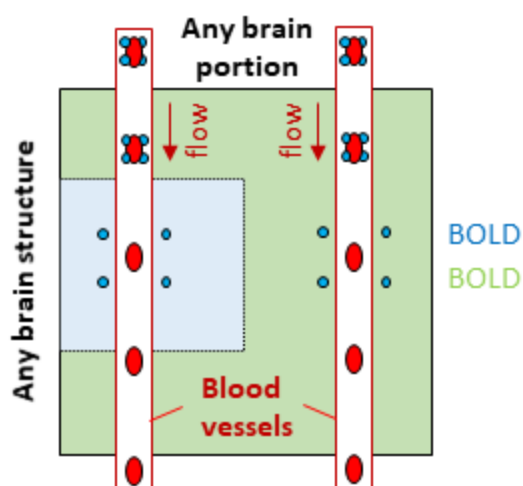




Figure 10

A fMRI, rest

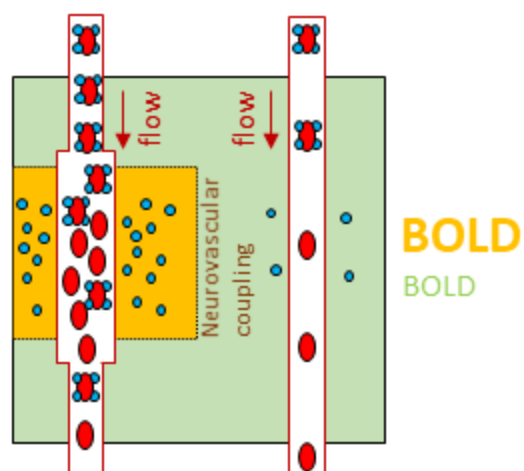


MRI image

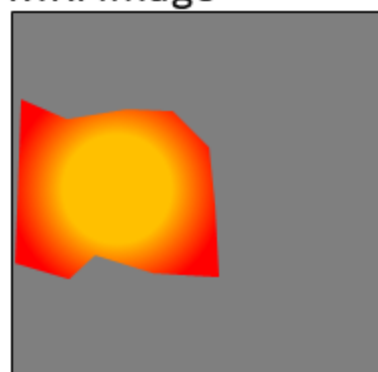


 oxyhemoglobin
 deoxyhemoglobin

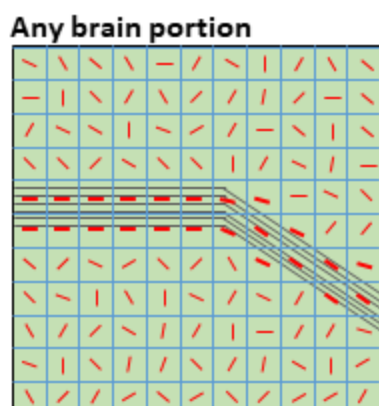
B fMRI, stimulation



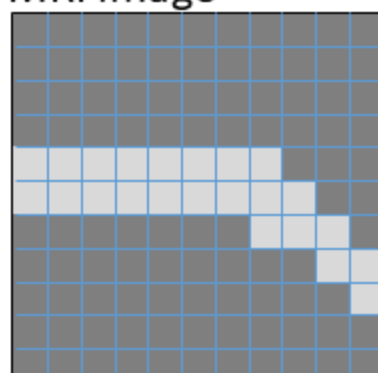
MRI image



C MRI, DTI



MRI image



Graphical abstract

A selection of milestones (in brown) on the route of the thalamus in the history of neuroanatomy from antiquity to the XXth century, with apologies from the authors for all omissions.

BCE		CE	
Edwyn Smith papyrus	~ -1600	Galenus	~ 170
Alcmeon of Croton	~ -600	Albertus Magnus	~ 1240
Herophilus of Chalcedon Herastrate of Ceos	~ -300	Andreas Vesalius	1543
		Thomas Willis	1664
		Karl Friedrich Burdach	1822
		Bartolomeo Panizza	1855
		Joseph Gerlach	1858
		Franz Nissl	1884
		Vittorio Marchi	1886
		Wilfrid Le Gros Clark	1932
		Walle Nauta	1954
		Michael Hendrickson	1970
		Krister Kristensson	1973
		Krister Kristensson	1974
		Gy. Somogyi Miles Herkenham	1978
		Rainer Guillery	1980
		Edward Jones	1985
		Various authors	~ 2000
Oldest trace of anatomofunctional knowledge in the brain		Name given to the thalamus = <i>thalamus nervorum optitorum</i>	
Dissections used to study brain organization		Resumes ventricular theory with functional localization	
Dissections/vivisections of humans used intensively		<i>De Humani Corporis Fabrica</i> Two illustrations of thalamus	
		<i>De Cerebri Anatome</i> Splendid drawings of thalamus	
		Subdivision of thalamus into 4 subregions	
		Degeneration method for study of connectivity : visual tracts	
		First stain for nervous tissue carmine = powder from cochineal	
		Nissl stain : thalamus subdivided in 16 regions (1889), then 18 (1913)	
		Ablation and staining combined Thalamic connections described	
		Important & influential synthesis of knowledge about thalamus	
		More details on connectivity: Degenerating amyelinic fibers stained	
		Autoradiography to study the retinothalamic pathways	
		First MRI image (water-filled tubes)	
		First use of herpes simplex virus to trace eye to dLGN projections	
		Thalamic connectivity investigated with retrograde tracing (HRP)	
		Eye-specific segregation of retinal input to dLGN using [³ H]proline	
		Publication of a monograph entirely devoted to the thalamus	
		MRI-based diffusion imaging describes human functional connectivity	