

# VU Research Portal

## Parcellation of Prefrontal Cortical Areas in Mouse and Rat Brain

van de Werd, H.J.J.M.

2013

### **document version**

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

### **citation for published version (APA)**

van de Werd, H. J. J. M. (2013). *Parcellation of Prefrontal Cortical Areas in Mouse and Rat Brain*.

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

### **E-mail address:**

[vuresearchportal.ub@vu.nl](mailto:vuresearchportal.ub@vu.nl)

# Chapter 5

## **Summary and General Discussion**

Chapter 5

R1  
R2  
R3  
R4  
R5  
R6  
R7  
R8  
R9  
R10  
R11  
R12  
R13  
R14  
R15  
R16  
R17  
R18  
R19  
R20  
R21  
R22  
R23  
R24  
R25  
R26  
R27  
R28  
R29  
R30  
R31  
R32  
R33  
R34

In the preceding chapters the cytoarchitectonics of the prefrontal cortex was studied in the ventral/orbital side and on the lateral side of the frontal lobe of the rat (chapter 2), and in all areas of the prefrontal cortex (PFC) in the mouse (chapter 3). The comparative study (chapter 4) examined to what extent prefrontal areas in the mouse differ between the atlases and other publications. The conclusions of these chapters are:

(a) Boundaries between subareas of the PFC can be assessed in rats and mice. (b) The criteria to define boundaries in Nissl stained sections should be based on features consistently observed and well described. (c) For detection of the boundaries between all subareas of the PFC the general cellular staining like Nissl staining is preferred to a histochemical or immunocytochemical staining, which stains specific features of a limited number of cells.

(d) Results of a specific (e.g. immunocytochemical) staining may support the location of a particular PFC subarea boundary defined in a Nissl stained section.

(e) Tracing studies have been important to focus on subdividing PFC regions into PFC subareas. These boundaries are assessable in Nissl stained sections.

(f) The PFC subarea boundaries have been described to facilitate the research and the communication on specific functional properties present.

This chapter will further discuss (1) stainings for PFC area parcellation; (2) differences in rat and mouse PFC areas; (3) to what extent the PFC areas in mouse brain are different in the very new edition of Paxinos and Franklin (2013); (4) the nomenclatures used for the PFC areas in rodents; and finally (5) future developments.

### *1. Stainings for PFC area parcellation*

In cytoarchitectonic studies, generally several factors will influence the delineation of the PFC and its subareas on the surface of the cerebral cortex or in coronal sections through the frontal lobe. These factors are e.g. the use of more than one staining method, the characteristics attributed to

R1 an area in other cytoarchitectonic descriptions, connectivity studies and  
R2 the number of animals examined within a defined strain of a species. In  
R3 defining the boundaries of the prefrontal cortex and its subareas in the  
R4 Figures that are shown in chapters 2 and 3, the characteristics found in  
R5 the Nissl staining have been the principal source for the determination of  
R6 boundaries. In addition, the examination of the immunocytochemically and  
R7 myelin-stained coronal sections supported the results found in the Nissl  
R8 staining. Thus the additional staining methods confirm the distinction of  
R9 the areas Ald1 and Ald2 in Nissl-stained sections of PFC areas in both rat  
R10 and mouse (chapters 2 and 3), and also the location of area DLO in the rat  
R11 (chapter 2). The location and size of areas Ald1 and Ald2, as defined in  
R12 the mouse PFC (chapter 3), agrees with the location and size of the areas  
R13 Ald and Alv in the immunocyto/myeloarchitectonic study of Paulussen  
R14 et al. (2011) who used the low-molecular-weight neurofilament protein  
R15 subunit (NF-L) for staining coronal sections of the mouse PFC. The authors  
R16 point to the superiority of this staining method over the combined use  
R17 of the different supporting staining methods employed in our study (Van  
R18 de Werd et al., 2010). The results of NF-L staining are impressive in their  
R19 ability to show the myelographic structure and cortical layers in low-power  
R20 micrographs, as well as fine fibres and the structure of neurons in high-  
R21 power micrographs. Especially in PFC areas, the NF-L is, however, inferior  
R22 to the traditional Nissl staining in showing cells in low-power micrographs.  
R23 In NF-L-stained coronal sections, the distinction of several PFC areas on the  
R24 medial side of the frontal lobe is not possible. The Nissl staining is superior  
R25 to additional staining methods as far as it allows viewing all cells without  
R26 being restricted to a special group of cells. Boundaries that are defined on  
R27 the basis of the combined results originating from multiple and various  
R28 methods are more likely to be reproducible /stable and to delineate areas  
R29 that are reliably functional units. For the evaluation of the prefrontal cortex  
R30 after experimental procedures [e.g., the effects of cortical or subcortical  
R31 lesions] the Nissl staining is often preferred for reasons mentioned above.  
R32 Delineating a boundary in Nissl-stained coronal sections will sometimes  
R33  
R34

