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EXPRESSION OF COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT IN THE RAT FOREBRAIN DURING POSTNATAL DEVELOPMENT

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Abstract

Cocaine- and amphetamine-regulated transcript (CART) is widespread in the rodent brain. CART has been implicated in many different functions including reward, feeding, stress responses, sensory processing, learning and memory formation. Recent studies have suggested that CART may also play a role in neural development. Therefore, in the present study we compared the distribution pattern and levels of CART mRNA expression in the forebrain of male and female rats at different stages of postnatal development: P06, P26 and P66. At 6 days of age (P06), male and female rats showed increased CART expression in the somatosensory and piriform cortices, indusium griseum, dentate gyrus, nucleus accumbens, and ventral premammillary nucleus. Interestingly, we found a striking expression of CART mRNA in the ventral posteromedial and ventral posterolateral thalamic nuclei. This thalamic expression was absent at P26 and P66. Contrastingly, at P06 CART mRNA expression was decreased in the arcuate nucleus. Comparing sexes, we found increased CART mRNA expression in the anteroventral periventricular nucleus of adult females. In other regions including the CA1, the lateral hypothalamic area and the dorsomedial nucleus of the hypothalamus, CART expression was not different comparing postnatal ages and sexes. Our findings indicate that CART gene expression is induced in a distinct temporal and spatial manner in forebrain sites of male and female rats. They also suggest that CART peptide participate in the development of neural pathways related to selective functions including sensory processing, reward and memory formation.

Keywords

cerebral cortex; somatosensory cortex; hippocampus; ventral premammillary nucleus; thalamus

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The cocaine- and amphetamine-regulated transcript (CART) was first described as an mRNA upregulated in the striatum of rodents in response to acute administration of cocaine or amphetamine (Douglass et al., 1995). Subsequently, studies from different laboratories showed that CART mRNA and peptides are widespread in the rodent and primate brains (Douglass et al., 1995; Couceyro et al., 1997; Koylu et al., 1997, 1998; Dall Vechia et al., 2000; Elias et al., 2001). In the forebrain, CART is highly expressed in the dorsal and ventral striatum, in the somatosensory and the piriform cortices, in the hippocampus, amygdala and paraventricular thalamic nucleus. CART is particularly enriched in hypothalamic nuclei, including the arcuate, the paraventricular, the supraoptic, the dorsomedial, the ventral premammillary and the lateral hypothalamic area (Couceyro et al., 1997; Koylu et al., 1997, 1998; Broberger, 1999; Vrang et al., 1999; Elias et al., 2001).

In spite of the observed effects of psychostimulants upon CART expression, its wide distribution in the brain suggested a role in a variety of functions including learning and memory, stress response, feeding, motivated behaviors and sensory processing (Kuhar et al., 2002; Dominguez et al., 2004). In fact, soon after its description it became clear that CART exhibit a potent anorexigenic effect. Intracerebroventricular delivery of CART peptides inhibits feeding, and immuno blockade of CART increases food intake (Kristensen et al., 1998; Lambert et al., 1998). Notably, CART is reduced in the arcuate nucleus of leptin-deficient (*ob/ob*) obese mice and in the leptin receptor-deficient (Zucker) rat (Kristensen et al., 1998). Leptin administration to leptin-deficient mice normalizes CART mRNA expression in the arcuate nucleus. Several lines of evidence indicate that leptin may act as a trophic factor in neuronal development (Ahima and Hileman, 2000; Bouret et al., 2004; Bouret and Simerly, 2004). Leptin-deficient *ob/ob* mice display structural and molecular neuronal abnormalities, which are rescued by leptin treatment (Bereiter and Jeanrenaud, 1979; Ahima et al., 1999; Steppan and Swick, 1999; Ahima and Hileman, 2000; Bouret et al., 2004; Pinto et al., 2004). Interestingly, various hypothalamic nuclei coexpress CART and leptin receptors (Elias et al., 1998, 2000, 2001). Thus, CART peptide might participate in some of leptin's effects in neuronal tone and development. Importantly, CART induces neurite elongation and ramification in a variety of primary cultured neurons (Louis, 1996). This includes dopaminergic, hippocampal, retinal and motoneurons. CART peptide is also detected early (E11) in the ventral plate along the neural tube of the developing rat embryo (Brischoux et al., 2002). Interestingly, studies on postnatal immunoreactive detection of CART peptide in two specific brain sites— dorsal motor nucleus of the vagus nerve and dentate gyrus—suggested a differential expression pattern. CART immunoreactivity in the dorsal motor nucleus of vagus nerve peaks at P5–P8, and in the dentate gyrus at P30 (Dun et al., 2001; Ábrahám et al., 2007). Several studies have also suggested that CART mRNA is differentially expressed in specific brain sites at different ages (Bai et al., 2005; Hunter et al., 2007). But it is not clear whether specific forebrain sites show any particular pattern of CART expression across neuronal development. Therefore, in the present study, we aimed at performing a systematic analysis of CART mRNA expression in the developing rat forebrain. We compared CART mRNA distribution and expression levels in the forebrain of male and female rats in three different postnatal stages: early post-natal (P06), post-weaning/juvenile (P26) and adult (P66).

EXPERIMENTAL PROCEDURES

Animals

Male and female Sprague–Dawley rats were maintained on a 12-h on/12-h off cycle (lights on at 7 AM) in a temperature-controlled environment (21±2 °C) and were given free access to food and water. All experiments were carried out in accordance with the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and by the University of São Paulo Committee for Research and Animal Care.

Male and female rats were perfused in three different post-natal ages: P06, P26 and P66. Rats were selected randomly from three different dams. These dams were primiparae and had litters culled to eight pups on postpartum day 2. Males at P26 (n=4) had not shown signs of sexual maturity and females at P26 were at peripubertal stage. Males and females at P66 (n=4 males and n=5 females) were classified as adults, since both groups had attained sexual maturation (assessed by the size of reproductive organs). Females had the estrous cycle monitored and were perfused on diestrus. The estrus cycle was defined through the analysis of the vaginal cytology and only rats showing at least two consecutive regular 4- to 5-day estrous cycles were used (Donato et al., 2009). Because of the asynchronism of estrous cycles, females were perfused at 66–70 days of age, but were all classified as P66. All rats were perfused at the same time of the day, between 11:00–13:00 h.

Perfusion and histology

Rats were anesthetized with 35% chloral hydrate (1 ml, i.p.; Sigma, St. Louis, MO, USA) and perfused via the ascending aorta with 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5 at 4 °C). The brains were removed, post-fixed in the same fixative for 4 h at 4 °C and cryoprotected in 0.1 M phosphate buffer pH 7.4 (PBS) containing 20% sucrose, prepared with diethylpyrocarbonate (DEPC)-treated water (Sigma). The brains of rats at P26 and P66 were cut (30-μm sections) in the frontal plane in a freezing microtome. Five series were collected in antifreeze solution and stored at −20 °C. The brains of rats at P06 were cut (30-μm sections) in the frontal plane in a cryostat. Two series were collected and mounted directly onto SuperFrost plus slides (Fisher Scientific, Pittsburgh, PA, USA).

