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NPY and its involvement in axon guidance, neurogenesis, and feeding

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Abstract

Objectives: The role of neuropeptides in nervous system function is still in many cases undefined. In the present study we examined a possible role of the 36–amino acid neuropeptide Y (NPY) with regard to three functions: axon guidance and attraction/repulsion, adult neurogenesis, and control of food intake.

Methods: Growth cones from embryonic dorsal root ganglion neurons were studied in culture during asymmetrical gradient application of NPY. Growth cones were monitored over a 60-min period, and final turning angle and growth rate were recorded. In the second part the NPY Y_1 and Y_2 receptors were studied in the subventricular zone, the rostral migratory stream, and the olfactory bulb in normal mice and mice with genetically deleted NPY Y_1 or Y_2 receptors. In the third part an anorectic mouse was analyzed with immunohistochemistry.

Results: 1) NPY elicited an attractive turning response and an increase in growth rate, effects exerted via the NPY Y_1 receptor. 2) The NPY Y_1 receptor was expressed in neuroblasts in the anterior rostral migratory stream. Mice deficient in the Y_1 or Y_2 receptor had fewer proliferating precursor cells and neuroblasts in the subventricular zone and rostral migratory stream and fewer neurons in the olfactory bulb expressing calbindin, calretinin or tyrosine hydroxylase. 3) In the anorectic mouse markers for microglia were strongly upregulated in the arcuate nucleus and in projection areas of the NPY/agouti gene-related protein arcuate system.

Conclusion: NPY participates in several mechanisms involved in the development of the nervous system and is of importance in the control of food intake.

Keywords: Anorexia; Dorsal root ganglia; Microglia; Neuropeptide; Trophism

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Introduction

Neuropeptide Y (NPY), a 36-amino acid peptide discovered by Tatemoto and collaborators [1,2], has been reported to be involved in a variety of neuronal functions, as presented e.g. in the Proceedings of the 8th International NPY Meeting in 2006 [3] and in a book edited by Zukowska and Feuerstein [4]. In the present report we briefly summarize the findings from our laboratories in three different areas: NPY's role as a trophic and guiding factor, its involvement in neurogenesis, and some new findings possibly related to anorexia.

Growth-promoting and guiding effects of NPY

Studies have shown that peripheral nerve injury induces a dramatic increase in NPY expression in adult dorsal root ganglion (DRG) neurons in the rat [5–8] and mouse [9]. These findings raised the possibility that NPY may be important for the survival and regenerative processes. In fact, White and Mansfield [10] and White [11] demonstrated that NPY promotes growth of DRG neurites in vivo and in vitro.

The observation that NPY is expressed in the ensheathing cells of the peripheral olfactory system in the rat [12] raised the possibility of a trophic action of these peptides in the olfactory system. Olfactory neurons are continuously renewed throughout life [13], and the ensheathing cells tightly surround and support the growing olfactory axons on their way from the olfactory mucosa to the glomeruli in the olfactory bulb [14]. In fact, Hansel et al. [15] demonstrated that NPY induces proliferation of neurons in the olfactory mucosa via the Y_1 receptor (Y1R).

In our laboratory we tested the hypothesis that NPY released from ensheathing cells could also be important for guiding the olfactory axons. For this purpose we crossed NPY-null mice [16] with mice in which a single olfactory receptor is genetically labeled with a Tau-LacZ construct [17]. When comparing the wild type with the resulting crossed mice, there was, disappointingly, no difference with regard to distribution of labeled glomeruli in the olfactory bulb [18]. Therefore, NPY does not seem to be essential for targeting of the olfactory axons to reach their proper termination in the olfactory system. Here, the key function of NPY apparently is the trophic effect shown by Hansel et al. [15].

More recently, we addressed the same question, but now in growth cones of DRGs and with an in vitro technique [19]. This approach is based on the knowledge that, during development, the advance of axons is controlled by molecules that affect the rate and direction of growth (see Tessier-Lavigne and Goodman [20]). Such molecules include nerve growth factor [21,22], brain-derived neurotrophic factor, netrin-1, and slit [23].

Individual growth cones of fetal rat DRG neurons were exposed to asymmetric gradients of NPY (10^{-9} M) and their final turning angles and growth rates were recorded. NPY elicited a strong attractive response (final turning angle of 15.1 ± 5.0 degrees, $P \leq 0.05$) and exerted a significant, but moderate, increase in growth rate (65.4 ± 5.2 μm/h, $P \leq 0.03$). A small-molecule non-peptide Y1R antagonist, H409/22 (at 10^{-7} M) [24–26], had no effect by itself on growth rate. However, when turning assays to NPY were performed in the presence of H409/22, NPY lost its ability to elicit an attractive turning response and an increase in rate of extension (final turning angle from 15.1 ± 5.0 to 3.0 ± 6.8 degrees, $P \leq 0.02$; growth rate from 65.4 ± 5.2 to 35.8 ± 8.4 μm/h, $P \leq 0.002$). The "sister" peptide galanin [27] had a significant effect on growth rate, which was somewhat stronger than that of NPY (85.5 ± 13.1 μm/h, $P \leq 0.008$). However, galanin had no effect on turning angle, that is, it caused neither an attractive nor a repulsive response. The effect on growth rate is in agreement with extensive studies [28–31], in particular by Hobson et al. [31] who showed that galanin promotes neurite outgrowth, an effect exerted via the galanin receptor-2.

Taken together, these findings indicate that NPY (and galanin) at the spinal level not only acts as a transmitter-like molecule but also can exert trophic actions, features similar to classic growth factors. Thus, NPY promotes growth and attracts growth cones of embryonic DRG neurons. In fact, its attractive effect is stronger than those by nerve growth factor or insulin-like growth factor-1, but less pronounced than hepatocyte growth factor [19]. The fact that the Y1R antagonist H409/22 completely blocked attraction and its effect on growth rate strongly suggests that the NPY effects are mediated via the Y1R, whereas the growth-promoting effect of galanin on DRG neurons shown in other studies is mediated via galanin receptor-2 (see above).