be hampered in case an expected change in a main characteristic of the boundary is not clearly visible, e.g. the difference in layer II of frontal area Fr2 compared with layer II of area ACd is sometimes very subtle. Nevertheless, the description of layer II of the medial precentral area, *PrCm*, as appearing 'lighter, being less densely packed, and more homogeneous than in the anterior cingulate area' (Krettek and Price, 1977) agrees well with the description of the corresponding area Fr2 in the studies of Van Eden and Uylings (1985), Van de Werd and Uylings (2008) and Van de Werd et al. (2010). Usually sufficient characteristics are present to delineate boundaries in such a way that they are consistent when repeatedly studied by different examiners and at different times. It is most probable that no single staining method is able to offer a parcellation that presents all possible functional units in the prefrontal cortex as the number of functions attributed to the prefrontal cortex is much higher than the areas distinguished in cytoarchitectonic studies. This view is supported in the review of the myeloarchitectonic studies of the Vogt - Vogt school by Nieuwenhuys (2013), reporting that in the human cerebral cortex some 200 different areas can be found, far more than in the commonly used Brodmann parcellation. Within a delineated area in a complicated structure such as the cerebral cortex no uniform structure can be expected, as is illustrated in layer II of the lateral orbital area LO. The clustering of cells in layer II is clearest in a small part of LO near the boundary with area Ald2, but less near the boundary with VLO. In the medial PFC a ventral-dorsal gradually narrowing of layer II can be seen passing different successive areas. In such an area the thickness of layer II is not constant. In order to be able to communicate adequately on the properties of areas it is therefore important that their boundaries are defined on agreed characteristics that are considered to be typical of a particular boundary. Studies of connections reveal that the cytoarchitectonically defined different PFC areas all have a particular set of connections with the thalamus, other cortical areas, basal ganglia, and amygdala (Hoover and Vertes, 2011; Groenewegen and Uylings, 2010; Schilman et al., 2008; Gabbott et al., 2005; Reep et al., 1996; Groenewegen, 1988).

R1  
R2  
R3  
R4  
R5  
R6  
R7  
R8  
R9  
R10  
R11  
R12  
R13  
R14  
R15  
R16  
R17  
R18  
R19  
R20  
R21  
R22  
R23  
R24  
R25  
R26  
R27  
R28  
R29  
R30  
R31  
R32  
R33  
R34

## 2. Comparison of PFC areas in rat and mouse

When the prefrontal areas on the ventral/orbital and the lateral side of the rat frontal lobe are compared with the prefrontal areas on the same sides of the frontal lobe in the mouse, a different number of prefrontal areas are found. In the rat, the ventral agranular insular area (Alv) is described in the posterior part of the dorsal bank of the rhinal fissure (Van de Werd and Uylings, 2008; Ray and Price, 1992), where it replaces the lateral orbital area that is visible in the part of the ventral/orbital side of the frontal lobe that is rostral to it. In the mouse, this replacement of the lateral orbital area (LO) by the ventral agranular insular area could not be distinguished in a reproducible way (Van de Werd et al., 2010; chapter 3). In the rat, Alv is distinguishable from LO in layer III, which is more cell-sparse in Alv than in LO. In the stereotactic atlas of the mouse brain of Franklin and Paxinos (2008), the ventral agranular insular area (AIV) is located on the lateral side of the frontal lobe, corresponding with the location of area Ald2 in chapters 2- 4. Thus, AIV is defined in this atlas at a different location than Alv in our study (Van de Werd and Uylings, 2008). In addition, the dorsal agranular insular area (AID) in this atlas corresponds with area Ald1 in chapters 2 - 4.