In situ hybridization histochemistry

In order to assess the distribution of CART mRNA in all groups, series of brain sections from each rat were processed for *in situ* hybridization. A ^{35}S-labeled CART riboprobe (Douglass et al., 1995) was used following a previously described procedure (Elias et al., 2000, 2001). Plasmids containing the CART cDNA were kindly provided by Drs. P. Couceyro and J. K. Elmquist. Prior to hybridization, sections were mounted onto SuperFrost plus slides and pretreated with proteinase K (37 °C for 30 min, Roche Diagnostics, Indianapolis, IN, USA) and triethanolamine/acetic anhydride (Sigma). For generation of antisense ^{35}S-labeled cRNA CART probes, the plasmid was linearized by digestion with *Hind*III and subjected to *in vitro* transcription with ^{35}S-UTP and T3 polymerase (Promega, Madison, WI, USA). The nucleotide mixture was then digested with DNAase and the labeled probe was purified and collected by using resin spin columns (GE Healthcare,

Uppsala, Sweden). The ^{35}S-labeled probe was diluted (10^6 dpm/ml) in hybridization solution. The solution consisted of 50% formamide, 10 mM Tris–HCl (Invitrogen, Carlsbad, CA, USA), 0.01% sheared salmon sperm DNA, 0.01% yeast tRNA, 0.05% total yeast RNA (Sigma), 10 mM dithiothreitol, 10% dextran sulfate, 0.3 M NaCl, 1 mM EDTA (pH 8.0) and 1× Denhardt's solution (Sigma). The hybridization solution (120 μl) and a coverslip were applied to each slide and sections were incubated at 56 °C for 12–16 h. On the following day, the coverslips were removed and the slides were rinsed in 2× sodium chloride/sodium citrate buffer (SSC). Sections were incubated for 30 min in a solution of 0.002% RNAase A (Roche Diagnostics, Indianapolis, IN, USA) in 0.5 M NaCl, 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA (Sigma). They were then rinsed in decreasing concentrations of SSC, dehydrated and placed in X-ray film cassettes with BMR-2 film (Kodak, Rochester, NY, USA) for 1–2 days. Subsequently, slides were dipped in NTB2 photographic emulsion (Kodak), dried and stored with desiccant in foil-wrapped slide boxes at 4 °C for 7 days. Slides were developed with Dektol developer (Kodak), counter-stained with Thionin, dehydrated, cleared in xylene and cover-slipped with DPX mounting medium (Sigma-Aldrich).

Immunohistochemistry

Series of sections from each group were pretreated with peroxide and blocked in 2% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 1 h. They were subsequently incubated in rabbit primary antisera against CART peptide (1:20,000, rat amino-acid sequence 55–102, Phoenix Pharmaceutical, Burlingame, CA, USA) diluted in 0.02 M potassium phosphate buffer pH 7.4 (KPBS) and 0.3% Triton X-100. The antisera specificity test and adsorption control have been described previously (Dun et al., 2000). Sections were then incubated for 1 h in biotin-conjugated IgG donkey anti-rabbit (1:1,000, Jackson Laboratories) and for 1 h in avidin–biotin complex (1:500, Vector Labs., Burlingame, CA, USA). This was followed by incubation in DAB with 0.01% hydrogen peroxide. After 2–3 min, the reaction was terminated with successive rinses in KPBS. Sections were mounted onto gelatin-coated slides (except sections from P06 which were processed on slides), dehydrated, delipidated and coverslipped with DPX mounting medium.

Quantification

Quantifications were performed by an observer unaware of the experimental groups. The hybridization signal was estimated by the analysis of the integrated optical density (IOD) using the ImagePro® Plus software (Media Cybernetics Inc., Bethesda, MD, USA). Darkfield photomicrographs were acquired using the same illumination and exposure time for every section. No image editing was processed before quantifications. The IOD values for each area were calculated as the total IOD of a constant area subtracting the background. The area of interest was defined using anatomical references (e.g. the wall of the third ventricle, the base of the brain, fiber tracts). The background was obtained from adjacent nuclei that do not express CART.

Data analysis and production of photomicrographs

Brain sections were analyzed in a Leica DMR microscope (Leica, Wetzlar, Germany). The photomicrographs were captured with a SPOT RT® digital camera (Diagnostic Instruments, Sterling Heights, MI, USA), adapted to a Leica DMR microscope and a Dell Dimension 4400 computer. Images were digitalized using ImagePro® Plus software. Adobe Photoshop CS3 image-editing software was used to integrate photomicrographs into plates. Only sharpness, contrast and brightness were adjusted.

Statistical analysis

Data are expressed as mean±SEM. Two-way ANOVA followed by Bonferroni post-test were used to compare two independent variables (sex and ages) and the interaction factor. Degree of freedom was constant across the brain sites analyzed (sex=1, ages=2 and interaction=2). Statistical analysis was performed using GraphPad Prism software, and an α value of 0.05 was considered in all analyses.

RESULTS

Distribution of CART mRNA in the developing forebrain

In general, we found that CART mRNA is highly expressed in the forebrain of male and female rats at 6 days of age (P06). We also observed virtually no difference in CART expression comparing males and females, and small or no differences comparing rats at 26 (P26) and those at 66 days of age (P66, Fig. 1). Because of the widespread distribution of CART mRNA in the rat forebrain, the description of the result will be focused only in the sites in which we found significant differences comparing ages and/or sexes. Results are summarized in Table 1.

Cerebral cortex—We found high expression of CART mRNA in the primary somatosensory cortex (S1) of male and female rats at P06. At this postnatal age, CART mRNA was particularly enriched in layers 3 and 4, while lower levels were also noticed in layers 2, 5 and 6. CART mRNA expression was very low in all cortical layers at P26 and P66 (Figs. 1, 2A–F and 3A), except in layer IV which displayed moderate expression. The piriform cortex expresses high CART mRNA across development and in the adult male and female rats (Fig. 1). Comparison among ages showed higher CART expression at P06, particularly at more rostral levels (Figs. 1, 3B and 4). We also found abundance of CART mRNA in the insular cortex of male and female rats at P06, while only moderate to low expression was observed at P26 and P66 (Fig. 1).

Hippocampal formation—We found high CART mRNA expression in the dentate gyrus and indusium griseum of rats at P06, compared to those at P26 and P66 (Figs. 1, 3C, D, 5 and 6). In contrast, we did not detect any difference in CART mRNA expression in CA1, CA2 or CA3 comparing ages and sexes (Fig. 6).

Amygdala—We found moderate CART mRNA expression in the central nucleus of the amygdala (CeA) of rats at P06 and P26 compared to those at P66 (Fig. 3E), which showed low expression levels. No difference between sexes was noticed across development.

Striatum—We found very low CART mRNA expression in the dorsal striatum of male and female rats across development (Fig. 1). In the ventral striatum, CART mRNA was very high in both sexes and in different postnatal ages. Quantitative analysis showed a higher expression of CART in the nucleus accumbens of rats at P06 compared to those at P26 and P66 (Figs. 1, 3F and 7).

Thalamus and hypothalamus—Low CART mRNA expression was observed in thalamic nuclei across development and in adult rats. However, we found a striking expression of CART mRNA in the ventral posteromedial and ventral posterolateral thalamic nuclei (VPM and VPL, respectively) only in rats at P06 (Figs. 8 and 9A). This expression pattern was completely absent in male and female rats at P26 and P66.

In the hypothalamus, we found moderate to high expression of CART mRNA in rats at all ages in previously described nuclei, including the parventricular, the supraoptic, the periventricular, the dorsomedial, the ventral premammillary and the lateral hypothalamic area (Douglass et al., 1995; Couceyro et al., 1997; Koylu et al., 1997, 1998; Elias et al., 2001). Several differences were noticed. CART mRNA was low in the arcuate nucleus of males and females at P06, compared to those at P26 and P66 (Figs. 9B and 10). In contrast, CART expression was high in the ventral premammillary nucleus at P06 and moderate in P26 and P66 (Figs. 9C and 11). In addition, we found high expression of CART mRNA in the posterior ependymal layer of the third ventricle in rats at P06 (Fig. 11). No significant difference was observed in other hypothalamic sites including the dorsomedial nucleus and the lateral hypothalamic area. Of note, we observed higher expression of CART mRNA in the anteroventral periventricular nucleus (AVPV) of females at P66 compared to males at same age, and to males and females at P06 and P26 (data not shown).

