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Birthdate of parvalbumin-neurons in the Parvafox-nucleus of the lateral hypothalamus

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The Parvafox-nucleus in the lateral hypothalamus is characterized by the presence of two distinct neural populations, the Parvalbumin (Parv) and the Foxb1-expressing ones. Foxb1-neurons are born at day 10 in the subventricular zone of the mouse mammillary region. It would be interesting to know if the subpopulation of Parv- neurons develop independently at different times and then meet the Foxb1- expressing neurons in the lateral hypothalamus, their final settling place. The aim of this study was to define the period of birth of the Parv-positive neurons using an in-vivo Bromodeoxyuridine-based method in rats. Parv-neurons are generated from embryonic day 10 to day 13, with a peak at day 12. Thus, it appears that the birthdates of the two subpopulations in these two species is similar, perhaps suggesting that they are born from the same neuroepithelial region.

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1. Introduction

The hypothalamus is known to play a pivotal role in the regulation of vital functions (Dearden and Ozanne, 2015; MacKay and Abizaid, 2014).

The Parvafox-nucleus is a cluster composed of two neuronal populations, lodged within the ventrolateral hypothalamus between the fornix and the optic tract, with axially-located Parvalbumin (Parv)-positive neurons surrounded by a cuff of Foxb1-expressing ones (Bilella et al., 2014; Celio, 1990). In mice, the Foxb1-expressing neurons are generated in the periventricular zone at embryonic day 10 and migrate tangentially towards the ventrolateral hypothalamus between the embryonic day 13 and 14 (Alvarez-Bolado et al., 2000). Since 10% of the Parv-positive cells also express *Foxb1* (Bilella et al., 2014), they probably derive from the same source during the same period of time. The period of birth and the provenance of the remaining 90% Parv-neurons are unknown. In this study, we used an in vivo Bromodeoxyuridine (BrdU) method in order to define the temporal pattern of genesis of the Parv-expressing neurons in Wistar rats. The study revealed that the date of birth of these neurons was between embryonic day 10 and embryonic day 13 with a peak at embryonic day 12, similar to that of the Foxb1-expressing neurons in mice (Alvarez-Bolado et al., 2000; Workman et al., 2013).

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2. Results

Horizontal sections through the Parvafox-nucleus of P18 rats obtained from pregnant females injected on E6 to E14 were analyzed by immunostaining for the presence of double labeled Parv/BrdU cells.

BrdU positive nuclei were found on all examined sections in different hypothalamic areas at all gestational days analyzed, but double labeled Parv/BrdU neurons in the Parvafox-nucleus were observed only from E10 until E13 (Fig. 1). Only few double positive cells started to appear at embryonic day 10 (E10, Fig. 1A). The number of the double stained nerve cells in the Parvafox nucleus increases at E11 (Fig. 1B). A peak of double-labeled neurons was observed at E12 (E12, Fig. 1C). The number of neurons generated at E13 was again reduced (Fig. 1D), and almost none were observed at E14 (not shown).