In the rat, the dorsolateral area (DLO) (Ray and Price, 1992), is cytoarchitectonically differentiated from the dorsal agranular area (Ald) by the difference in layer II that is broader in DLO than in Ald, by the less densely packed cells in layer III of DLO in comparison with Ald and the absence of a deep claustral layer in DLO (Van de Werd, 2008; Chapter 2). DLO is located in front of, and adjacent to, area Ald on the lateral side of the frontal pole. In addition to the cytoarchitectonic difference of DLO with Ald, Ray and Price (1992) based their distinction of a separate area DLO in the rostral part of the frontal lobe on its distinctive connection with the thalamus. The difference of connections with the mediodorsal nucleus of the thalamus between areas DLO and Ald has been demonstrated in anterograde and retrograde tracer experiments (Groenewegen, 1988; Ray and Price, 1992). Area DLO appears to be connected mainly with the ventrolateral caudal

part of the lateral segment of the mediodorsal nucleus (MD), but the dorsal agranular insular area (Ald) is mainly connected with the medial segment of MD (Ray and Price, 1992). In the mouse, a differentiation in the cytoarchitecture of Ald that would justify the distinction of a separate dorsolateral orbital area DLO on the lateral side of the rostral part of the frontal lobe, is not found (chapter 3 of this thesis; Van de Werd et al., 2010). Connectivity studies are needed to definitely confirm the absence of area DLO in the mouse.

On the ventral/orbital side of the frontal lobe of both rat and mouse three or four orbital areas are usually distinguished. In some groups (e.g. Hof et al., 2000; Franklin and Paxinos, 2008; Paxinos and Franklin, 2013; Paulussen et al., 2011) only one area is defined between the lateral orbital area (LO) and the medial orbital area (MO). This area is called either the ventral orbital area (VO) or the ventrolateral orbital area (VLO). In other studies two areas have been defined, i.e., both VO and VLO, by e.g. Krettek and Price, 1977; Groenewegen, 1988; Ray and Price, 1992; Reep et al., 1996; Van de Werd et al., 2008, 2010. Several authors noted that the orbital prefrontal areas are difficult to distinguish on the basis of only their cytoarchitectonic characteristics (Krettek and Price, 1977; Ray and Price, 1992; Reep et al., 1996). They mention that additional information about their thalamic connections is needed to define these boundaries. Our own studies (Van de Werd and Uylings (2008) and Van de Werd et al. (2010) support the view that a distinction between the orbital areas can be solely based on cytoarchitectonic characteristics if a set of reproducible characteristics is used to define each orbital boundary. The boundary between the orbital areas LO and VLO is mainly defined upon the different aspect of the layers II and III, i.e. the transition of the clustered cell-groups of layer II, sharply separated from the more homogeneous layer III of LO, into the homogeneous columns of cells in layers II and III of VLO. Reep et al. (1996) defined the boundary between LO and VLO mainly upon the difference in their connections with the mediodorsal nucleus (MD) and the submedial nucleus of the thalamus. They report that LO is connected with

R1  
R2  
R3  
R4  
R5  
R6  
R7  
R8  
R9  
R10  
R11  
R12  
R13  
R14  
R15  
R16  
R17  
R18  
R19  
R20  
R21  
R22  
R23  
R24  
R25  
R26  
R27  
R28  
R29  
R30  
R31  
R32  
R33  
R34



R1 the central segment of MD, whereas VLO is supposed to be connected  
R2 mainly with the submedial thalamic nucleus, and to a lesser degree with  
R3 the lateral segment of MD. Reep et al. (1996) locate the lateral boundary  
R4 of VLO in the 'orbital notch', the indentation in the middle of the ventral/  
R5 orbital side of the frontal lobe, in agreement with the lateral boundary  
R6 of VLO in Ray and Price (1992), but somewhat more medial than in the  
R7 studies of Krettek and Price (1977) and Groenewegen (1988). The lateral  
R8 boundaries of VLO, delineated in chapters 2-4, based on cytoarchitectonic  
R9 characteristics, are in agreement with the lateral boundary of VLO in Ray  
R10 and Price (1992) and Reep et al. (1996).

R11  
R12 The medial orbital area (MO) as delineated in chapters 2 - 4 and in the  
R13 studies of Reep et al. (1996), Ray and Price (1992), Uylings and Van Eden  
R14 (1990), Groenewegen (1988), Krettek and Price (1977) is a small area on  
R15 the ventral part of the medial prefrontal cortex with a little extension to  
R16 the ventral/orbital side of the frontal lobe. In the stereotactic atlases of  
R17 the mouse brain (Franklin and Paxinos, 2008; Hof et al., 2000), area *MO*  
R18 is larger and reaches, in the rostral coronal sections, halfway the medial  
R19 prefrontal cortex. Cytoarchitectonically the *MO* area is distinguished by its  
R20 localization between the more easily distinguishable ventral orbital area  
R21 *VO* on one side, and either the infralimbic area (*IL*) or the prelimbic area  
R22 (*PL*) on the other side. In the rostral part of the frontal lobe, layer II of *MO*  
R23 is broad and contains rather evenly dispersed cells, but posteriorly layer II is  
R24 narrower, contains darker cells and has a sharp border with layers I and II  
R25 (Van de Werd and Uylings, 2008; Van de Werd et al., 2010). When present,  
R26 fine cellular columns that involve all layers will be distinctive for *MO*.