Distribution of CART peptide in the developing forebrain

In order to determine whether CART mRNA is translated into CART peptide in neurons of developing rat forebrain, we used immunohistochemistry to detect CART immunoreactive fibers and cell bodies in the forebrain of male and female rats at P06. Both sexes showed weak but clear CART immunoreactivity in the sites in which we found high expression of CART mRNA. These included the S1 (Fig. 12A), the indusium griseum, the dentate gyrus, the nucleus accumbens, the VPM/VPL (Fig. 12B) and the ventral premammillary nucleus (Fig. 12C, D). CART immunoreactive cell bodies were suggestive of neurons as they showed characteristic dendritic arborization and cell shape (e.g. pyramidal in S1 and multipolar in VPM/VPL and hypothalamic nuclei). We also observed CART-immunoreactive fibers in cerebral cortex, hippocampus, thalamus, hypothalamus and amygdala (Fig. 12E, F), suggesting the production and transport of CART peptide to the terminals. Small numbers of CART-immunoreactive cell bodies were also found in the dorsal striatum, in the piriform cortex, in the lateral hypothalamic area, in the arcuate nucleus and in the periventricular nucleus of the hypothalamus of male and female rats at P06. Virtually no difference in the distribution of CART immunoreactivity comparing males and females at P26 and P66 was noticed. In those postnatal groups, distribution pattern of CART immunoreactivity is in accordance with previous publications (Couceyro et al., 1997; Koylu et al., 1997, 1998; Broberger, 1999; Vrang et al., 1999; Elias et al., 2001).

DISCUSSION

In the present study, we performed a systematic analysis of the distribution of CART mRNA in the developing fore-brain of male and female rats. We found virtually no differences in CART mRNA expression comparing sexes. In both male and female, CART mRNA was increased at P06 in several brain sites, including the S1, the piriform and the insular cortices, the indusium griseum, the dentate gyrus, the nucleus accumbens, the thalamic VPM/VPL and the ventral premammillary nucleus. In the hypothalamus of rats at P06, CART mRNA was decreased in the arcuate nucleus of both males and females. We also noticed that CART mRNA was low in the AVPV of males and females at early postnatal and juvenile ages, and also in the adult male rats, whereas it was increased in adult females. The AVPV is a sexually dimorphic nucleus— bigger in female—well-known to play a role in the luteinizing hormone (LH) surge that occurs in the afternoon of the proestrus day (Wiegand and Terasawa, 1982; Herbison, 2008). The CART neurons in the AVPV projects to the adjacencies of gonadotropin-releasing hormone (GnRH) cell bodies and, therefore, may be involved in the modulation of preovulatory LH surge (Rondini et al., 2004). We have also shown that CART mRNA is increased in the AVPV of female rats at late pregnancy (Valera et al., 2006). Studies are in progress to determine the role played by CART from AVPV neurons in the control of the female reproductive function.

In general, we found that CART mRNA expression decreases across postnatal development, but CART expression in the thalamic VPM/VPL of rats at early postnatal ages particularly called our attention. CART mRNA and peptide were abundant in these sites but were completely absent in juvenile and adult rats. The VPM and VPL are key relays of somatosensory pathways (Andersen et al., 1964; Nicolelis and Fanselow, 2002). Neurons in these sites display massive reciprocal connections with those in the primary somatosensory cortex/S1 (Jensen and Killackey, 1987; Steriade and Llinás, 1988; Killackey and Sherman, 2003; Jones, 2009). Importantly, studies have suggested that the establishment of thalamo–cortical–thalamic circuitry requires specific neuronal signaling during development (Allendoerfer and Shatz, 1994; Molnár and Blakemore, 1995). For example, deletion of neurotropin receptor p75, endocannabinoid receptor (CB1) and several transcription factors disrupts the development of this pathway (Hevner et al., 2002; McQuillen et al., 2002; Wu et al., 2010). The role played by CART is not known, but it is interesting that also layer IV of S1 express high levels of CART mRNA at P06. Our findings that CART is expressed in the VPM/VPL only during early postnatal ages indicate that CART is involved in the neuronal development of thalamo–cortical–thalamic somatosensory circuitry, but not in neuronal communication/neurotransmission in adult life. Further studies will be necessary to test our model.

CART mRNA is also highly expressed in the primary olfactory piriform cortex of rats at P06. The piriform cortex receives direct input from the main olfactory bulb (Haberly, 1985). Interestingly, studies have shown that neurons in this site are not completely developed until P14 (Schwob and Price, 1984; Walz et al., 2006). Thus, as for the somatosensory pathways, CART may also play a role in the development and establishment of olfactory circuitry in the rodent brain.

Previous studies have shown that CART peptide promotes cellular differentiation and prolong the viability of hippocampal neurons in culture (Louis, 1996). The hippocampal neurons express high levels of CART and also of brain-derived neurotrophic factor (BDNF) (Koylu et al., 1998; Ivanova and Beyer, 2001; Ábrahám et al., 2007). The BDNF role in neuronal development is well defined. It regulates axonal and dendritic elongation, synaptic plasticity, and a variety of cellular and molecular aspects related to neuronal development (Barde et al., 1982; Murphy et al., 1998; Sherwood and Lo, 1999; Binder and Scharfman, 2004). The BDNF expression is modulated by a series of neuropeptides, including CART, which induces BDNF expression in hippocampal cell culture (Wu et al., 2006). Neurogenesis of hippocampal cells starts relatively late in embryonic development (E15–17), and high proliferation of dentate gyrus neurons occurs in early postnatal age (Reznikov, 1991). In agreement with a role for CART in neuronal development, we found very abundant expression of CART mRNA in the dentate gyrus of rats at P06. Of note, previous studies have found that CART peptide is detected in the granular layer of the dentate gyrus at postnatal day P12 and that its levels increase until reach the adult levels, at P30 (Ábrahám et al., 2007). Our studies used different time points and methodologies, but we found a somewhat similar result. At P06, CART mRNA was very high, but CART immunoreactivity was low. Moreover, at P26 CART mRNA and peptide were not different from adult levels. Studies comparing the expression of CART mRNA in the dentate gyrus and central amygdala of rats at two different ages (prepubertal P21 and adult P70) have identified higher CART expression in prepubertal rats (Hunter et al., 2007). In the present study we observed a similar trend concerning the central amygdala but we did not detect a significant difference in the dentate gyrus at P26 and P66. This apparent conflicting data are probably due to a gradual change or lack of a clear contrast across development. In favor of this argument is the fact that when analyzed in pairs (P26 vs. P66, Student *t*-test, data not shown) a similar result as described previously can be obtained; higher CART mRNA expression in prepubertal rats (P=0.02) in both sexes. Additionally, studies in human tissue have found higher levels of CART expression in the adult compared to fetal hippocampus (Bai et al., 2005). Because of species differences (human vs. rat), distinct developmental stages (fetal vs. postnatal) and methodology (hippocampal blocks vs. site-specific quantification of hybridization signal), the findings are not comparable. However, further studies assessing different time points (including embryonic development) may generate additional insights.

We also observed high levels of CART in the anterior aspects of the hippocampal formation —the indusium griseum—which displays similarities with the granular layer of the dentate gyrus (Wyss and Sripanidkulchai, 1983). Thus, our findings suggest the existence of a common postnatal developmental drive in both neuronal populations.

