Increasing neuron numbers in the vasopressin and oxytocin containing nucleus of the adult female pig hypothalamus

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Neuron number and volume of the recently described vasopressin and oxytocin containing nucleus (VON) in the pig hypothalamus have been studied into adulthood. Following a 2.5-fold increase in both sexes around puberty, both neuron number and volume of the VON continued to increase in the female pig between 1 and 2.5 years of age. Since no such increase occurred in the male, the VON exhibits a pronounced sexual dimorphism in adulthood of 260%.

In general, neurons are considered to be postmitotic early postnatally [7, 9, 11, 13], especially in species such as man, monkey and pig, which have a relatively mature brain at birth [5, 13]. Recently [19] however, we found a 2.5-fold increase in the neuron count as well as in the volume of a magnocellular hypothalamic cell group in the pig, the vasopressin and oxytocin containing nucleus (VON), around the time of puberty (i.e., between 16 and 30 weeks) in both males and females. This finding was even more surprising since the closely related magnocellular vasopressin and oxytocin neurons of the supraoptic nucleus (SON) and paraventricular nucleus (PVN) occur early in development [3, 7, 9, 11]. The VON as described in the pig may be homologous to structures described in sheep, cow, horse, rabbit, cat and mouse (for an extensive description see Van Eerdenburg et al. [19]), however, cell counts are not available in these species. Because the increase in neuron number occurred much later during development than ever reported, we decided to investigate whether this unexpected process would even continue into adulthood. Therefore pigs of 40, 52 and 130 (= adult) weeks of age were added to the series and neuron counts were performed in the present study.

The head of the animal was perfused under general

anesthesia as previously described [19]. Morphometric analysis was performed on 5–7 Nissl stained (Cresyl violet) 10 μ m paraffin sections per pig selected through systematic sampling; neuron number was estimated on the basis of nucleolar counts [19]. Briefly, this semiautomatic method outlined the nucleus using the distance between the nuclei of magnocellular neurons in the central part of the VON. Within this outline all nucleoli were counted. Analysis of variance was used to compare for sex and age. Homogeneity of variances was evaluated using Bartlett's test. Subsequent multiple comparisons were performed using the Kruskal-Wallis and Mann-Whitney *U* test. A *P* value < 0.05 (2-tailed) was considered to be statistically significant.

In order to show the complex sexually dimorphic pattern of the development of the VON, the earlier [19] and new data have been combined in Figs. 1 and 2. Photomicrographs of the VON at 16 and 130 weeks of age in a male and female are given in Fig. 3.

The number of neurons and the volume of the VON at the age of 52 weeks were not significantly different from those at 30 weeks in males and females. These parameters did not change significantly in males between 30 and 130 weeks of age (i.e., at 40 and 52 weeks). Only a trend (*P* < 0.06) towards lower VON volumes was observed in males. However, a striking finding was that at an age of 130 weeks the VON of females had almost 3 times the number of neurons as in males of the same age (*P* < 0.01). This number was 1.6 times the number

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Fig. 1. Number of neurons (mean + S.E.M.) of the vasopressin and oxytocin containing nucleus (VON) in the pig hypothalamus during postnatal development, from day 1 to 130 weeks (= 2.5 years). *n* is the number of animals in each group. (The S.E.M. in the female group of 0 weeks was too small to be drawn.) Data of 0–30 weeks have been published previously [19].

observed in the VON of females of one year of age ($P < 0.05$) and up to 7 times the number of neurons at the beginning of puberty (viz. 16 weeks, the age at which the VON is at its smallest). The volume of the VON in females at 130 weeks was also more than twice that of the males at the same age ($P < 0.05$), and more than twice the volume of both males and females at the age of 1 year ($P < 0.05$).

The continuing strong increase in neuron number after puberty in female pigs results in a pronounced sexual dimorphism of the VON in young adults. Sexual dimorphism has been reported in the brain of other mammals [4, 6], including man [2, 15, 16] but males generally have larger cell counts than females in sexually dimorphic nuclei. One exception to this is the parastrial nucleus of the rat, in which the volume is larger in females [1]. Up till now, most sex differences in the brain are believed to result from a decrease in the number of neurons in females, leaving the males with more [6, 8, 14, 16]. Since in the VON of the pig, sexual dimorphism does not seem to result from a decrease in cell number but from an increase in the female, sexual differentiation of the VON in the pig takes place in an entirely different, not earlier reported way.

It seems as if in the VON of the pig neurogenesis continues far longer than reported so far in any other mammal [3, 7, 9, 11, 13]. In songbirds seasonal changes in neuron number of the song-controlling nuclei have already been reported [12]. Late neurogenesis is, however, not the only possible explanation for our observations. Alternatives are: (1) migration of neurons from the surrounding structures or, (2) 'activation' of immature neurons in the VON, which in earlier stages of develop-

Fig. 2. Volume (mean + S.E.M.) of the VON in the pig hypothalamus during postnatal development, from day 1 to 130 weeks (= 2.5 years). *n* is the number of animals in each group. Data of 0–30 weeks have been published previously [19].

ment have a glia-like appearance and are thus not recognized as neurons. Until now we did not observe any cells that could be migrating neurons in the area surrounding the VON. We also do not have another indication that there is a massive migration of neurons into the area of the VON around puberty, nor has migration in adulthood of pre- or neonatally formed neurons been reported. We also do not see a larger proportion of small glia-like cells in the VON at early ages than in adulthood. Since neurons are generally easily recognizable already early in development, because of their clear nucleus with nucleolus [17], this explanation is also unlikely. However, future experiments with compounds that can be incorporated into DNA during mitosis could establish definitively whether or not the increased size of the VON is indeed due to neurogenesis.

The function of the VON is still not known, but because of the timing of its neuronal alterations and since these lead to sexual dimorphism, it is presumably involved in reproduction. In songbirds, seasonal neurogenesis is related to the learning of new songs [12]. The continued increase in neuron number in females even after one year of age could be related to the fact that the 2.5 year old females, although these animals were not pregnant at the time of sacrifice, all had delivered and nursed several litters. In contrast, the females of 1 year old had not yet been pregnant. Pigs tend to have fast growing, large litters (15 piglets is no exception), which may be of interest in relation to the oxytocin content of the VON. At this moment no comparable morphometric data are available for the rat, an animal that has large litters too, but it is known that the hypothalamic neurosecretory activity is altered during parturition and lac-

Fig. 3. Photomicrograph of the VON in the pig hypothalamus at 16 weeks of age in a male (A) and a female (B) and at 130 weeks of age in a male (C) and a female (D). Note the increase in neuron number and volume. Bar = 100 μ m.

tation [17, 18]. Further research will be necessary to determine whether indeed parturition and/or lactation are causal factors of the increase in neuron number and volume of the VON in the hypothalamus of the female pig in adulthood.

In conclusion, the present study shows the development of sexual dimorphism in a hypothalamic nucleus that seems to result from an increase in neuron number in adolescence in female pigs, leading to a nucleus that has almost 3 times the number of neurons and is twice as large as that of males of the same age.

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