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Radial derivatives of the mouse ventral pallium traced with *Dbx1*-LacZ reporters

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ABSTRACT

The progeny of *Dbx1*-expressing progenitors was studied in the developing mouse pallium, using two transgenic mouse lines: (1) $Dbx1^{nlslacZ}$ mice, in which the gene of the β-galactosidase reporter (LacZ) is inserted directly under the control of the *Dbx1* promoter, allowing short-term lineage tracing of *Dbx1*-derived cells; and (2) $Dbx1^{CRE}$ mice crossed with a Cre-dependent reporter strain ($ROSA26^{loxP\text{-}stop\text{-}loxP\text{-}LacZ}$), in which the *Dbx1*-derived cells result permanently labeled (Bielle et al., 2005). We thus examined in detail the derivatives of the postulated longitudinal ventral pallium (VPall) sector, which has been defined among other features by its selective ventricular zone expression of *Dbx1* (the recent ascription by Puelles, 2014 of the whole olfactory cortex primordium to the VPall was tested). Earlier notions about a gradiental caudorostral reduction of *Dbx1* signal were corroborated, so that virtually no signal was found at the olfactory bulb and the anterior olfactory area. The piriform cortex was increasingly labeled caudalwards. The only endopiriform grisea labeled were the ventral endopiriform nucleus and the bed nucleus of the external capsule. Anterior and basolateral parts of the whole pallial amygdala also were densely marked, in contrast to the negative posterior parts of these pallial amygdalar nuclei (leaving apart medial amygdalar parts ascribed to subpallial or extratelencephalic sources of *Dbx1*-derived GABAergic and non-GABAergic neurons). Alternative tentative interpretations are discussed to explain the partial labeling obtained of both olfactory and amygdaloid structures. This includes the hypothesis of an as yet undefined part of the pallium, potentially responsible for the posterior amygdala, or the hypothesis that the VPall may not be wholly characterized by *Dbx1* expression (this gene not being necessary for VPall molecular distinctness and histogenetic potency), which would leave a dorsal *Dbx1*-negative VPall subdomain of variable size that might contribute partially to olfactory and posterior amygdalar structures.

1. Introduction

The ventral pallium (VPall) was first defined as a primary histogenetic subdivision of the mouse and chicken pallial telencephalon by Puelles et al. (1999, 2000), in their original proposal of a tetrapartite pallium model (MPall, DPall, LPall, VPall). The VPall domain lies next to the striatal subpallium and was first distinguished molecularly from the neighboring lateral pallium domain (LPall) on the basis of its minimal expression of *Emx1*, a marker otherwise widespread in the other parts of the pallium (see also Smith-Fernández et al., 1998; Gorski et al., 2002). The VPall division was later further characterized during early embryonic development by its distinct expression of *Dbx1* in the ventricular zone (Yun et al., 2001; Medina et al., 2004) and *Lhx9* in the mantle

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(García-López et al., 2008; Abellán et al., 2009). Comparative neuroanatomical studies had used previously a simpler tripartite model of the pallium, in which VPall and LPall were lumped as 'lateral pallium', supposedly forming an olfactory pallial region that included the olfactory cortical areas and the claustroamygdaloid complex held to lie deep to them (reviewed by Striedter, 1997). Consistently with the new concept of molecularly distinct VPall and LPall sectors within the earlier olfactory 'lateral pallium' region, Puelles et al. (1999, 2000) and Medina et al. (2004) suggested that both divisions might contribute radially migrated cells to adjacent subsets of olfactory, claustral and amygdalar formations, in line with the classic postulates of Holmgren (1925). The cortical areas ascribed to the VPall included the whole olfactory bulb, plus the ventromedial part of the anterior olfactory area and the ventromedial prepiriform/piriform cortex (covered by the lateral olfactory tract), plus the anterior and posteromedial amygdaloid corticoid formations (Medina et al., 2004). The VPall also was thought to produce some deep (so-called hypopallial) nuclear formations, including a ventromedial part of the claustrum-endopiriform nuclear complex and the lateral/basomedial parts of the pallial amygdala (Medina et al., 2004). In contrast, the dorsolateral anterior olfactory area, the dorsolateral prepiriform/piriform cortex, plus the larger dorsolateral part of the claustrum/endopiriform complex, jointly with the basolateral amygdaloid nucleus and the posterolateral cortical part of the amygdala, were ascribed to the LPall (Puelles et al., 1999, 2000, 2007; Puelles, 2001; Medina et al., 2004). This classification was employed in later studies in mouse studying *Lhx9* expression in the VPall mantle (García-López et al., 2008; Abellán et al., 2009), and was used as well in various other reports (e.g., Hirata et al., 2002, 2009; Guirado and Dávila, 2002; Legaz et al., 2005b; Martínez-García et al., 2007, 2012). The proposal of a distinct VPall also received support from data on genetic fate mapping of the Dbx1-positive progenitors (found in the VPall but not in other pallial sectors) using Cre/loxP technology (Bielle et al., 2005; Hirata et al., 2009; Teissier et al., 2010; Waclaw et al., 2010). Nevertheless, such Dbx1 fate mapping data also raised concerns on the description of VPall derivatives, since ventropallial Dbx1-lineage cells appeared to occupy most of the basal amygdalar complex (Waclaw et al., 2010), and not only part of it, as suggested previously (Medina et al., 2004).