CART also plays a role in the regulation of dopamine reuptake and development of dopaminergic neurons in cell culture (Louis, 1996; Rogge et al., 2008). Some anatomical and pharmacological data collected in rodents and primates have shown evidence for a reciprocal connection between dopamine and CART in brain pathways related to reward and motivational processes. The CART neurons from the nucleus accumbens coexpress dopamine receptors and receive apparent synaptic contact from dopaminergic terminals (Smith et al., 1999; Hubert and Kuhar, 2006). These CART neurons also project to

dopaminergic cells in the midbrain (Dallvechia-Adams et al., 2001, 2002). Interestingly, CART administration into the ventral striatum caused no changes in the locomotor activity, but blunted the effects of dopamine and amphetamine (Jaworski et al., 2003; Kim et al., 2003). Following the same line, intra-accumbal administration of CART reduced the effects of cocaine in a drug self-administration paradigm (Jaworski et al., 2008). Thus, CART may play a complex homeostatic role in reward processes at the level of the nucleus accumbens (Rogge et al., 2008). Our findings of increased CART mRNA expression in this site in rats at early postnatal age may indicate a role in the establishment of reward pathways and in the neuronal development of the ventral striatum circuitry. Because of the reciprocal connections and intimate relationship between CART and dopaminergic neurons, it would be interesting to assess the relative correspondence of their maturational process across development.

As mentioned, CART is reduced in the arcuate nucleus of leptin-deficient (*ob/ob*) obese mice. Leptin administration to leptin-deficient mice normalizes CART mRNA expression in the arcuate nucleus (Kristensen et al., 1998). We have previously shown that CART and leptin receptors are colocalized in a variety of hypothalamic nuclei in adult rats (Elias et al., 2000, 2001). Thus, in the present study, we gave special attention to changes in CART mRNA expression in these specific hypothalamic sites. Contrary to our expectations, we found that only the ventral premammillary nucleus expressed high levels of CART mRNA in rats at early postnatal ages. The ventral premammillary nucleus is activated following conspecific odor stimulation and is highly connected with sexually dimorphic areas and with those related to the vomeronasal system and to the control of the reproductive function (Canteras et al., 1992; Rondini et al., 2004; Boehm et al., 2005; Cavalcante et al., 2006a,b; Leshan et al., 2009; Donato et al., 2010). Importantly, female odors stimulate CART mRNA expression in the ventral premammillary nucleus of adult male rats (Cavalcante et al., 2006a). Odor discrimination is a vital faculty for a newborn's recognition of their food source, the lactating dams (Shair, 2007; Moriceau et al., 2010). Whether the ventral premammillary nucleus is involved in this process is not known, but we interpreted our findings as suggestive of high transcriptional activity of neurons related to odor discrimination in rats at early postnatal development. However, if CART drives neuronal developmental changes in this specific hypothalamic site is also a matter for further investigation.

As shown before in mice (Ahima and Hileman, 2000), CART mRNA levels were very low in the arcuate nucleus of rats at early postnatal ages and increased at P26 and P66. During early postnatal development, leptin levels are high and are not directly regulated by food restriction or satiety (Ahima et al., 1998). Thus, CART mRNA levels in the arcuate nucleus may not be directly correlated with nutritional state or leptin levels during development. However, the decreased expression of CART mRNA may bring about beneficial effects on the offspring's feeding behavior, as it may facilitate or drive the increased milk consumption.

The present study showed that CART mRNA is increased in several forebrain nuclei of rats at P06. Particularly, we found increased CART expression in sites related to sensorial processing (primary somatosensory and olfactory—piriform— cortices and thalamic VPM

and VPL nuclei), reward (accumbens), learning and memory formation (dentate gyrus and indusium griseum). Additional studies will be necessary to systematically evaluate CART peptide expression in these sites as well as their target areas, but our findings suggest that CART may play a relevant role in the postnatal development of selective brain pathways.

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Abbreviations

AVPV	anteroventral periventricular nucleus
BDNF	brain-derived neurotrophic factor
CART	cocaine- and amphetamine-regulated transcript
IOD	integrated optical density
S1	primary somatosensory cortex
VPL	ventral posterolateral thalamic nucleus
VPM	ventral posteromedial thalamic nucleus

References

Ábrahám H, Orsi G, Seress L. Ontogeny of cocaine- and amphetamine-regulated transcript (CART) peptide and calbindin immunoreactivity in granule cells of the dentate gyrus in the rat. Int J Dev Neurosci. 2007; 25:265–274. [PubMed: 17616293]

Ahima RS, Bjorbaek C, Osei S, Flier JS. Regulation of neuronal and glial proteins by leptin: implications for brain development. Endocrinology. 1999; 140:2755–2762. [PubMed: 10342866]

Ahima RS, Hileman SM. Postnatal regulation of hypothalamic neuropeptide expression by leptin: implications for energy balance and body weight regulation. Regul Pept. 2000; 92:1–7. [PubMed: 11024558]

Ahima RS, Prabakaran D, Flier JS. Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. J Clin Invest. 1998; 101:1020–1027. [PubMed: 9486972]

Allendoerfer KL, Shatz CJ. The Subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. Annu Rev Neurosci. 1994; 17:185–218. [PubMed: 8210173]

Andersen P, Eccles JC, Sears TA. The ventrobasal complex of the thalamus: types of cells, their responses and their functional organization. J Physiol. 1964; 174:370–399. [PubMed: 14232399]

Bai F, Sözen MA, Lukiw WJ, Argyropoulos G. Expression of AgRP, NPY, POMC and CART in human fetal and adult hippocampus. Neuropeptides. 2005; 39:439–443. [PubMed: 15885775]

Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1982; 1:549–553. [PubMed: 7188352]

Bereiter DA, Jeanrenaud B. Altered neuroanatomical organization in the central nervous system of the genetically obese (ob/ob) mouse. Brain Res. 1979; 165:249–260. [PubMed: 421139]

Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors. 2004; 22:123–131. [PubMed: 15518235]

Boehm U, Zou Z, Buck LB. Feedback loops link odor and pheromone signaling with reproduction. Cell. 2005; 123:683–695. [PubMed: 16290036]

Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. Science. 2004; 304:108–110. [PubMed: 15064420]

Bouret SG, Simerly RB. Minireview: leptin and development of hypothalamic feeding circuits. Endocrinology. 2004; 145:2621–2626. [PubMed: 15044371]

Brischoux F, Griffond B, Fellmann D, Risold PY. Early and transient ontogenetic expression of the cocaine- and amphetamine-regulated transcript peptide in the rat mesencephalon: correlation with tyrosine hydroxylase expression. J Neurobiol. 2002; 52:221–229. [PubMed: 12210105]

Broberger C. Hypothalamic cocaine- and amphetamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. Brain Res. 1999; 848:101–113. [PubMed: 10612702]

Canteras NS, Simerly RB, Swanson LW. Projections of the ventral premammillary nucleus. J Comp Neurol. 1992; 324:195–212. [PubMed: 1430329]

Cavalcante JC, Bittencourt JC, Elias CF. Female odors stimulate CART neurons in the ventral premammillary nucleus of male rats. Physiol Behav. 2006a; 88:160–166. [PubMed: 16687159]

Cavalcante JC, Sita LV, Mascaro MB, Bittencourt JC, Elias CF. Distribution of urocortin 3 neurons innervating the ventral premammillary nucleus in the rat brain. Brain Res. 2006b; 1089:116–125. [PubMed: 16638605]

Couceyro PR, Koylu EO, Kuhar MJ. Further studies on the anatomical distribution of CART by in situ hybridization. J Chem Neuroanat. 1997; 12:229–241. [PubMed: 9243343]

Dall Vechia S, Lambert PD, Couceyro PC, Kuhar MJ, Smith Y. CART peptide immunoreactivity in the hypothalamus and pituitary in monkeys: analysis of ultrastructural features and synaptic connections in the paraventricular nucleus. J Comp Neurol. 2000; 416:291–308. [PubMed: 10602089]

Dallvechia-Adams S, Kuhar MJ, Smith Y. Cocaine- and amphetamine-regulated transcript peptide projections in the ventral midbrain: colocalization with γ-aminobutyric acid, melanin-concentrating hormone, dynorphin, and synaptic interactions with dopamine neurons. J Comp Neurol. 2002; 448:360–372. [PubMed: 12115699]