R27 Although the stereotactic atlases of the mouse brain (Franklin and  
R28 Paxinos, 2008; Hof et al., 2000) do not provide the cytoarchitectonic  
R29 considerations that led them to delineate the dorsal boundary of area  
R30 *MO* on the place they did, it can be seen there that layer II, ventral to  
R31 this boundary, is broad with evenly spread, not very densely packed cells,  
R32 whereas the aspect of layer II dorsal to this boundary is different inasmuch  
R33  
R34

here the cells of layer II are more concentrated at the border of layer I, and also as layer II is narrower. In our parcellation, this typical change in the aspect of layer II marks the boundary between the dorsal and ventral part of the prelimbic area (PL).

Contrary to the Franklin and Paxinos (2008) and Hof et al. (2000) parcellations, the localization of the infralimbic area (IL) in rat and mouse is delineated more anteriorly in our studies (Van de Werd and Uylings, 2008; Van de Werd et al., 2010). The homogeneity of all cortical layers in IL distinguishes this area at its dorsal boundary from the prelimbic area (PL) and ventrally from the medial orbital area (MO). In some studies, area *IL* is positioned just posterior to area *MO*, which it suddenly replaces (e.g. Franklin and Paxinos, 2008; Hof et al. 2000; Reep et al., 1996; Swanson, 1992).

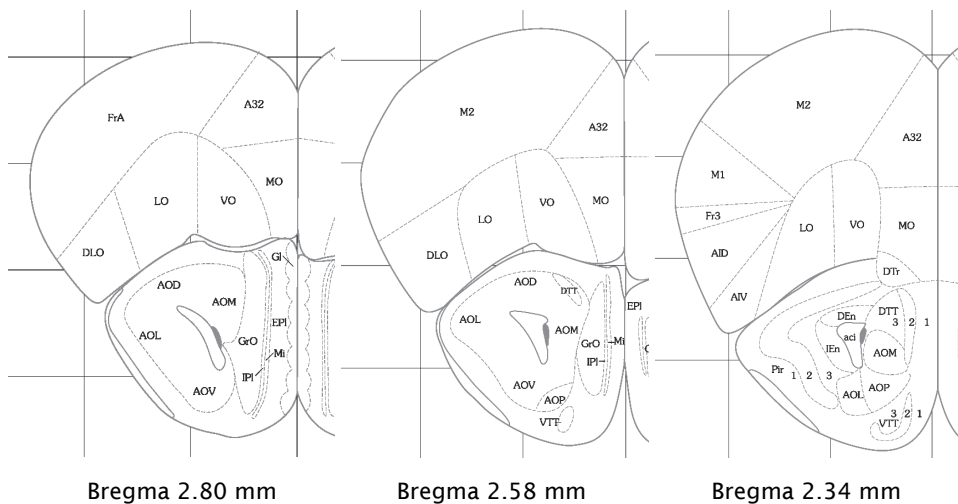
The distinction of PFC areas in the frontal pole is difficult in coronal sections because of the small surface and a restricted number of visible cortical layers. This only leaves the characteristics of the superficial cortical layers available and therefore it is partly necessary to extrapolate from more posterior boundaries. In the rat Ray and Price (1992) divided the frontal pole of the rat into two portions, the medial frontal polar cortex, *Fpm*, and the lateral frontal polar cortex, *FPl*, based on the different aspect of layer II in *Fpm* in comparison with layer II in *FPl* as well as upon a different pattern of connections. Groenewegen (1988) and Uylings and Van Eden (1990) also based the rostral parcellation on connections with the MD, but came to a different rostral parcellation with among others Fr2, ACd and PL, supported by the features of the superficial layers.