An interesting question is, under which circumstances, if any, are the growth and turning properties of NPY of physiological significance. Under normal circumstances NPY is not expressed in DRG neurons, but at embryonic day 16 the NPY-related peptide tyrosine tyrosine [2,32] is transiently expressed in DRGs [33], and this peptide has an affinity for the Y1R [34]. In fact, the Y1R has been detected in trigeminal neurons at embryonic day 16.5 [35], and one could hypothesize that during this window NPY-related peptide tyrosine tyrosine released from primary afferent neurons could influence the turning response and growth rate of growth cones. In contrast to DRG neurons, there is an abundant expression of NPY in the dorsal horn [36] and NPY is already present prenatally, at embryonic day 15 [37]. Thus, NPY released from dorsal horn interneurons could influence how the primary afferents, carrying Y1Rs, are guided and grow, once they have arrived in the superficial dorsal horn.

It has been shown that the Y1R is virtually always expressed in neurons that produce the neuropeptide calcitonin gene-related peptide [38,39], and at least some of these neurons are involved in pain processing. The marked upregulation of NPY in DRG neurons after peripheral nerve injury mainly occurs in large neurons associated with

Aβ/Aδ fibers [5–8], that is, in non-nociceptive neurons. Thus, during regeneration NPY released from these large axons could perhaps promote growth and guide regenerating Y1R/calcitonin gene-related peptide–positive nociceptive neurons. Because peripheral nerve injury may be associated with plasticity changes and wiring in the dorsal horn [40], it is possible that NPY released from dorsal horn interneurons may be involved in such plasticity processes.

It may also be mentioned in this context that, in another project, we studied the outgrowth of embryonic neurons of wild-type mice transplanted into the hypothalamus of adult NPY knockout (KO) mice. We found that NPY-positive fibers innervated normal target areas of the NPY neurons, including the hypothalamic paraventricular nucleus and amygdala. These results show that directional cues for NPY neurons are present in the adult brain, suggesting a possibility of functional remodeling of arcuate NPY projections, which can be relevant to the regulation of feeding [41].

NPY and its receptors in adult neurogenesis

It has been recognized that there is substantial neurogenesis also in adulthood [42–49]. This occurs in particular brain areas, the subgranular zone of the dentate gyrus in the hippocampal formation and the subventricular zone (SVZ), from where neurons migrate via the rostral migratory stream (RMS) to the olfactory bulb. Thus, it is now accepted that adult neurogenesis, long thought to exclusively be a characteristic of the olfactory neurons in the nasal mucosa projecting to the glomeruli of the olfactory bulb [13] (see above), also occurs in other forebrain areas. Many molecules are involved in the neurogenesis in the SVZ and subgranular zone, including NPY, as shown in particular by Howell et al. [50–52]. Thus, NPY is a potent proliferative factor acting via the Y1R for neuroblast and nestin-positive sphere-forming stem cells in hippocampal cultures from early postnatal rats [50]. Moreover, this Y1R-mediated proliferative effect occurs on neuroblasts isolated from the dentate gyrus [51]. Howell et al. [52] also found that NPY is involved in seizure-induced precursor cell proliferation not only in the dentate gyrus but also in the caudal SVZ.

We recently studied a possible role of NPY on neuroblast proliferation, migration and placement in the adult mouse forebrain [53]. A similar topic was addressed at the 9th Annual NPY Meeting by another group that reported that NPY promotes neurogenesis in SVZ cell cultures [54].

Our interest in this topic started with a detailed mapping of the Y_2 receptor (Y2R) in the mouse brain by making the "accidental" observation that Y2R-like immunoreactivity (LI) was present in a thin band close to the SVZ along the medial aspects of the lateral ventricle extending toward the olfactory bulb, which is in the RMS [55]. Y1R-LI was also found in ependymal cells along the third brain ventricle [56].

In a follow-up study [53] we analyzed the expression of Y1R-LI and Y2R-LI in the adult mouse brain by performing double-labeling with the double-cortin (DCx) antibody. Most DCx-immunoreactive cells were present on the striatal side, with lower levels along the septum. Although there was a close relationship between the NPY system markers and DCx, we could not detect any coexistence at caudal levels (Fig. 1A–C). However, in the anterior RMS in the olfactory bulb, DCx-immunoreactive cells were also labeled with the Y1R antibody to a high extent (Fig. 1D–L), suggesting a possible involvement of the Y1R in migration. NPY, Y1R, and Y2R did not coexist with glial fibrillary acidic protein (Fig. 1M–O). The extent to which the Y1R is present in DCx cells also at more caudal levels, but below the detection level of our methodology, remains to be analyzed. Nevertheless, the finding suggests increasing Y1R levels in neuroblasts in the rostral direction.

We then turned to an analysis of mice with one of the receptors deleted, namely Y1R and Y2R KO mice [57]. These adult mice were analyzed for immunohistochemical expression of DCx and Ki67, the latter a marker for proliferating cells in the adult brain [58]. There was a marked reduction in the SVZ of Y1R and Y2R KO mice (Fig. 2A–C) and a decrease in DCx-labeled neuroblasts migrating through the SVZ and RMS (Fig. 2D–F). In fact, neuroblast assembly appeared disrupted in the Y1R KO mice, in contrast to the more chain-like appearance in the wild-type mouse.

In the olfactory bulb there was a reduction in several of the interneuron subtypes originating from the migrating RMS neuroblasts (Fig. 2G–I). Thus, fewer calbindin-, calretinin-, and tyrosine hydroxylase-immunoreactive neurons were seen in the glomerular layer of the olfactory bulb, and in the granular layer there was a reduction of calretinin-immunoreactive cells. Whereas the effect on Ki67-immunoreactive cells in the SVZ and on DCx cells in the RMS were more pronounced in the Y1R KO mouse, the three populations in the olfactory bulb were not distinctly different between the two KO transgenes analyzed. Interestingly, in the same study we also analyzed the effect of deletion of the cholecystokinin 1(A) receptor, and these mice also showed reductions very similar to those seen in the NPY receptor KO mice [53].

Because NPY terminals are comparatively rare in the RMS, peptide released from nearby nerve endings could diffuse via so-called volume transmission [59] to reach the receptor-positive cells. The Y2R is not expressed in proliferating cells, and we could not establish what type of cell expresses this receptor. For example, double-labeling for glial fibrillary acidic protein showed cells forming tubes along the migrating neuroblasts, but they were not positive for Y1R or Y2R. Thus, the role of Y2R remains unclear, but the distinct loss of DCx-immunoreactive cells seen in Y2R KO mice suggests an involvement.