Dallvechia-Adams S, Smith Y, Kuhar MJ. CART peptide-immunoreactive projection from the nucleus accumbens targets substantia nigra pars reticulata neurons in the rat. J Comp Neurol. 2001; 434:29–39. [PubMed: 11329127]

Dominguez G, Vicentic A, Del Giudice EM, Jaworski J, Hunter RG, Kuhar MJ. CART peptides: modulators of mesolimbic dopamine, feeding, and stress. Ann N Y Acad Sci. 2004; 1025:363–369. [PubMed: 15542737]

Donato J Jr, Cavalcante JC, Silva RJ, Teixeira AS, Bittencourt JC, Elias CF. Male and female odors induce Fos expression in chemically defined neuronal population. Physiol Behav. 2010; 99:67–77. [PubMed: 19857504]

Donato J Jr, Silva RJ, Sita LV, Lee S, Lee C, Lacchini S, Bittencourt JC, Franci CR, Canteras NS, Elias CF. The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. J Neurosci. 2009; 29:5240–5250. [PubMed: 19386920]

Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci. 1995; 15:2471–2481. [PubMed: 7891182]

Dun NJ, Dun SL, Wong PY, Yang J, Chang J. Cocaine- and amphetamine-regulated transcript peptide in the rat epididymis: an immunohistochemical and electrophysiological study. Biol Reprod. 2000; 63:1518–1524. [PubMed: 11058560]

Dun SL, Castellino SJ, Yang J, Chang JK, Dun NJ. Cocaine-and amphetamine-regulated transcript peptide-immunoreactivity in dorsal motor nucleus of the vagus neurons of immature rats. Brain Res Dev Brain Res. 2001; 131:93–102.

Elias CF, Kelly JF, Lee CE, Ahima RS, Drucker DJ, Saper CB, Elmquist JK. Chemical characterization of leptin-activated neurons in the rat brain. J Comp Neurol. 2000; 423:261–281. [PubMed: 10867658]

Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, Elmquist JK. Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron. 1998; 21:1375–1385. [PubMed: 9883730]

Elias CF, Lee CE, Kelly JF, Ahima RS, Kuhar M, Saper CB, Elmquist JK. Characterization of CART neurons in the rat and human hypothalamus. J Comp Neurol. 2001; 432:1–19. [PubMed: 11241374]

Haberly LB. Neuronal circuitry in olfactory cortex: anatomy and functional implications. Chem Senses. 1985; 10:219–238.

Herbison AE. Estrogen positive feedback to gonadotropin-releasing hormone (GnRH) neurons in the rodent: the case for the rostral periventricular area of the third ventricle (RP3V). Brain Res Rev. 2008; 57:277–287. [PubMed: 17604108]

Hevner RF, Miyashita-Lin E, Rubenstein JL. Cortical and thalamic axon pathfinding defects in Tbr1, Gbx2, and Pax6 mutant mice: evidence that cortical and thalamic axons interact and guide each other. J Comp Neurol. 2002; 447:8–17. [PubMed: 11967891]

Hubert GW, Kuhar MJ. Colocalization of CART peptide with prodynorphin and dopamine D1 receptors in the rat nucleus accumbens. Neuropeptides. 2006; 40:409–415. [PubMed: 17064765]

Hunter RG, Bellani R, Bloss E, Costa A, Romeo RD, McEwen BS. Regulation of CART mRNA by stress and corticosteroids in the hippocampus and amygdala. Brain Res. 2007; 1152:234–240. [PubMed: 17434149]

Ivanova T, Beyer C. Pre- and postnatal expression of brain-derived neurotrophic factor mRNA/protein and tyrosine protein kinase receptor B mRNA in the mouse hippocampus. Neurosci Lett. 2001; 307:21–24. [PubMed: 11516565]

Jaworski JN, Hansen ST, Kuhar MJ, Mark GP. Injection of CART (cocaine- and amphetamine-regulated transcript) peptide into the nucleus accumbens reduces cocaine self-administration in rats. Behav Brain Res. 2008; 191:266–271. [PubMed: 18485497]

Jaworski JN, Kozel MA, Philpot KB, Kuhar MJ. Intra-accumbal injection of CART (cocaine-amphetamine regulated transcript) peptide reduces cocaine-induced locomotor activity. J Pharmacol Exp Ther. 2003; 307:1038–1044. [PubMed: 14551286]

Jensen KF, Killackey HP. Terminal arbors of axons projecting to the somatosensory cortex of the adult rat. I. The normal morphology of specific thalamocortical afferents. J Neurosci. 1987; 7:3529–3543. [PubMed: 3316525]

Jones EG. Synchrony in the interconnected circuitry of the thalamus and cerebral cortex. Ann N Y Acad Sci. 2009; 1157:10–23. [PubMed: 19351352]

Killackey HP, Sherman SM. Corticothalamic projections from the rat primary somatosensory cortex. J Neurosci. 2003; 23:7381–7384. [PubMed: 12917373]

Kim JH, Creekmore E, Vezina P. Microinjection of CART peptide 55–102 into the nucleus accumbens blocks amphetamine-induced locomotion. Neuropeptides. 2003; 37:369–373. [PubMed: 14698680]

Koylu EO, Couceyro PR, Lambert PD, Kuhar MJ. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. J Comp Neurol. 1998; 391:115–132. [PubMed: 9527537]

Koylu EO, Couceyro PR, Lambert PD, Ling NC, DeSouza EB, Kuhar MJ. Immunohistochemical localization of novel CART peptides in rat hypothalamus, pituitary and adrenal gland. J Neuroendocrinol. 1997; 9:823–833. [PubMed: 9419833]

Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature. 1998; 393:72–76. [PubMed: 9590691]

Kuhar MJ, Adams S, Dominguez G, Jaworski J, Balkan B. CART peptides. Neuropeptides. 2002; 36:1–8. [PubMed: 12147208]

Lambert PD, Couceyro PR, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ. CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse. 1998; 29:293–298. [PubMed: 9661247]

Leshan RL, Louis GW, Jo Y-H, Rhodes CJ, Münzberg H, Myers MG Jr. Direct innervation of GnRH neurons by metabolic- and sexual odorant-sensing leptin receptor neurons in the hypothalamic ventral premammillary nucleus. J Neurosci. 2009; 29:3138–3147. [PubMed: 19279251]

Louis, JCM. Methods of preventing neuron degeneration and promoting neuron regeneration. Amgen WO 96/34619. 1996.

McQuillen PS, DeFreitas MF, Zada G, Shatz CJ. A novel role for p75NTR in subplate growth cone complexity and visual thalamocortical innervation. J Neurosci. 2002; 22:3580–3593. [PubMed: 11978834]

Molnár Z, Blakemore C. How do thalamic axons find their way to the cortex? Trends Neurosci. 1995; 18:389–397. [PubMed: 7482804]

Moriceau S, Roth TL, Sullivan RM. Rodent model of infant attachment learning and stress. Dev Psychobiol. 2010; 52:651–660. [PubMed: 20730787]

Murphy DD, Cole NB, Segal M. Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. Proc Natl Acad Sci U S A. 1998; 95:11412–11417. [PubMed: 9736750]

Nicolelis MA, Fanselow EE. Thalamocortical optimization of tactile processing according to behavioral state. Nat Neurosci. 2002; 5:517–523. [PubMed: 12037519]

Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL. Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science. 2004; 304:110–115. [PubMed: 15064421]

Reznikov KY. Cell proliferation and cytogenesis in the mouse hippocampus. Adv Anat Embryol Cell Biol. 1991; 122:1–74. [PubMed: 1927657]