3. Discussion

Foxb1 is a winged helix transcription factor involved in the development of the diencephalon during the embryogenesis and to a lesser extent during the postnatal development (Altman and Bayer, 1978; Alvarez-Bolado et al., 2000; Croizier et al., 2014; Iball et al., 1966; Markakis and Swanson, 1997; Szabo et al., 2015). *Foxb1* is expressed in a "patch" of the caudal hypothalamic neuroepithelium that gives rise to neurons later forming defined hypothalamic nuclei. The *Foxb1* pattern of expression evolves with time with widespread expression in early stages of development and very restricted one in the adult. Therefore, it is possible to make a distinction between E9/E10, (Alvarez-Bolado et al., 2000), when *Foxb1* expressing neurons are born (Zhao et al., 2008) and are confined to the posterior hypothalamus and E13/14 when *Foxb1*-expressing cells leave the mammillary region and migrate in rostral direction. They reach the tuberal region of the lateral hypothalamus, giving rise to the *Foxb1*-expressing neurons belonging to the Parvafox-nucleus observed postnatally (Bilella et al., 2014). Of the total number of neurons of the Parvafox-nucleus, 10% manifest double staining for both *Foxb1* and Parv- (Bilella et al., 2014), thereby implying that these cells stem from the caudal periventricular zone (Alvarez-Bolado et al., 2000). Our study indicates that in rats the Parv-positive cells are born first around E10, their number peaks at E12 and decreases at E13, confirming what was already observed for the *Foxb1*-neurons in mice (Alvarez-Bolado et al., 2000; Szabó et al., 2015; Workman et al., 2013). This is a time-interval in which the majority of the lateral hypothalamic nerve cells are born (Croizier et al., 2014). Indeed, the melanin-concentrating hormone (MCH) producing neurons, which form an abundant cell population widespread in the diencephalon and also present in the lateral hypothalamus show a similar spatio-temporal ontogeny also peaking between E12 and E13 (although the MCH-expressing neurons are generated throughout the entire time-period of genesis of the whole hypothalamus, i.e. E10-E17) (Altman and Bayer, 1978; Altman and Bayer, 1986; Amiot et al., 2005; Brischoux et al., 2001; Khachaturian et al., 1985). The hypocretin/orexin positive neurons in the lateral hypothalamus, which are involved in the regulation of sleep/waking states (Chemelli et al., 1999; Nishino et al., 2000; Peyron et al., 2000; Thannickal et al., 2000) and feeding behavior (Qu et al., 1996; Rossi et al., 1997), display also a similar ontogenic pattern. These neurons are generated between the E11 and E14 with a peak at E12 (Amiot et al., 2005). They start to express orexin around E18 and complete the development during post-natal stages (Steininger et al., 2004; Van Den Pol et al., 2001).

The Parvafox-nucleus is located in the most lateral portion of the hypothalamus. According to the classical view of hypothalamic ontogeny, the lateral part is laid down first (Altman and Bayer, 1986) and therefore would be expected to show very early birthdates. However, the birthdate of the two neural populations of the Parvafox coincides with the ontogeny of lateral hypothalamic neurons situated at all mediolateral levels. This is in agreement with previous studies (Alvarez-Bolado et al., 2012) showing that the postulated "three waves" of hypothalamic neurogenesis (Altman and Bayer, 1986) can be considered more a useful model than a consistent description of all hypothalamic neurons.

4. Experimental procedures

4.1 *Animals*

Male and female Wistar rats, were used in the present study. The animals were maintained on a 12-hour light/12-hour dark cycle at a constant temperature of 24 °C and fed *ad libitum*. The study was approved by the Fribourg Cantonal Committee for Animal Experimentation.

The time of conception was documented by sperm-positive vaginal smear examined on the morning following the mating night (Embryonic day 0, E0) (Brischoux et al., 2001).

4.2 *Bromodeoxyuridine injections*

BrdU is a thymidine analog that is incorporated into the DNA as 5-bromouracil during the S phase of the cell cycle of any cells, and is widely used in vivo as a mitotic marker (Iball et al., 1966). The BrdU method used in this study was similar to that already described for the MCH neurons in the hypothalamus (Brischoux et al., 2001).

Nine pregnant animals, from p.c. gestational day (E) 6, 7, 8, 9, 10, 11, 12, 13 and 14 (E6-E14) were given an i.p. injection of 5-bromo-2'-deoxyuridine (BrdU; Roche, Germany) 160 mg/Kg body weight dissolved in 0.9% sterile NaCl, warmed to 60 °C for complete solubilization and filtered before injection (Brischoux et al., 2001; Markakis and Swanson, 1997).