Taken together, genetic modification of mice resulting in deletion of Y1R or Y2R shows a dramatic reduction in neuroblast proliferation, migration, and placement compared with wild-type mice, suggesting involvement of the NPY system in the regulation of adult neurogenesis. Our

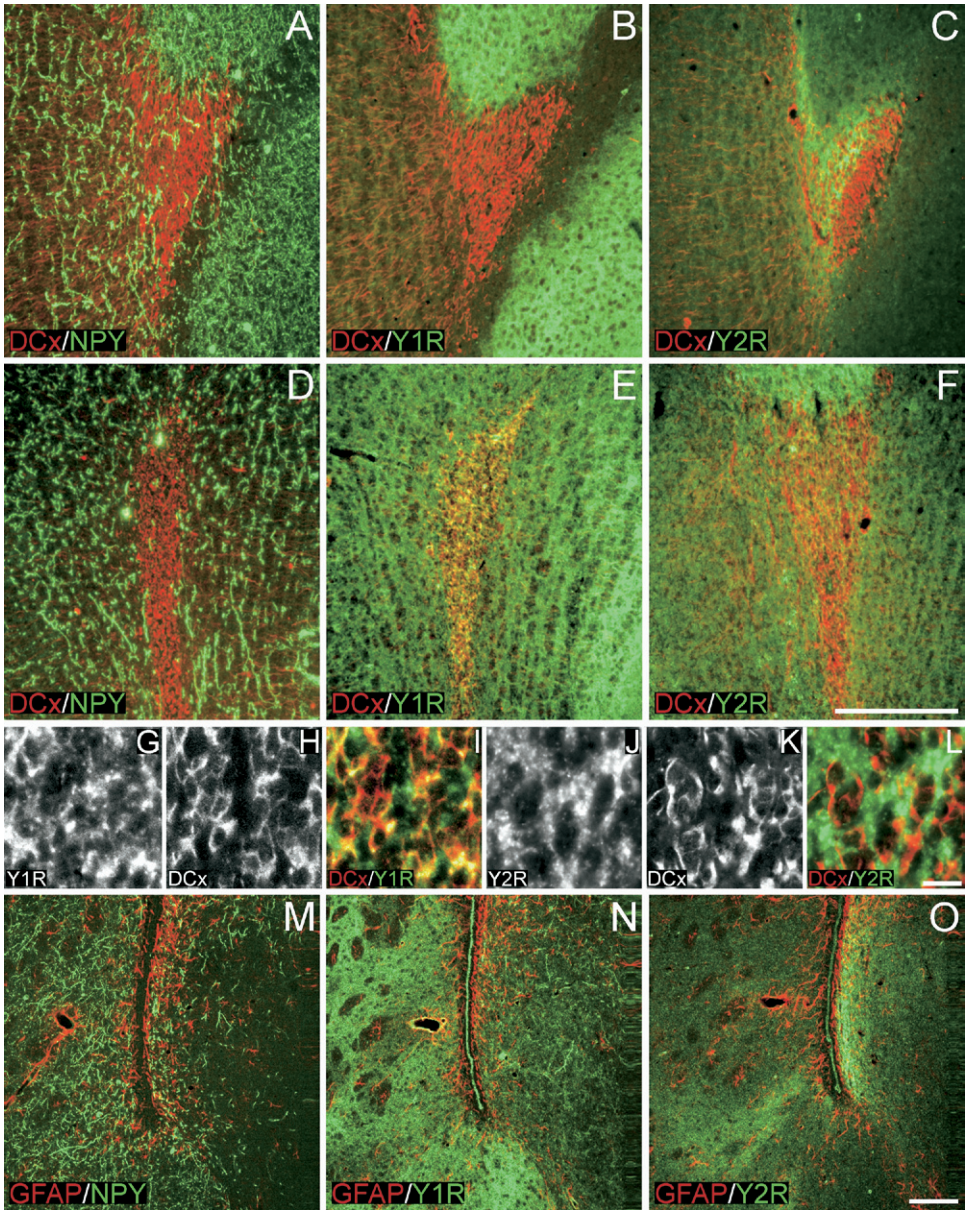

Fig. 1. Double-immunolabeling of NPY, Y1R, or Y2R with DCx or GFAP in the wild-type mouse subventricular zone and rostral migratory stream. (A–C) Rostral migratory stream. (D–F) Rostral migratory stream at the level of the olfactory bulb: (A, D) NPY (green) and DCx (red), (B, E) Y1R (green) and DCx (red), (C, F) Y2R (green) and DCx (red). (G–I) Confocal images of colocalization (I) between Y1R (G) and DCx (H) in the rostral migratory stream at the level of the olfactory bulb. (J–L) Rostral migratory stream showing no certain colocalization (L) between the Y2R (J) and DCx (K). (M–O) Subventricular zone: (M) NPY (green) and GFAP (red), (N) Y1R (green) and GFAP (red), (O) Y2R (green) and GFAP (red). Scale bars = 200 μm in F and applies to A–F, 10 μm in L and applies to G–L, 100 μm in O and applies to M–O. DCx, double-cortin; GFAP, glial fibrillary acidic protein; NPY, neuropeptide Y; Y1R, neuropeptide Y Y_1 receptor; Y2R, neuropeptide Y Y_2 receptor.

results thus support previous studies on the role of NPY in the proliferation of neurons shown in the olfactory and hippocampal systems [15,50–52,54].

The mechanisms involved include the extracellular signal-regulated kinase (ERK 1/2) subgroup of mitogen-activated protein kinases [51], molecules that already have been implicated in the proliferation of neural progenitors [60]. The Y2R-related effects may involve phosphotyrosine-containing proteins [61] and protein kinase C–dependent pathways [62]. Our observation of a change in migration patterns toward individual cell groups suggests participation of molecules such as integrins [63], reelin [64], and the transmembrane-4 superfamily protein CD63 [65].