Rogge G, Jones D, Hubert GW, Lin Y, Kuhar MJ. CART peptides: regulators of body weight, reward and other functions. Nat Rev Neurosci. 2008; 9:747–758. [PubMed: 18802445]

Rondini TA, Baddini SP, Sousa LF, Bittencourt JC, Elias CF. Hypothalamic cocaine- and amphetamine-regulated transcript neurons project to areas expressing gonadotropin releasing hormone immunoreactivity and to the anteroventral periventricular nucleus in male and female rats. Neuroscience. 2004; 125:735–748. [PubMed: 15099687]

Schwob JE, Price JL. The development of axonal connections in the central olfactory system of rats. J Comp Neurol. 1984; 223:177–202. [PubMed: 6200518]

Shair HN. Acquisition and expression of a socially mediated separation response. Behav Brain Res. 2007; 182:180–192. [PubMed: 17379325]

Sherwood NT, Lo DC. Long-term enhancement of central synaptic transmission by chronic brain-derived neurotrophic factor treatment. J Neurosci. 1999; 19:7025–7036. [PubMed: 10436057]

Smith Y, Kieval J, Couceyro PR, Kuhar MJ. CART peptide-immunoreactive neurones in the nucleus accumbens in monkeys: ultrastructural analysis, colocalization studies, and synaptic interactions with dopaminergic afferents. J Comp Neurol. 1999; 407:491–511. [PubMed: 10235641]

Steppan CM, Swick AG. A role for leptin in brain development. Biochem Biophys Res Commun. 1999; 256:600–602. [PubMed: 10080944]

Steriade M, Llinás RR. The functional states of the thalamus and the associated neuronal interplay. Physiol Rev. 1988; 68:649–742. [PubMed: 2839857]

Valera AG, Cavalcante JC, Elias CF, Felício LF. Cocaine- and amphetamine-regulated transcript is overexpressed in the anteroventral periventricular nucleus of pregnant rats. J Neuroendocrinol. 2006; 18:711–714. [PubMed: 16879170]

Vrang N, Larsen PJ, Clausen JT, Kristensen P. Neurochemical characterization of hypothalamic cocaine-amphetamine-regulated transcript neurons. J Neurosci. 1999; 19:RC5. [PubMed: 10234051]

Walz A, Omura M, Mombaerts P. Development and topography of the lateral olfactory tract in the mouse: imaging by genetically encoded and injected fluorescent markers. J Neurobiol. 2006; 66:835–846. [PubMed: 16673392]

Wiegand SJ, Terasawa E. Discrete lesions reveal functional heterogeneity of suprachiasmatic structures in regulation of gonadotropin secretion in the female rat. Neuroendocrinology. 1982; 34:395–404. [PubMed: 6808412]

Wu B, Hu S, Yang M, Pan H, Zhu S. CART peptide promotes the survival of hippocampal neurons by upregulating brain-derived neurotrophic factor. Biochem Biophys Res Commun. 2006; 347:656–661. [PubMed: 16842741]

Wu C-S, Zhu J, Wager-Miller J, Wang S, O'Leary D, Monory K, Lutz B, Mackie K, Lu H-C. Requirement of cannabinoid CB1 receptors in cortical pyramidal neurons for appropriate development of corticothalamic and thalamocortical projections. Eur J Neurosci. 2010; 32:693–706. [PubMed: 21050275]

Wyss JM, Sripanidkulchai K. The indusium griseum and anterior hippocampal continuation in the rat. J Comp Neurol. 1983; 219:251–272. [PubMed: 6619339]

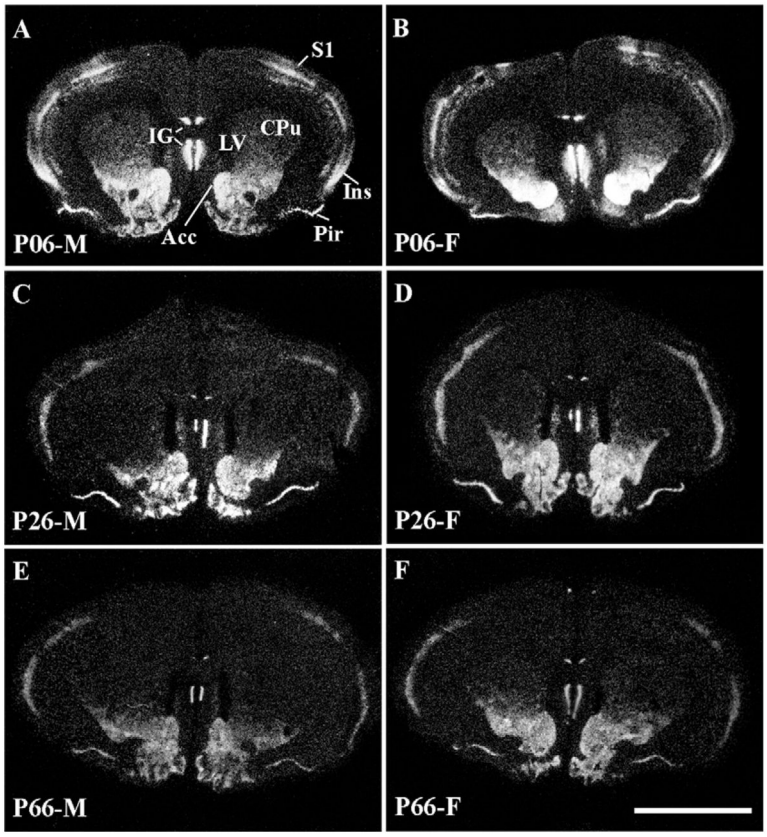

Fig. 1.

Distribution of CART mRNA in the rostral forebrain of male and female rats. (A–F) Darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the striatum, indusium griseum (IG), somatosensory cortex (S1), piriform cortex (Pir) and insular cortex (Ins) of male (M) and female (F) rats at P06 (A, B), at P26 (C, D) and at P66 (E, F). Abbreviations: Acc, nucleus accumbens; LV, lateral ventricle. Scale bar: 1200 μm.

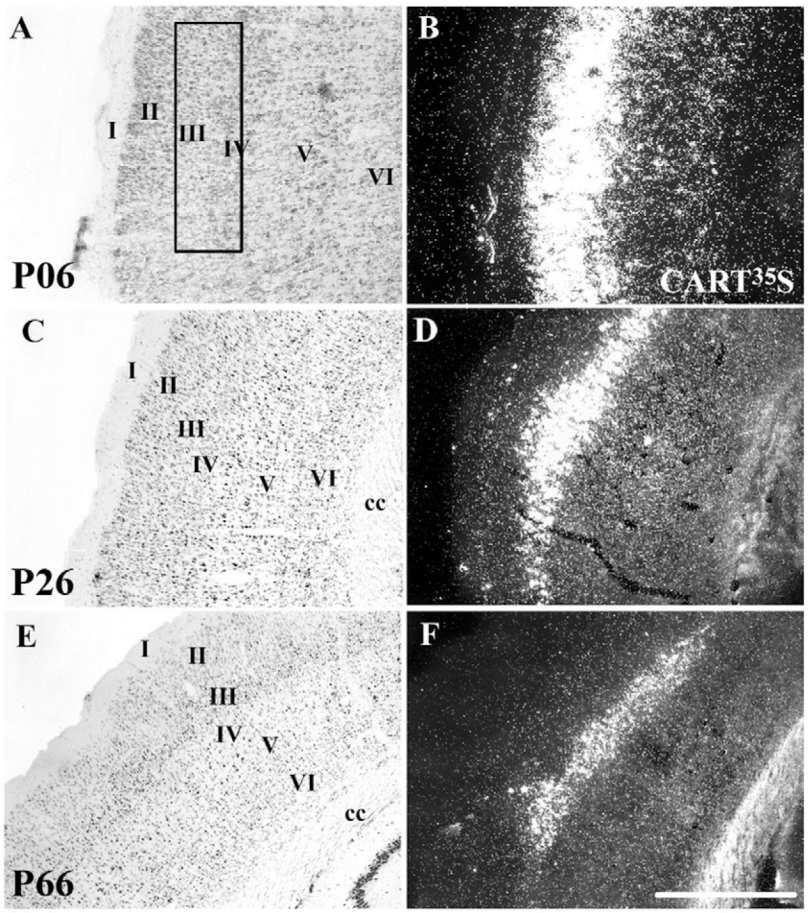

Fig. 2.