### 3. Changes in the parcellations of Paxinos and Franklin (2013)

In the very recent fourth edition of the stereotactic atlas of the mouse brain (Paxinos and Franklin, 2013), delineations of prefrontal cortical areas (Fig. 1 of this chapter) differ from delineations in the third edition (visible in Figs. 1-11 of chapter 4). As indicated in the introduction of the fourth edition of their atlas, in order to facilitate the comparison of different

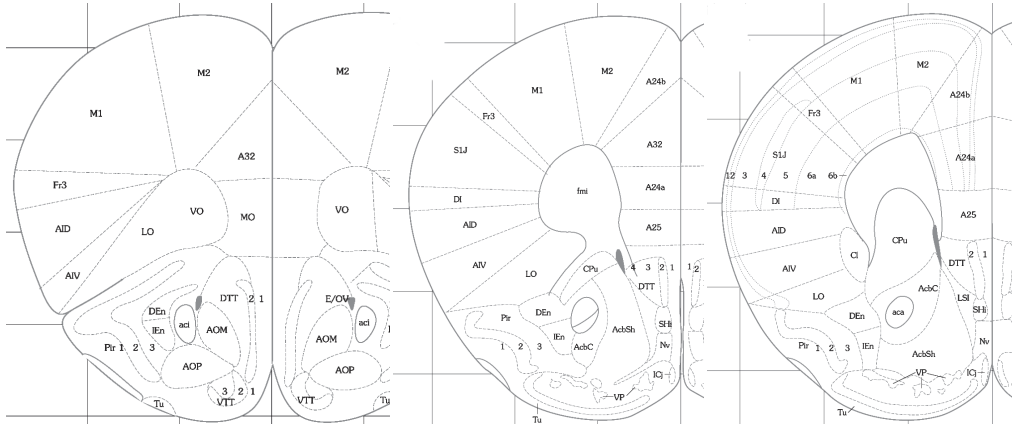
R1 species, the authors adapted the nomenclature of the cingulate areas  
R2 and the prelimbic and infralimbic areas to the Brodmann nomenclature  
R3 used in the non-human and human primate (Paxinos and Franklin, 2013).  
R4 The names of other prefrontal cortical areas have remained the same in  
R5 both editions. The cingulate areas in the fourth edition generally show  
R6 some minor changes in size and position in comparison with their size  
R7 and position in the third edition. As is generally the case in stereotactic  
R8 atlases the specific reasons for these differences are not specified. For  
R9 this new fourth edition, the cingulate areas have been parcellated by B.A.  
R10 Vogt. Therefore, we might assume that Vogt extrapolated his former rat  
R11 parcellations (Vogt and Peters, 1981; Vogt et al., 2004) into the mouse  
R12 brain. In the new scheme at Bregma 2.34 mm in Fig. 1 of this chapter (i.e.,  
R13 Fig 3 in Chapter 4), area *Cg1* is taken together with *PrL* into the new area  
R14 *A32*. In the new scheme at Bregma 2.10 mm, see Fig.1 (i.e. Fig 4 in Chapter  
R15 4), however, the former area *Cg1* is included in *M2*, and no longer in *A32*,  
R16 whereas in the new scheme at Bregma 1.94 mm (i.e. Fig. 5 in Chapter 4)  
R17 *Cg1* is recognized as a separate entity, now called *A24b*. The former areas  
R18 *PrL*, *IL* and *DP* have been replaced in the new scheme at Bregma 1.94 mm by  
R19 areas *A32*, *A24a* and *A25*, respectively, with some change in their mutual  
R20 boundaries. Area *A25* includes the former dorsopeduncular area (*DP*) and  
R21 a ventral part of former Franklin and Paxinos' area *IL*. This can be a source  
R22 of misunderstanding because *A25* will now be easily considered as part of  
R23 the prefrontal cortex, whereas no tracing studies have indicated until now  
R24 that *DP* is a part of PFC. A major difference from the former third edition  
R25 is visible in the parcellation of the medial PFC at new Bregma 1.95 (Fig. 5 in  
R26 chapter 4). In this figure area *A32* is wedged in between the areas *A24b* and  
R27 *A24a*. More posteriorly, above the corpus callosum, at Bregma 1.42 mm,  
R28 areas *A24b* and *A24a* largely conform to former areas *Cg1* and *Cg2* (Fig. 7  
R29 in chapter 4). In a comparison of the parcellations of the prefrontal cortex  
R30 in the two editions of the Franklin and Paxinos atlas (2008 resp. 2013)  
R31 with the Van de Werd et al. (2010) parcellation (chapter 4), less accordance  
R32 is found with the new parcellation in the fourth (2013) edition than with  
R33  
R34