There is now evidence for a relationship between neurogenesis and depression. Thus, it has been demonstrated in many studies that, with a few exceptions, treatment with antidepressants is associated with increased neurogenesis [66–70]. Also, NPY is one of many peptides thought to be involved in mood regulation with an anxiolytic/antidepressive action, as reported earlier [71] and at this meeting by

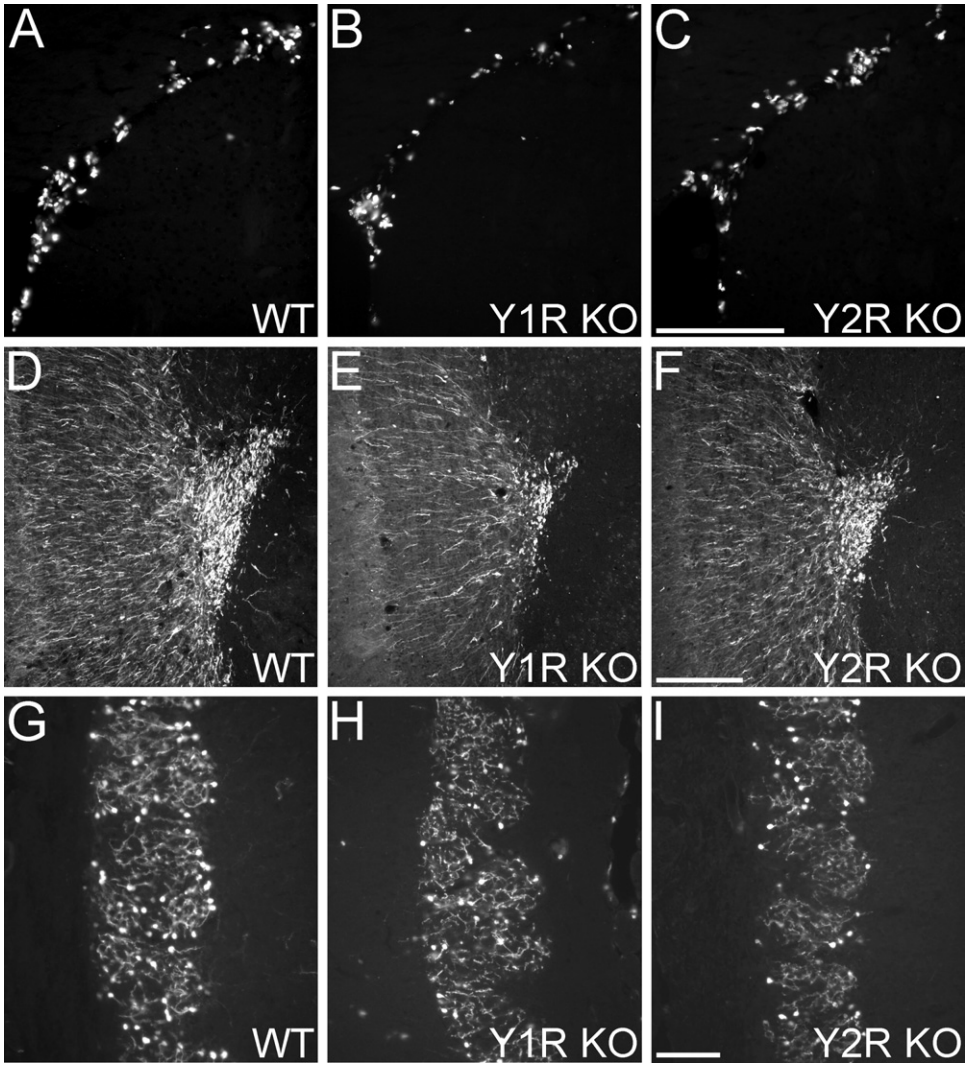

Fig. 2. Reduced number of Ki67-, double-cortin-, and calbindin-immunoreactive cells in the subventricular zone, rostral migratory stream, and olfactory bulb of Y1R KO and Y2R KO mice. (A–C) Ki67-like immunoreactivity in the subventricular zone of WT (A), Y1R KO (B), and Y2R KO (C) mice. (D–F) Double-cortin–like immunoreactivity in the rostral migratory stream of WT (D), Y1R KO (E), and Y2R KO (F) mice. (G–I) Calbindin-like immunoreactivity in the glomerular cell layer of WT (G), Y1R KO (H), and Y2R KO (I) mice. Scale bars = 200 μm in C and applies to A–C, 200 μm in F and applies to D–F, 100 μm in I and applies to G–I. KO, knockout; WT, wild type; Y1R, neuropeptide Y Y_1 receptor; Y2R, neuropeptide Y Y_2 receptor.

Quirion and collaborators, as well as by many other groups. In general terms, it has been reported that NPY has an anxiolytic antidepressive behavior profile and that this action is exerted, among others, at the hippocampal level. Any type of derangement in the hippocampal neurogenesis process may therefore influence mood regulation and the efficacy of antidepressant treatment. It is possible that these effects of NPY involve formation of new forebrain neurons.

Arcuate NPY/agouti gene-related protein system in the anorectic mouse

Almost 25 y ago Maltais et al. [72] described a mouse with an autosomal recessive mutation, the *anx/anx* mouse, which appeared normal at birth but developed growth failure and neurological abnormalities 3–5 wk postnatally with an emaciated appearance, resulting in death due to malnutrition. Subsequent analysis of these mice revealed increased guanosine triphosphate hydroxylase activity and tetrahydrobiopterin[73], increased cell proliferation, apoptosis [74], and changes in transmitter systems [75–82]. In our laboratory we focused on the changes in the hypothalamic arcuate NPY/agouti gene-related protein (AGRP) system, which showed strongly increased NPY/AGRP levels in the arcuate cell bodies and apparent loss of these two immunoreactivities in their nerve terminal projections (Fig. 3). Moreover, there is evidence for neurodegenerative changes not only in the NPY/AGRP neurons but also in the α-melanocyte–stimulating hormone (α-MSH) system [76,82]. Mechanisms