4.3 *Immunohistochemistry*

After being anaesthetized at postnatal day (P) 18 (Pentobarbital; 20 µl/10 g per body weight), two rats/injection time-point (= total of 18 rats), constituting the progeny of the BrdU injected mothers, were perfused first with physiological (0.9%) saline and then with chilled 4% paraformaldehyde (4 °C) dilute in phosphate (0.1 M, pH7.3). The brains were removed, immersed overnight in the same chemical fixative, and then transferred to a chilled (4 °C) 30% solution of

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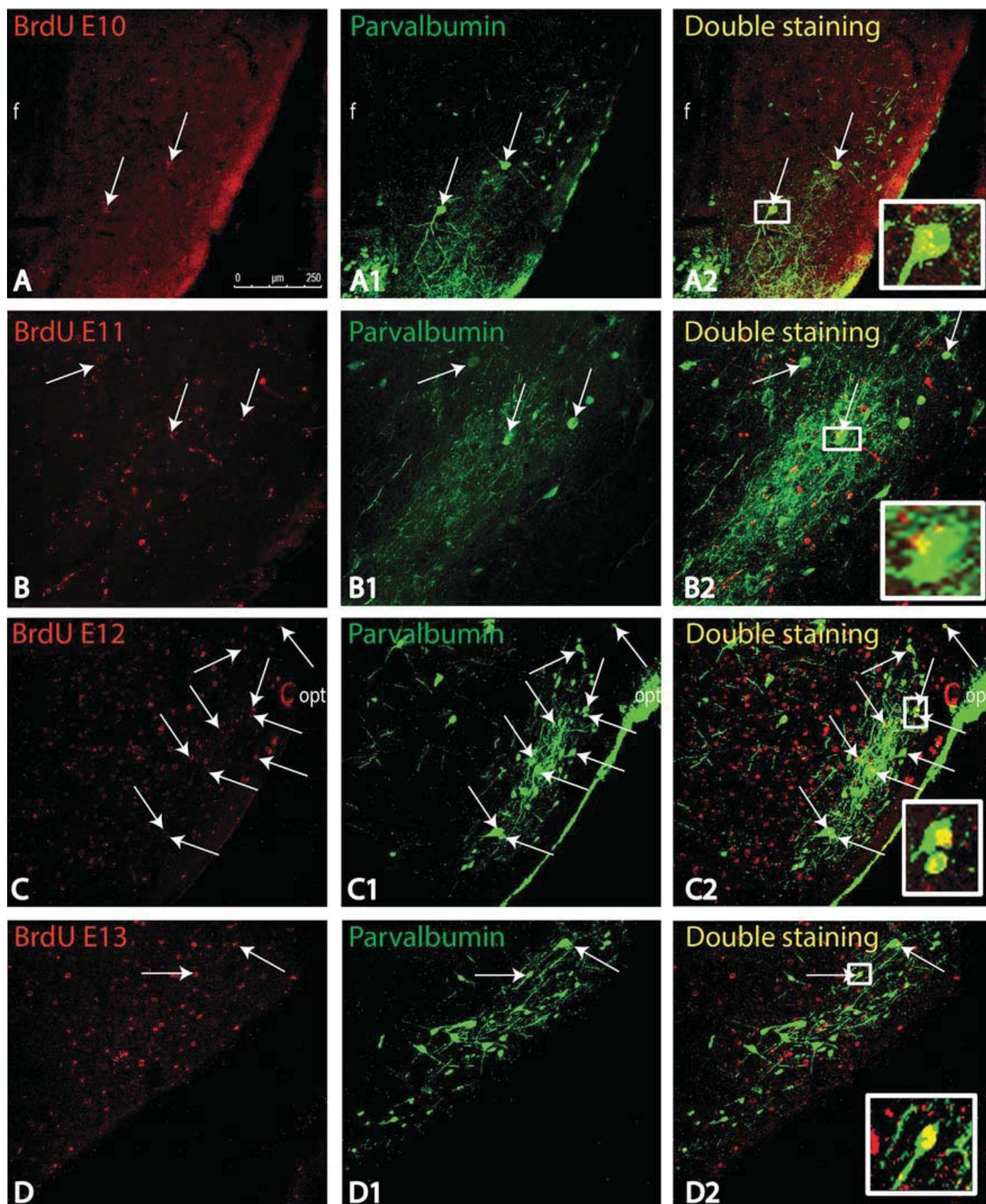