the older parcellation in the third (2008) edition of the atlas. This is best illustrated by comparing the Van de Werd et al. (2010) parcellation (chapter 4, Figs.3-9) with the corresponding new Bregma images presented in Fig.1 of this chapter. The dorsal anterior cingulate area (ACd) and the prelimbic area (PLd–PLv) in the parcellation of Van de Werd et al. (2010) (chapter 4) agree more with the parcellation in the Franklin and Paxinos (2008) edition than with the Franklin and Paxinos (2013) edition. Whether the change in the nomenclature of the cingulate areas and the prelimbic and infralimbic areas in the fourth edition of the Paxinos and Franklin (2013) mouse brain atlas will prove to be really important for the communication between researchers working in the field of the primate brain and those in the field of the rodent brain remains to be seen. The difference in the position of boundaries in the two editions is illustrative of the common problem met in delineating boundaries in cytoarchitectonic studies. As will be discussed in the next paragraph, Lashley and Clark (1946) already called attention to this problem, as was more recently done by Uylings (2007; Botha and Swanson, 2008).



R1  
R2  
R3  
R4  
R5  
R6  
R7  
R8  
R9  
R10  
R11  
R12  
R13  
R14  
R15  
R16  
R17  
R18  
R19  
R20  
R21  
R22  
R23  
R24  
R25  
R26  
R27  
R28  
R29  
R30  
R31  
R32  
R33  
R34

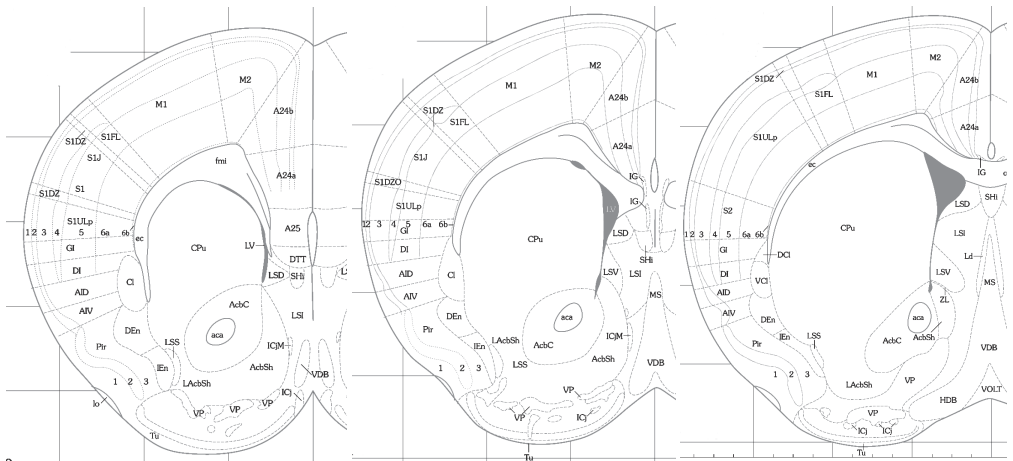
Chapter 5



Bregma 2.10 mm

Bregma 1.94 mm

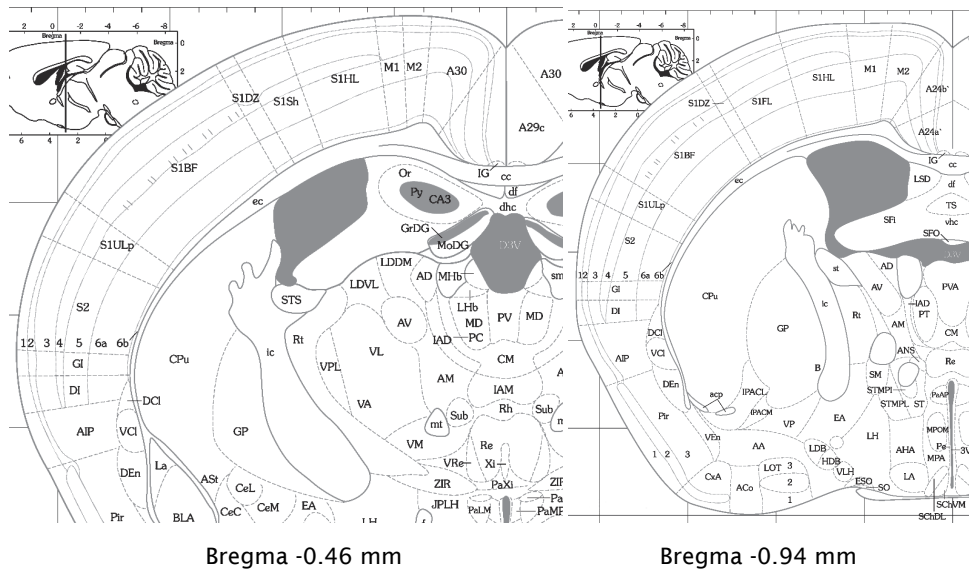
Bregma 1.70 mm



Bregma 1.42 mm

Bregma 1.18 mm

Bregma 0.74 mm



**Fig.1.** Series of the new schemes from the Paxinos and Franklin (2013) mouse brain atlas, "The Mouse Brain in Stereotaxic Coordinates, Academic Press, Elsevier, N.Y., USA" corresponding with the series of images from the Franklin and Paxinos (2008) mouse brain atlas, used in the Van de Werd and Uylings study (chapter 4, submitted). Permission granted by Elsevier.