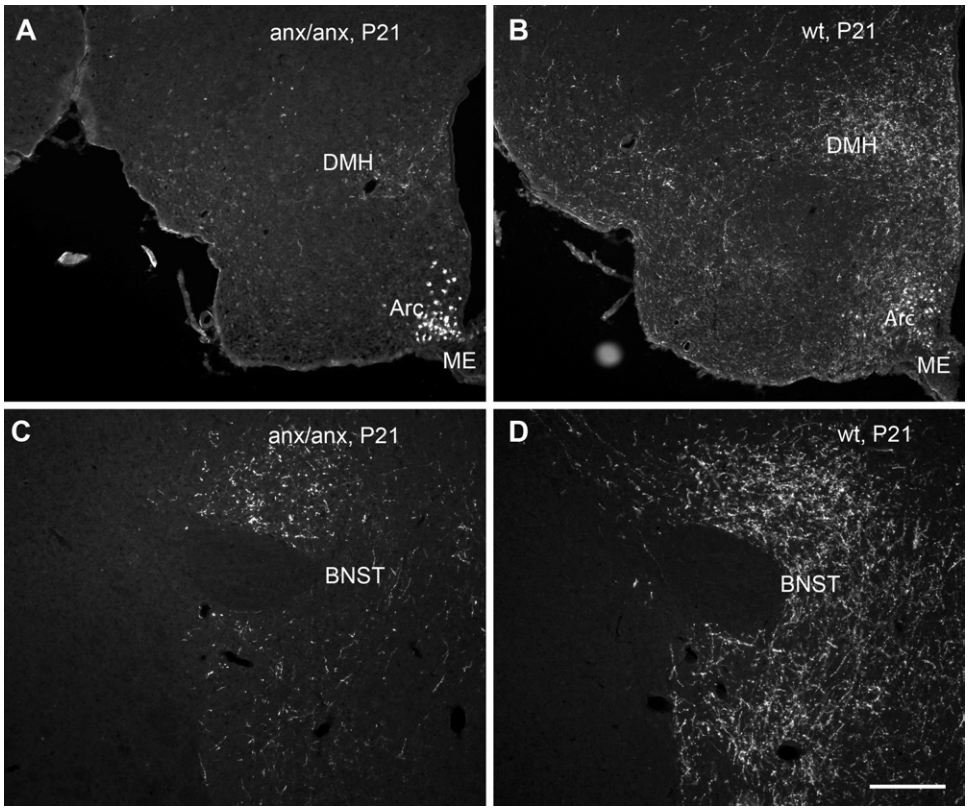

Fig. 3. Immunofluorescence micrographs showing agouti-related protein in *anx/anx* (A, C) and wt (B, D) mice in the Arc (A, B) and BNST (C, D) at P21. Arc, arcuate nucleus, BNST, bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamus; ME, median eminence; P21, postnatal day 21; wt, wild-type. Scale bar = 200 μm.

underlying these changes and the identity of the spontaneous mutation have still not been verified.

Even if, initially, the NPY mRNA levels in the arcuate neurons seemed unchanged [80], a subsequent study indicated increased transcript levels [77]. One possible explanation is that, during postnatal development, the NPY neurons in these mice have been stimulated and increased their NPY/AGRP release, resulting in "empty" nerve terminals and a compensatory increase in mRNA levels/synthesis. Another line of thought is involvement of neurodevelopmental/neurodegenerative changes resulting in impaired centrifugal transport of the peptides and subsequent depletion of NPY in nerve terminals. A hypothetical feedback system could then activate these neurons and their peptide synthesis.

Developmental effects are also suggested by the fact that a similar phenotype concerning body weight and NPY/AGRP expression has been seen in a contactin-KO mouse [77]. Contactin is a molecule implicated in axon guidance processes [83], and neurodegenerative changes could therefore be related to deranged development of the NPY/AGRP projections.

We compared the postnatal development of the NPY/AGRP system in wild-type and *anx/anx* mice [84]. We found a similar development up to postnatal day (P) 12, after which the increase in AGRP-like immunoreactivity (LI) in fibers started to lag behind that seen in wild-type mice. At P21 the AGRP fiber density was significantly reduced versus that at P15, and at P21 strongly AGRP-positive cell bodies appeared in the *anx/anx* arcuate nucleus, which was not seen in wild-type mice. (We studied AGRP because this peptide is exclusively expressed in arcuate neurons, whereas NPY also is present in afferent systems, making it more difficult to dissect the changes specific for the arcuate system.)

Furthermore, we stained sections with two markers for microglia activation, the ionized calcium-binding adapter molecule Iba1 and the toll-like receptor-2 (TRL-2). The results showed that Iba1 was diffusely distributed at low levels throughout the brain in *anx/anx* and control mice at P1, with increasing levels up to P10. However, starting at P12/P15 there were increased numbers and more intensely stained cells, the latter often having a bushy-shaped morphology. This is indicative of activation, in specific brain regions, largely overlapping with those areas showing loss of AGRP-LI in fibers. The bushy-shaped Iba1 cells were often closely apposed to the NPY/AGRP neurons in the arcuate nucleus and their projection areas (Fig. 4). Incubation with TRL-2 antiserum revealed a very parallel pattern of appearance, and in fact TRL-2-LI coexisted with Iba1.

These findings indicate that inflammatory/neurodegenerative processes may be involved, because Iba1 is ex-

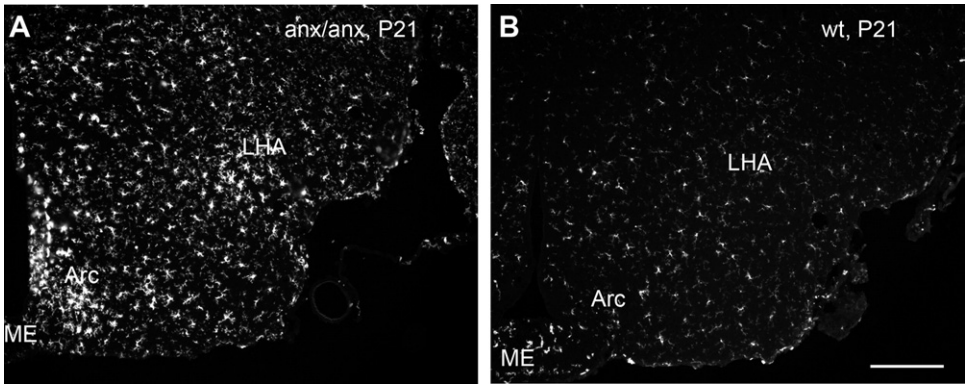

Fig. 4. Immunofluorescence micrographs showing Iba1 in the Arc and LHA of an *anx/anx* (A) compared with a wt (B) mouse. Arc, arcuate nucleus; LHA, lateral hypothalamus; ME, median eminence; P21, postnatal day 21; wt, wild-type. Scale bar = 200 μm.

pressed by microglial cells [85] and is an accepted marker for activated glia in the injured or inflamed nervous system [86–91]. We have interpreted that the changes in the NPY/AGRP neurons occur as a result of microglia-associated inflammation/neurodegeneration. This is supported by the fact that, as indicated above, the α-MSH neurons, which are close to the NPY/AGRP neurons in the arcuate nucleus, show signs of neurodegeneration when stained with antibodies against the Y1R. In normal rats/wild-type mice these antibodies decorate the dendritic processes of the α-MSH neurons [38,92], but in the *anx/anx* mouse the Y1R-labeled dendrites were short/retracted with a degenerative morphology [82]. Moreover, the fact that these changes in Iba1 and TRL-2-LI also occur in the regions innervated by AGRP/NPY nerve terminals suggests processes with a close relation not only to the cell bodies in the arcuate nucleus but also to the innervated area.