Fig. 1 – Images of horizontal sections of the Parvafox-nucleus of progenies from pregnant females injected on embryonic day 10 (E10) to embryonic day 13 (E13), stained for BrdU and Parv. The insets show at higher magnification the results of merging the Parv and BrdU staining; a single neuron, for each embryonic day is represented. Panels A, B, C, D show BrdU-staining (red); panels A1, B1, C1, D1 show the Parv-staining (green), and panels A2, B2, C2, D2 show the merged images. White arrows mark double labeled BrdU/Parv cells. A, A1, A2: BrdU-injection at E10. Only few cells are immunopositive for both BrdU and Parv (A2), while the Parvafox nucleus already contains numerous Parv-postive BrdU-negative cells (A1). B, B1, B2: BrdU injection at E11. BrdU staining is more widespread through the lateral hypothalamus compared to E10 (A). Nevertheless, only few Parv-positive cells in the Parvafox are also BrdU-immunoreactive (B2). C, C1, C2: BrdU injection at E12. BrdU labeled cells are visible throughout the ventral hypothalamus. Most of the Parv-positive neurons of the Parvafox nucleus show BrdU immunoreactivity. D, D1, D2: BrdU injection at E13. E13 marks the cessation of the generation of Parv-positive cells, as only few double labeled BrdU/Parv are visible.

phosphate (0.1 M) – buffered sucrose, for cryoprotection. Horizontal, 40 μm-thick cryo-sections were prepared using a freezing microtome (Frigomobil Reichert-Jung, Vienna, Austria) and collected in 0.1 M phosphate buffer (pH 7.3). Free-floating sections were treated for 1 day at room temperature with a monoclonal antibody against parvalbumin (PV 235 (Swant, Marly, Switzerland) diluted 1:1'000 in TBS+10% bovine serum) and then with an anti-mouse Alexa 488 antibody (Jackson ImmunoResearch, Laboratories, INC, USA) diluted 1:500 in Tris–HCl (pH 8.2). Cell nuclei were revealed by counterstaining with DAPI (diluted 1:2'000 in phosphate-buffered saline) for 5 minutes at room temperature. Afterwards, the sections were mounted on glass slides, treated for 30 min at 37 °C in 2NHCl followed by rinsing in 0.1 M borate buffer pH8.5, and then exposed for 1 day at room temperature (RT) to a rat anti-BrdU antiserum (Abcam, Cambridge Science Park Cambridge CB4 0FL UK, diluted 1:200 in TBS+0.2% Triton-X 100+10% bovine serum). The sections were rinsed in TBS and then incubated for 2 hours at room temperature with biotinylated goat anti-rat antibody (Vector Laboratories, Burlingame, CA, USA, diluted 1:200 in TBS+10% bovine serum). After rinsing once in TBS and twice in Tris–HCl (pH 8.2), the sections were exposed for 2 hours to Cy3-conjugated streptavidin (Jackson ImmunoResearch, Laboratories, INC, USA) diluted 1:200 in Tris–HCl (pH 8.2).

The sections were evaluated with either a Leica 6'000 epifluorescence microscope (equipped with a Hamamatsu C4742-95 camera), a digital slide scanner (Nanozoomer, Hamamatsu) or a Leica TCS SP5 confocal laser microscope.

Images were post-processed for brightness and contrast using Adobe Photoshop CS5. The image stacks were prepared with ImageJ 1.44p software and the figures collated using the Adobe Illustrator CS6.

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REFERENCES

Altman, J., Bayer, S.A., 1978. Development of the diencephalon in the rat. II. Correlation of the embryonic development of the hypothalamus with the time of origin of its neurons. J. Comp. Neurol. 182, 973–993.