Distribution of CART mRNA in the primary somatosensory cortex (S1) of male rats. (A–F) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the S1 of male rats at P06 (A, B), at P26 (C, D) and at P66 (E, F). (A, C, E) Adjacent sections submitted to Thionin staining for reference. Square in (A) represents the area of interest used for quantification. Abbreviations: I–VI, cortical layers; cc, corpus callosum. Scale bar=400 μm.

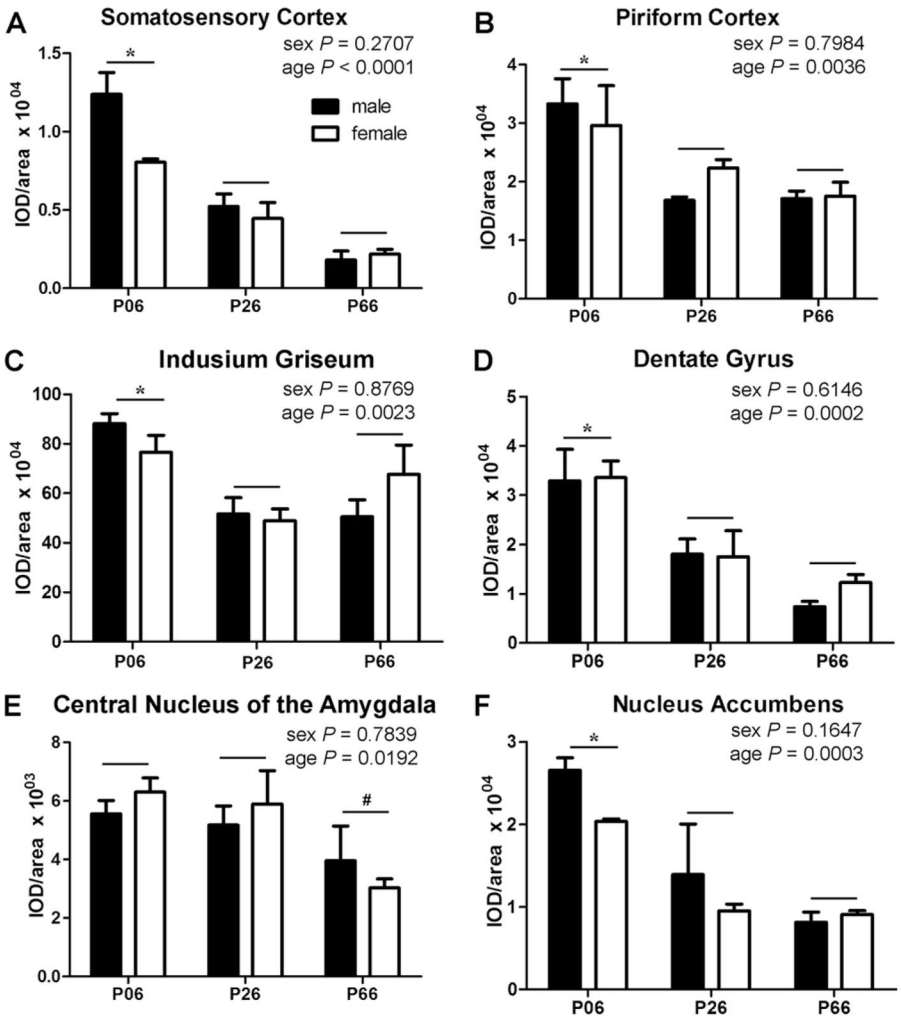

Fig. 3.

Bar graphs showing the quantification analysis of hybridization signal in the somatosensory cortex (A), the piriform cortex (B), indusium griseum (C), dentate gyrus (D), central nucleus of the amygdala (E), and nucleus accumbens (F) of male and female rats in different postnatal developmental stages (P06, P26 and P66). Quantification was performed using integrated optical density (IOD). Data are expressed as mean±SEM (black bars = male, white bars = female). * Statistically different from P26 and P66. # Statistically different from P06 and P26. *P*-values for both factors considered in the statistical analysis (sex and ages) are illustrated in each graph. No interaction between factors was observed ($P>0.05$).

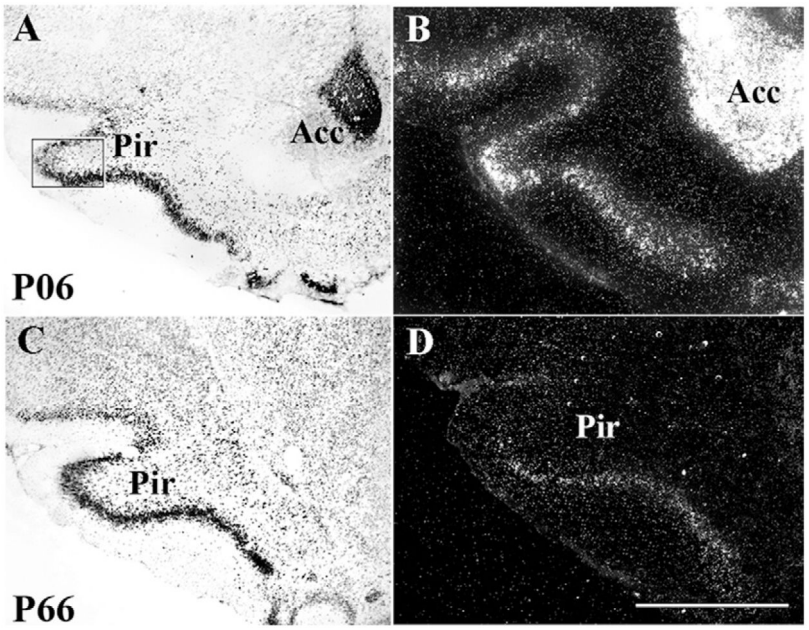

Fig. 4.

Distribution of CART mRNA in the piriform cortex (Pir) of male rats. (A–D) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the rostral aspect of the piriform cortex of male rats at P06 (A, B) and at P66 (C, D). (A, C) Adjacent sections submitted to Thionin staining for reference. Square in (A) represents the area of interest used for quantification. Abbreviations: Acc, nucleus accumbens. Scale bar=400 μm.