Concluding remarks

The results summarized above give rise to some speculations concerning the role of the Y1R in neurogenesis. Thus, our work on prenatal DRG neurons suggests that NPY via the Y1R can affect growth rate and growth angle, that is, exert trophic and attraction effects. The fact that NPY is produced in the RMS and that the Y1R, at least at the more anterior levels of the migratory stream, is expressed by proliferating neuroblasts suggests the possibility that NPY-ergic mechanisms may be involved in guidance and neurite outgrowth in migrating cells, a possibility that should be pursued in further studies. In general terms, the results summarized in this report underline the multifaceted roles of NPY in nervous system functions, not only being an important transmitter-like mediator in the hypothalamic feeding circuitry but also playing an important role in neurodevelopmental processes. Interestingly, Kokoeva et al. [93] reported that new cells are continuously born in the adult murine hypothalamus, and that ciliary neurotrophic factor may play an important role in this process. Perhaps this represents a bridge to the evidence presented above for a role of NPY in neurogenesis in the olfactory bulb and hippocampal formation. A derangement of NPY-ergic mechanisms may hypothetically be involved in various disorders affecting the nervous system.

References

[1] Tatemoto K. Neuropeptide Y; complete amino acid sequence of the brain peptide. Proc Natl Acad Sci U S A 1982;79:5485–9.
[2] Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y, a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982;296:659–60.
[3] Kalra SP, Kalra PS, editors. NPY and cohorts in human disease. Proceedings of the 8th International NPY meeting 2006. Peptides 2006;28:197–484.
[4] Zukowska Z, Feuersteing GZ, editors. NPY family of peptides in neurobiology, cardiovascular and metabolic disorders: from genes to therapeutics. New York: Springer; 2006.
[5] Wakisaka S, Kajander KC, Bennett GJ. Increased neuropeptide Y (NPY)–like immunoreactivity in rat sensory neurons following peripheral axotomy. Neurosci Lett 1991;124:200–3.
[6] Wakisaka S, Kajander KC, Bennett GJ. Effects of peripheral nerve injuries and tissue inflammation on the levels of neuropeptide Y–like immunoreactivity in rat primary afferent neurons. Brain Res 1992; 598:349–52.
[7] Nahin RL, Ren K, De Leon M, Ruda M. Primary sensory neurons exhibit altered gene expression in a rat model of neuropathic pain. Pain 1994;58:95–108.
[8] Zhang X, Meister B, Elde R, Verge VM, Hökfelt T. Large calibre primary afferent neurons projecting to the gracile nucleus express neuropeptide Y after sciatic nerve lesions: an immunohistochemical and in situ hybridization study in rats. Eur J Neurosci 1993;5:1510–9.
[9] Corness J, Shi TJ, Xu ZQ, Brulet P, Hökfelt T. Influence of leukemia inhibitory factor on galanin/GMAP and neuropeptide Y expression in mouse primary sensory neurons after axotomy. Exp Brain Res 1996; 112:79–88.
[10] White DM, Mansfield K. Vasoactive intestinal polypeptide and neuropeptide Y act indirectly to increase neurite outgrowth of dissociated dorsal root ganglion cells. Neuroscience 1996;73:881–7.
[11] White DM. Contribution of neurotrophin-3 to the neuropeptide Y–induced increase in neurite outgrowth of rat dorsal root ganglion cells. Neuroscience 1998;86:257–63.