Altman, J., Bayer, S.A., 1986. The development of the rat hypothalamus. Adv. Anat. Embryol. Cell. Biol. 100, 1–178.

Alvarez-Bolado, G., et al., 2000. Expression of Foxb1 reveals two strategies for the formation of nuclei in the developing ventral diencephalon. Dev. Neurosci. 22, 197–206.

Alvarez-Bolado, G., Paul, F.A., Blaess, S., 2012. Sonic hedgehog lineage in the mouse hypothalamus: from progenitor domains to hypothalamic regions. Neural Dev. 7, 4.

Amiot, C., et al., 2005. Hypocretin/orexin-containing neurons are produced in one sharp peak in the developing ventral diencephalon. Eur. J. Neurosci. 22, 531–534.

Bilella, A., Alvarez-Bolado, G., Celio, M.R., 2014. Coaxiality of Foxb1- and parvalbumin-expressing neurons in the lateral hypothalamic PV1-nucleus. Neurosci. Lett. 566, 111–114.

Brischoux, F., Fellmann, D., Risold, P.Y., 2001. Ontogenetic development of the diencephalic MCH neurons: a hypothalamic'MCH area' hypothesis. Eur. J. Neurosci. 13, 1733–1744.

Celio, M.R., 1990. Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience. 35, 375–475.

Chemelli, R.M., et al., 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 98, 437–451.

Croizier, S., et al., 2014. Characterization of a mammalian prosencephalic functional plan. Front. Neuroanat. 8, 161.

Dearden, L., Ozanne, S.E., 2015. Early life origins of metabolic disease: developmental programming of hypothalamic pathways controlling energy homeostasis. Front. Neuroendocr..

Iball, J., Morgan, C.H., Wilson, H.R., 1966. Structures of 5-bromodeoxyuridine and 5-bromouridine. Nature 209, 1230–1232.

Khachaturian, H., et al., 1985. Time of origin of opioid peptide-containing neurons in the rat hypothalamus. J. Comp. Neurol. 236, 538–546.

MacKay, H., Abizaid, A., 2014. Embryonic development of the hypothalamic feeding circuitry: transcriptional, nutritional, and hormonal influences. Mol. Metab. 3, 813–822.

Markakis, E.A., Swanson, L.W., 1997. Spatiotemporal patterns of secretomotor neuron generation in the parvicellular neuroendocrine system. Brain Res. Brain Res. Rev. 24, 255–291.

Nishino, S., et al., 2000. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 355, 39–40.

Peyron, C., et al., 2000. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat. Med. 6, 991–997.

Qu, D., et al., 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature 380, 243–247.

Rossi, M., et al., 1997. Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight. Endocrinology 138, 351–355.

Steininger, T.L., et al., 2004. Comparison of hypocretin/orexin and melanin-concentrating hormone neurons and axonal projections in the embryonic and postnatal rat brain. J. Chem. Neuroanat. 27, 165–181.

Szabó, N., et al., 2015. Cadherins mediate sequential roles through a hierarchy of mechanisms in the developing mammillary body. Front. Neuroanat., 9.

Szabo, N.E., et al., 2015. Cadherins mediate sequential roles through a hierarchy of mechanisms in the developing mammillary body. Front. Neuroanat. 9, 29.

Thannickal, T.C., et al., 2000. Reduced number of hypocretin neurons in human narcolepsy. Neuron 27, 469–474.

Van Den Pol, A.N., et al., 2001. Lateral hypothalamus: early developmental expression and response to hypocretin (orexin). J. Comp. Neurol. 433, 349–363.

Workman, A.D., et al., 2013. Modeling transformations of neurodevelopmental sequences across mammalian species. J. Neurosci. 33, 7368–7383.

Zhao, T., et al., 2008. Genetic mapping of Foxb1-cell lineage shows migration from caudal diencephalon to telencephalon and lateral hypothalamus. Eur. J. Neurosci. 28, 1941–1955.