Apart of the VPall and LPall, the tetrapartite pallial model of Puelles et al. (1999, 2000) also contained the dorsal pallium (DPall; the isocortex) and the medial pallium (MPall; hippocampal and perihippocampal allocortex), which essentially conformed to earlier conventions (as in the tripartite pallial model; review in Striedter, 1997). Due to corroborating evidence obtained by mappings of pallial *Emx1* expression in a diversity of gnathostome species, the new tetrapartite pallial model –and, accordingly, the new VPall sector- found wide acceptance in recent developmental and comparative neuroanatomical literature (e.g., Smith-Fernández et al., 1998; Pombal and Puelles, 1999; Puelles et al., 1999, 2000, 2007; Redies et al., 2001; Puelles, 2001; Bachy et al., 2002; Brox et al., 2003, 2004; Wullimann and Rink, 2002; Lindsay et al., 2005; Bielle et al., 2005; Medina et al., 2004, 2005; Moreno and González, 2006; Bardet et al., 2008; Causeret et al., 2011; De Carlos et al., 1996; Fan et al.,1996; Ferreiro-Galve et al., 2008; Tole et al., 2005; Remedios et al., 2007; Abellán et al., 2009; Medina and Abellán, 2009; Nomura et al., 2009; García-Calero and Puelles, 2009; Kerwin et al., 2010; Teissier et al., 2010), as well as in various book chapters and reviews (Martínez-García et al., 2007, 2012; Aboitiz et al., 2002; Aboitiz and Montiel, 2007; Aboitiz and Zamorano, 2013).

However, recent expression studies on the developing mouse and chicken claustrum in which the *Nr4a2* marker gene was mapped (Puelles, 2014; Puelles et al., 2015) have suggested a modification of the earlier tetrapartite model: the claustrum, which is rather selectively identified by this marker, is not divided into ventropallial and lateropallial parts, and emerges as an exclusively lateropallial derivative formed deep to the insular cortex (rather than deep to the olfactory cortex, as was assumed previously). If the old tripartite pallium model is used, the new data imply (against Holmgren, 1925) that the claustrum develops outside of the olfactory pallium, and would be associated to the insula within the classic dorsal pallium. However, since the VPall is still evident, and is supported by the Dbx1 genetic fate mapping results mentioned above, there is advantage in reformulating the tetrapartite model (see Puelles, 2014), so that the newly defined LPall contains exclusively the claustrum and the insular cortex (specifically its agranular/dysgranular portion; Palomero-Gallagher and Zilles, 2004).

An 'insular claustrum' concept had been initially conjectured by most pioneering investigators of the mammalian cortex, such as Meynert (1868, 1872a,b) and Brodmann (1909). but had fallen into disuse subsequently, due to what now turns out to be misguided embryologic analysis (de Vries, 1910; Landau, 1923; Holmgren, 1925; Kuhlenbeck, 1927, 1973, 1977; see Puelles, 2014 for details). The newly reformulated nucleo-cortical complex formed by the mammalian claustrum (an early generated nuclear mass, initially present at the brain surface) and the insula (which secondarily adopts a superficial position via radial migration across the pre-existent claustrum), redefines the LPall concept relative to the original definition cited above (Puelles et al., 1999, 2000; Medina et al., 2004). This implies that the agranular insula falls out of the DPall and enters the LPall, and the whole olfactory cortex results ascribed to the VPall. There is no part of the claustrum proper that originates within the VPall. However, there apparently exist migrated claustral populations that secondarily invade tangentially the VPall and settle down deep to the olfactory cortex, forming the dorsal endopiriform nucleus (Puelles, 2014). Another novel aspect in the recently modified pallial model is that the reformulated LPall ends caudally at mid-telencephalic levels, as does the insula; the perirhinal cortex may be considered to represent the caudalmost part of the insular cortex, as it covers the caudalmost claustrum cells (Puelles, 2014). This implies that the reformulated LPall does not contribute to the amygdalar pallial nuclear complex. As a consequence, the earlier argument that adduced the presence of *Emx1* expression at the basolateral amygdaloid nucleus as an index of its lateropallial nature (Puelles et al., 2000; Gorski et al., 2002; Medina et al., 2004) results weakened in the new scenario, and an alternative explanation seems required for this genoarchitectonic peculiarity (no tangential migration has been observed so far that might account for these data).

As a result of these changes in the LPall, the VPall is now conjectured to encompass among its radial derivatives the olfactory bulb and the *whole* anterior olfactory area, the *whole* prepiriform and piriform olfactory cortex, and *most* of the pallial amygdala (Waclaw et al., 2010; Puelles, 2014; we will discuss below in the light of present data the possibility that additional regions, such as a part of the entorhinal cortex, may also be VPall derivatives). As regards the hypopallial deep nuclei, the larger part of the endopiriform population, forming the conventional *dorsal endopiriform nucleus* (EPd; present both rostrally and caudally, deep to the olfactory cortex) consists of lateropallial cells apparently migrated tangentially from the claustral hypopallium into the VPall, and only the smaller *ventral endopiriform nucleus*, plus a newly defined *bed nucleus of the external capsule*, are held to be radial ventropallial derivatives, leaving aside the pallial amygdala (EPv, BEC; Puelles, 2014). The BEC corresponds in topography to the *reservoir* of Bayer and Altman (1991) and Altman and Bayer (1995), but seems a definitive structure, rather than a transient cell aggregate as these authors conjectured. These rather