[12] Ubink R, Halasz N, Zhang X, Dagerlind A, Hökfelt T. Neuropeptide tyrosine is expressed in ensheathing cells around the olfactory nerves in the rat olfactory bulb. Neuroscience 1994;60:709–26.
[13] Graziadei PPC, Monti Graziadei GA. Continuous nerve cell renewal in the olfactory system. In: Jacobsen M, editor. Handbook of sensory physiology. Volume 9. Berlin-Heidelberg-New York: Springer; 1978, p. 55–82.
[14] Doucette R. Glial influences on axonal growth in the primary olfactory system. Glia 1990;3:433–49.
[15] Hansel DE, Eipper BA, Ronnett GV. Neuropeptide Y functions as a neuroproliferative factor. Nature 2001;410(6831):940–4.
[16] Erickson JC, Clegg KE, Palmiter RD. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 1996; 381(6581):415–21.
[17] Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, et al. Visualizing an olfactory sensory map. Cell 1996;87:675–86.
[18] Ubink R, Calza L, Hökfelt T. 'Neuro'-peptides in glia: focus on NPY and galanin. Trends Neurosci 2003;26:604–9.
[19] Sanford SD, Gatlin JC, Hökfelt T, Pfenninger KH. Growth cone responses to growth chemotropic factors. Eur J Neurosci 2008(in press).
[20] Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. Science 1996;274(5290):1123–33.
[21] Letourneau PC. Chemotactic response of nerve fiber elongation to nerve growth factor. Dev Biol 1978;66:183–96.
[22] Gundersen RW, Barrett JN. Characterization of the turning response of dorsal root neurites toward nerve growth factor. J Cell Biol 1980; 87(3, pt 1):546–54.
[23] Stein E, Tessier-Lavigne M. Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. Science 2001;291(5510):1928–38.
[24] Malmström RE, Alexandersson A, Balmer KC, Weilitz J. In vivo characterization of the novel neuropeptide Y Y1 receptor antagonist H 409/22. J Cardiovasc Pharmacol 2000;36:516–25.
[25] Bergman NA, D'Ambra T, Pilling G. New NPY agonist. International patent application WO 99/15498 1999.
[26] Gedda K, Berglund M-L, Larefalk A. H409/2, a potent and selective antagonist for the NPY Y1 receptor (abstract). Presented at the 5th International NPY Meeting; Cayman Islands; 1999.
[27] Tatemoto K, Rökaeus Å, Jörnvall H, McDonald TJ, Mutt V. Galanin—a novel biologically active peptide from porcine intestine. FEBS Lett 1983;164:124–8.
[28] Mahoney SA, Hosking R, Farrant S, Holmes FE, Jacoby AS, Shine J, et al. The second galanin receptor GalR2 plays a key role in neurite outgrowth from adult sensory neurons. J Neurosci 2003;23:416–21.
[29] Shi TJ, Hua XY, Lu X, Malkmus S, Kinney J, Holmberg K, et al. Sensory neuronal phenotype in galanin receptor 2 knockout mice: focus on dorsal root ganglion neurone development and pain behaviour. Eur J Neurosci 2006;23:627–36.
[30] Pirondi S, Fernandez M, Schmidt R, Hökfelt T, Giardino L, Calza L. The galanin-R2 agonist AR-M1896 reduces glutamate toxicity in primary neural hippocampal cells. J Neurochem 2005;95:821–33.
[31] Hobson SA, Holmes FE, Kerr NC, Pope RJ, Wynick D. Mice deficient for galanin receptor 2 have decreased neurite outgrowth from adult sensory neurons and impaired pain-like behaviour. J Neurochem 2006;99:1000–10.
[32] Tatemoto K, Mutt V. Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. Nature 1980;285(5764):417–8.
[33] Jazin EE, Zhang X, Soderstrom S, Williams R, Hökfelt T, Ebendal T, Larhammar D. Expression of peptide YY and mRNA for the NPY/PYY receptor of the Y1 subtype in dorsal root ganglia during rat embryogenesis. Brain Res Dev Brain Res 1993;76:105–13.
[34] Gehlert DR. Multiple receptors for the pancreatic polypeptide (PP-fold) family: physiological implications. Proc Soc Exp Biol Med 1998;218:7–22.
[35] Ubink R, Kopp J, Wong H, Walsh JH, Pedrazzini T, Hökfelt T. Transient prenatal expression of NPY-Y1 receptor in trigeminal axons innervating the mystacial vibrissae. J Comp Neurol 2001;429: 183–91.
[36] Gibson SJ, Polak JM, Allen JM, Adrian TE, Kelly JS, Bloom SR. The distribution and origin of a novel brain peptide, neuropeptide Y, in the spinal cord of several mammals. J Comp Neurol 1984;227:78–91.
[37] Marti E, Biffo S, Fasolo A. Neuropeptide Y m-RNA and peptide are transiently expressed in the developing rat spinal cord. Neuroreport 1992;3:401–4.
[38] Zhang X, Bao L, Xu ZQ, Kopp J, Arvidsson U, Elde R, Hökfelt T. Localization of neuropeptide Y Y1 receptors in the rat nervous system with special reference to somatic receptors on small dorsal root ganglion neurons. Proc Natl Acad Sci U S A 1994;91:11738–42.
[39] Zhang X, Tong YG, Bao L, Hökfelt T. The neuropeptide Y Y1 receptor is a somatic receptor on dorsal root ganglion neurons and a postsynaptic receptor on somatostatin dorsal horn neurons. Eur J Neurosci 1999;11:2211–25.
[40] Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science 2000;288(5472):1765–9.
[41] Fetissov SO, Kuteeva E, Hökfelt T. Directional cues for arcuate NPY projections are present in the adult brain. Exp Neurol 2003; 183:116–23.
[42] Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 1965; 124:319–35.
[43] Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ. Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 1999;2:260–5.
[44] Lois C, Garcia-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. Science 1996;271(5251):978–81.
[45] Luskin MB. Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 1993;11:173–89.
[46] Ghashghaei HT, Lai C, Anton ES. Neuronal migration in the adult brain: are we there yet? Nat Rev Neurosci 2007;8:141–51.
[47] Alvarez-Buylla A, Garcia-Verdugo JM. Neurogenesis in adult subventricular zone. J Neurosci 2002;22:629–34.
[48] Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008;132:645–60.
[49] Saghatelyan A, Carleton A, Lagier S, de Chevigny A, Lledo PM. Local neurons play key roles in the mammalian olfactory bulb. J Physiol Paris 2003;97:517–28.
[50] Howell OW, Scharfman HE, Herzog H, Sundstrom LE, Beck-Sickinger A, Gray WP. Neuropeptide Y is neuroproliferative for post-natal hippocampal precursor cells. J Neurochem 2003;86:646–59.
[51] Howell OW, Doyle K, Goodman JH, Scharfman HE, Herzog H, Pringle A, et al. Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. J Neurochem 2005;93:560–70.
[52] Howell OW, Silva S, Scharfman HE, Sosunov AA, Zaben M, Shatya A, et al. Neuropeptide Y is important for basal and seizure-induced precursor cell proliferation in the hippocampus. Neurobiol Dis 2007; 26:174–88.
[53] Staenic D, Paratcha G, Ledda F, Herzog H, Kopin AS, Hökfelt T. Peptidergic influences on proliferation, migration, and placement of neural progenitors in the adult mouse forebrain. Proc Natl Acad Sci USA 2008;105:3610–15.
[54] Agasse F, Bernardino L, Kristiansen H, Christiansen SH, Ferreira R, Silva B, et al. Neuropeptide Y promotes neurogenesis in murine subventricular zone. Stem Cells 2008;26:1636–45.
[55] Stanic D, Brumovsky P, Fetissov S, Shuster S, Herzog H, Hökfelt T. Characterization of neuropeptide Y2 receptor protein expression in the mouse brain. I. Distribution in cell bodies and nerve terminals. J Comp Neurol 2006;499:357–90.
[56] Fetissov SO, Kopp J, Hökfelt T. Distribution of NPY receptors in the hypothalamus. Neuropeptides 2004;38:175–88.