#### 4. Nomenclature of PFC areas in rodents

In the previous paragraph we have seen that even within the same group of authors the same areas (e.g., cingulate areas) have a different size in successive publications.

Another example of this kind of problem is that dorsally to the corpus callosum the boundary between the dorsal and ventral part of the cingulate cortex is defined more dorsally by one group of authors, and more ventrally by another group of authors. In the studies of Krettek and Price (1977), Groenewegen (1988), Zilles and Wree (1995), Palomero-Gallagher and Zilles (2004), Vogt and Peters (1981), and Vogt et al. (2004), this boundary is more ventral than the one in the studies of Franklin and Paxinos (2008), Paxinos and Franklin (2013), Hof et al. (2000), Wree et al. (1983), Swanson (1992), Paulussen et al. (2011). This difference in the

R1 localization of the dorsal and ventral part of the cingulate areas can be  
R2 explained by the recognition of two different cingulate boundaries in this  
R3 part of the cingulate region: the boundary between area ACd and area ACv,  
R4 and within ACv the boundary between ACvd and ACvv in the parcellation of  
R5 Van de Werd and Uylings (2008) and Van de Werd et al. (2010).

R6 These are two examples of one kind of problem (i.e., boundaries are  
R7 different) met when studies on cortical areas are compared. Another kind of  
R8 problem in comparing parcellations of the brain, even in the same species,  
R9 is that ‘the same terms are used for different areas and/or different terms  
R10 are used for same areas’ (Uylings, 2007; Botha and Swanson, 2008).  
R11 An example of this kind of problem is area Alv. The ventral agranular  
R12 insular area (Alv) is in some studies restricted to the dorsal bank of the  
R13 rhinal fissure and/or the dorsal lip of the rhinal fissure (Groenewegen,  
R14 1988; Berendse and Groenewegen, 1991; Swanson, 1992; Krettek and  
R15 Price, 1977), while other studies have located Alv on the lateral side of  
R16 the frontal lobe ventrally from area Ald (Zilles and Wree, 1995; Palomero-  
R17 Callagher and Zilles, 2004; Paulussen et al., 2011). As a consequence, the  
R18 type of area, indicated by the term Alv, is quite different for both groups  
R19 of authors and as a result communication can be confusing.

R20 Furthermore, the nomenclature on the ventral/orbital side of the frontal  
R21 lobe in rat and mouse appears to be based upon historical grounds instead  
R22 of the real position of an area. For instance, the ventrolateral orbital area  
R23 is not located in the lateral half of the ventral side, as the name suggests,  
R24 but in the medial half of the ventral side of the frontal lobe. It remains to  
R25 be seen if changing the name into a term that is more in agreement with  
R26 its location will improve communication about such an area. In the new  
R27 edition of the Mouse Brain Atlas in stereotactic coordinates, Paxinos and  
R28 Franklin (2013) have decided to replace the nomenclature for areas of  
R29 the cingulate region of the mouse by the Brodmann (1909) nomenclature,  
R30 which was introduced by Brodmann in human and primate cortex and  
R31 applied by Vogt and Peters, (1981) for the rat cingulate areas. In their  
R32 study on the ‘cingulate cortex and disease models’ Vogt et al. (2004)

advocate this change on account of the great relevance of the rat cingulate region in serving as model for human diseases such as schizophrenia, Alzheimer's disease, and chronic pain. They expect that use of the rat version of the Brodmann (1909) nomenclature (Vogt and Peters, 1981) will be of advantage in comparing the effects of changes in the cingulate region in human disease with changes in the rat model of such a disease. The use of Brodmann (1909) nomenclature in rodents, however, runs the risk of suggesting identification of human brain areas as being homologous with rodent brain areas, while rat cortices are not exactly identical to primate cortices (Uylings, 2007; Uylings et al, 2003).