[57] Sainsbury A, Schwarzer C, Couzens M, Fetissov S, Furtinger S, Jenkins A, et al. Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. Proc Natl Acad Sci U S A 2002;99:8938–43.
[58] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182:311–22.
[59] Fuxe K, Agnati L, editors. Volume transmission in the brain. New York: Raven Press, 1991.
[60] Li BS, Ma W, Zhang L, Barker JL, Stenger DA, Pant HC. Activation of phosphatidylinositol-3 kinase (PI-3K) and extracellular regulated kinases (Erk1/2) is involved in muscarinic receptor-mediated DNA synthesis in neural progenitor cells. J Neurosci 2001;21:1569–79.
[61] Shigeri Y, Fujimoto M. Y2 receptors for neuropeptide Y are coupled to three intracellular signal transduction pathways in a human neuroblastoma cell line. J Biol Chem 1994;269:8842–8.
[62] Herring N, Lokale MN, Danson EJ, Heaton DA, Paterson DJ. Neuropeptide Y reduces acetylcholine release and vagal bradycardia via a Y2 receptor-mediated, protein kinase C–dependent pathway. J Mol Cell Cardiol 2008;44:477–85.
[63] Jacques TS, Relvas JB, Nishimura S, Pytela R, Edwards GM, Streuli CH, ffrench-Constant C. Neural precursor cell chain migration and division are regulated through different beta1 integrins. Development 1998;125:3167–77.
[64] Pesold C, Liu WS, Guidotti A, Costa E, Caruncho HJ. Cortical bitufted, horizontal, and Martinotti cells preferentially express and secrete reelin into perineuronal nets, nonsynaptically modulating gene expression. Proc Natl Acad Sci U S A 1999;96:3217–22.
[65] Hallden G, Hadi M, Hong HT, Aponte GW. Y receptor-mediated induction of CD63 transcripts, a tetraspanin determined to be necessary for differentiation of the intestinal epithelial cell line, hBRIE 380i cells. J Biol Chem 1999;274:27914–24.
[66] Sahay A, Drew MR, Hen R. Dentate gyrus neurogenesis and depression. Prog Brain Res 2007;163:697–722.
[67] Duman RS, Nakagawa S, Malberg J. Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 2001; 25:836–44.
[68] Paizanis E, Kelai S, Renoir T, Hamon M, Lanfumey L. Life-long hippocampal neurogenesis: environmental, pharmacological and neurochemical modulations. Neurochem Res 2007;32:1762–71.
[69] Martinowich K, Lu B. Interaction between BDNF and serotonin: role in mood disorders. Neuropsychopharmacology 2008;33:73–83.
[70] Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 2008;33: 88–109.
[71] Redrobe JP, Dumont Y, Quinon R. Neuropeptide Y (NPY) and depression from animal studies to the human condition. Life Sci 2002;71:2921–37.
[72] Maltais LJ, Lane PW, Beamer WG. Anorexia, a recessive mutation causing starvation in preweanling mice. J Hered 1984;75:468–72.
[73] Duch DS, Bowers SW, Woolf JH, Davisson MT, Maltais LJ, Nichol CA. Differences in the metabolism of the aromatic amino acid hydroxylase cofactor, tetrahydrobiopterin, in mutant mice with neurological and immunological defects. Biochem Genet 1986;24:657–68.
[74] Kim MJ, Kim Y, Kim SA, Lee HJ, Choe BK, Nam M, et al. Increases in cell proliferation and apoptosis in dentate gyrus of anorexia (anx/anx) mice. Neurosci Lett 2001;302:109–12.
[75] Son JH, Baker H, Park DH, Joh TH. Drastic and selective hyperinnervation of central serotonergic neurons in a lethal neurodevelopmental mouse mutant, Anorexia (anx). Brain Res Mol Brain Res 1994;25:129–34.
[76] Johansen JE, Broberger C, Lavebratt C, Johansson C, Kuhar MJ, Hökfelt T, Schalling M. Hypothalamic CART and serum leptin levels are reduced in the anorectic (anx/anx) mouse. Brain Res Mol Brain Res 2000;84:97–105.
[77] Fetissov SO, Bergstrom U, Johansen JE, Hökfelt T, Schalling M, Ranscht B. Alterations of arcuate nucleus neuropeptidergic development in contactin-deficient mice: comparison with anorexia and food-deprived mice. Eur J Neurosci 2005;22:3217–28.
[78] Jahng JW, Houpt TA, Joh TH, Son JH. Differential expression of monoamine oxidase A, serotonin transporter, tyrosine hydroxylase and norepinephrine transporter mRNA by anorexia mutation and food deprivation. Brain Res Dev Brain Res 1998;107:241–6.
[79] Jahng JW, Houpt TA, Kim SJ, Joh TH, Son JH. Neuropeptide Y mRNA and serotonin innervation in the arcuate nucleus of anorexia mutant mice. Brain Res 1998;790:67–73.
[80] Broberger C, Johansen J, Schalling M, Hökfelt T. Hypothalamic neurohistochemistry of the murine anorexia (anx/anx) mutation: altered processing of neuropeptide Y in the arcuate nucleus. J Comp Neurol 1997;387:124–35.
[81] Broberger C, Johansen J, Johansson C, Schalling M, Hökfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc Natl Acad Sci U S A 1998;95:15043–8.
[82] Broberger C, Johansen J, Brismar H, Johansson C, Schalling M, Hökfelt T. Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus. J Neurosci 1999;19:7130–9.
[83] Berglund EO, Murai KK, Fredette B, Sekerkova G, Marturano B, Weber L, et al. Ataxia and abnormal cerebellar microorganization in mice with ablated contactin gene expression. Neuron 1999;24:739–50.
[84] Nilsson I, Lindfors C, Fetissov SO, Hökfelt T, Johansen JE. Aberrant agouti-related protein system in the hypothalamus of the anx/anx mouse is associated with activation of microglia. J Comp Neurol 2008;507:1128–40.
[85] Imai Y, Ibata I, Ito D, Ohsawa K, Kohsaka S. A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. Biochem Biophys Res Commun 1996;224:855–62.
[86] Streit WJ, Graeber MB, Kreutzberg GW. Functional plasticity of microglia: a review. Glia 1988;1:301–7.
[87] Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience 1985;15:313–26.
[88] Hwang IK, Yoo KY, Kim DW, Choi SY, Kang TC, Kim YS, Won MH. Ionized calcium-binding adapter molecule 1 immunoreactive cells change in the gerbil hippocampal CA1 region after ischemia/reperfusion. Neurochem Res 2006;31:957–65.
[89] Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci 1996;19:312–8.
[90] Nakajima K, Kohsaka S. Response of microglia to brain injury. In: Kettenmann H, Ransom BR, editors. Neuroglia. New York: Oxford University Press; 2005, p. 443–53.
[91] Streit WJ. Microglial cells. In: Kettenmann H, Ransom BR, editors. Neuroglia. New York: Oxford University Press; 2005, p. 60–71.
[92] Kopp J, Xu ZQ, Zhang X, Pedrazzini T, Herzog H, Kresse A, et al. Expression of the neuropeptide Y Y1 receptor in the CNS of rat and of wild-type and Y1 receptor knock-out mice. Focus on immunohistochemical localization. Neuroscience 2002;111:443–532.
[93] Kokoeva MV, Yin H, Flier JS. Evidence for constitutive neural cell proliferation in the adult murine hypothalamus. J Comp Neurol 2007; 505:209–20.