The great variability in cortical boundaries specified in a review of then existing literature brought Lashley and Clark (1946) to a quite pessimistic view on cytoarchitectonical studies. After this publication, cytoarchitectonic studies were considered to be superfluous and misleading for a long period. Zeki (2005) has reviewed the history of the changing appreciation of cytoarchitectonic descriptions during the period 1905 - 2005. Among other things, he pointed out that efforts to combine cytoarchitecture with function have been restored to favor, due to the convincing support for the relation between cytoarchitecture and function coming from highly developed computerized procedures of data processing and from functional brain recording and scanning. Still, the pitfalls in delineating boundaries, expressed in the study of Lashley and Clark (1946) stress the necessity of careful investigations into what characteristics can define a boundary most reliably and reproducibly. In addition, especially when a particular term is used for different regions, it is necessary to specify the criteria by which boundaries are defined.

##### *5. Future developments*

Today, especially for the mouse brain, very detailed web-based anatomical information is available. Recent advances in tracing technics, in visualizing cell-specific and genome-wide properties, in automated



R1 collecting and processing of huge amounts of data, and making them  
R2 accessible for interactive communication, are revolutionizing the research  
R3 on cytoarchitecture and connections of mouse and rat brain. Hintiryan  
R4 et al. (2012) describe the results of exploring the connectivity of the  
R5 olfactory bulb with other parts of the olfactory region by the use of two  
R6 non-overlapping co-injections, each with a mixture of an anterograde  
R7 and retrograde fluorescent tracer. In this manner reciprocal connections  
R8 are viewed easily and clearly distinguished from one-way connections.  
R9 The study is part of the Mouse Connectome Project at UCLA, which aims  
R10 to construct a very detailed connectivity atlas for the whole C57BL/  
R11 J6 mouse brain. Images are available for interactive use through the  
R12 online interactive iConnectome in [www.mouseconnectome.org](http://www.mouseconnectome.org). Chung  
R13 et al. (2011) developed the “Knife-Edge Scanning Microscope Brain Atlas”  
R14 (KESMBA), a web-based neuroinformatics platform for the visualization of  
R15 multi-scale, high-resolution images of connective patterns and cells. The  
R16 scale ranges from the level of the whole brain to the sub-micrometer level  
R17 without loss of resolution. The images can be customized to the demands  
R18 of the user by e.g. changing the number of sheets the image is built on,  
R19 or placing empty sheets between image sheets to enable the choice of  
R20 thickness of the image and the depth view. Although the images are very  
R21 sharp and detailed in showing axons as well as cells, the authors admit  
R22 that it is not yet possible to give an exact assessment of the connectivity  
R23 in local circuits and that on global scale only ‘certain fiber tracts show up  
R24 prominently in the KESM Golgi data’. The fact that the Golgi stain shows  
R25 only about 1% of cells has not been found to influence the information  
R26 negatively. The high resolution of the images enables the distinction of  
R27 different cell-types and patterns of dendrites and axon bundles. The Allen  
R28 brain atlas (<http://www.brain-map.org>) provides a genome-wide 3D gene  
R29 expression atlas (Markham, 2007; Lein et al., 2007), showing the regions  
R30 of activity for each of the genes that are active in the mouse brain and  
R31 also showing the degree of activity by means of different colors. In situ  
R32 hybridization is used to distinguish those cells in which a particular gene  
R33  
R34

is active. On a global level the atlas enables the visualization of the density of cells expressing a particular gene in the different regions of the brain as well as, for some genes, their specific location in the cell. Thus e.g., genes involved in synaptic plasticity are found localized in dendrites (Lein et al., 2007). Nissl-stained sections, however, are still needed to link the locations of gene expression with the general anatomy of the mouse brain. The information yielded by the Allen Brain Atlas is likely to advance the knowledge of the relation between structure and function in the brain. In the study of Chung et al. (2011), the existence of various other web-sites in the field of the mouse brain architecture are mentioned: The Mouse Atlas Project (MAP); The Mouse Brain Library (MBL); BrainMaps.org; Whole-Brain Catalog (WBC). The impressive increase in the available tools in brain research, bringing together specialists from many different, highly specialized fields of science will perhaps fulfill the expectation, found in a textbook of neurophysiology (Somjen, 1983) that 'in the future disciplines such as psychiatry and neurology, psychology and neurophysiology will all become a single science'.

R1  
R2  
R3  
R4  
R5  
R6  
R7  
R8  
R9  
R10  
R11  
R12  
R13  
R14  
R15  
R16  
R17  
R18  
R19  
R20  
R21  
R22  
R23  
R24  
R25  
R26  
R27  
R28  
R29  
R30  
R31  
R32  
R33  
R34