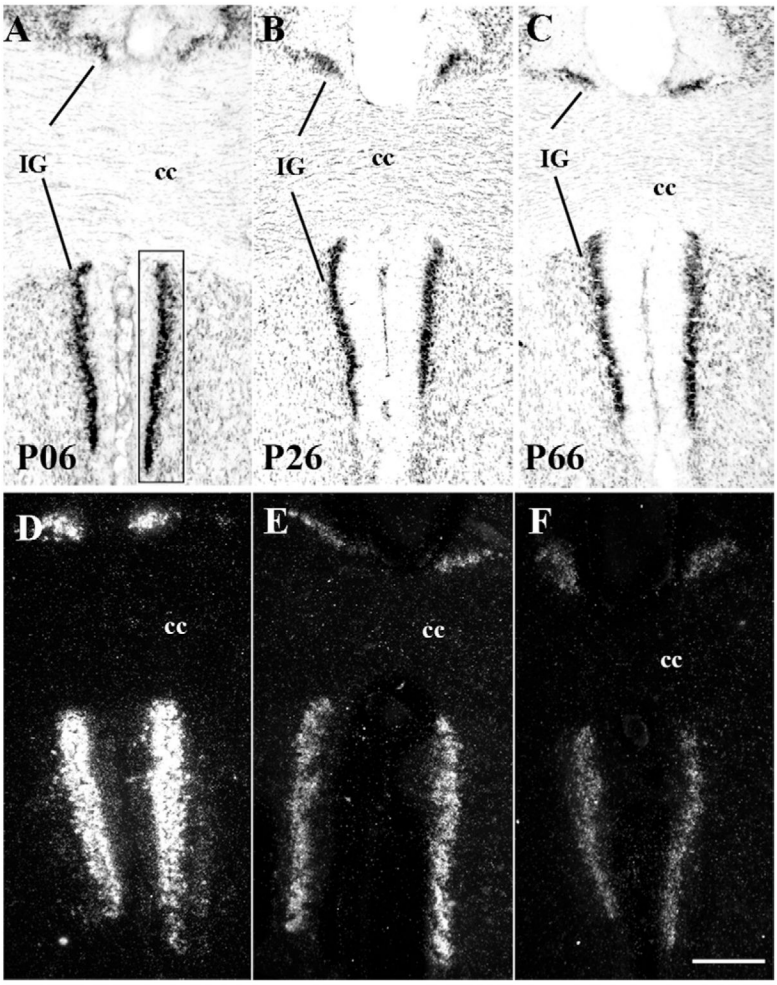

Fig. 5.

Distribution of CART mRNA in the indusium griseum (IG) of male rats. (A–F) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the IG of male rats at P06 (A, D), at P26 (B, E) and at P66 (C, F). (A–C) Adjacent sections submitted to Thionin staining for reference. Square in (A) represents the area of interest used for quantification. Abbreviations: cc, corpus callosum. Scale bar=200 μm.

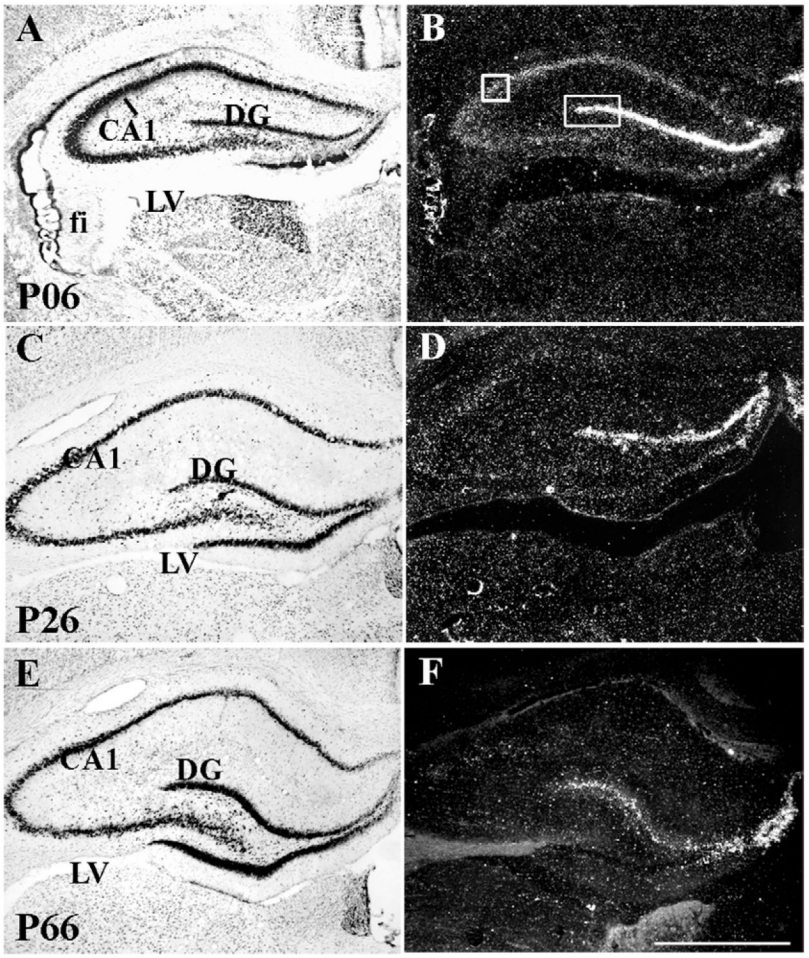

Fig. 6.

Distribution of CART mRNA in the hippocampus of male rats. (A–F) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the hippocampus of male rats at P06 (A, B), at P26 (C, D) and at P66 (E, F). (A, C, E) Adjacent sections submitted to Thionin staining for reference. Squares in (B) represent the areas of interest used for quantification of hybridization signal in the dentate gyrus (DG) and CA1 field. Abbreviations: fi, fimbria; LV, lateral ventricle. Scale bar=400 μm.

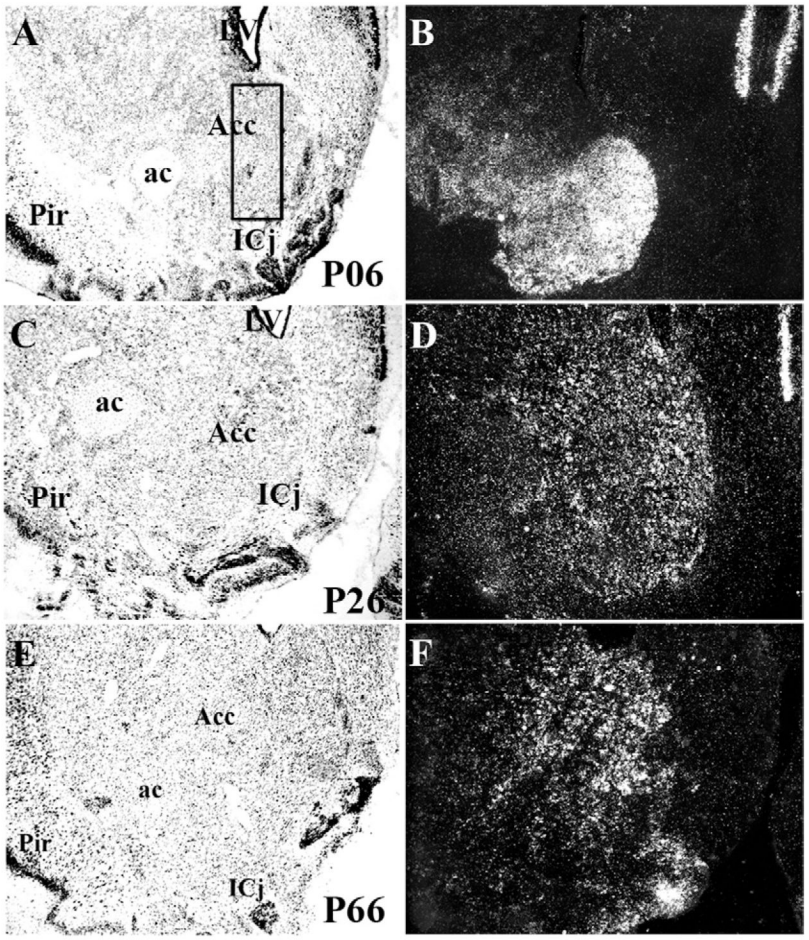

Fig. 7.

Distribution of CART mRNA in the nucleus accumbens (Acc) of male rats. (A–F) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the Acc of male rats at P06 (A, B), at P26 (C, D) and at P66 (E, F). (A, C, E) Adjacent sections submitted to Thionin staining for reference. Square in (A) represents the area of interest used for quantification. Abbreviations: ac, anterior commissure; ICj, islands of Calleja; LV, lateral ventricle; Pir, piriform cortex. Scale bar=400 μm.

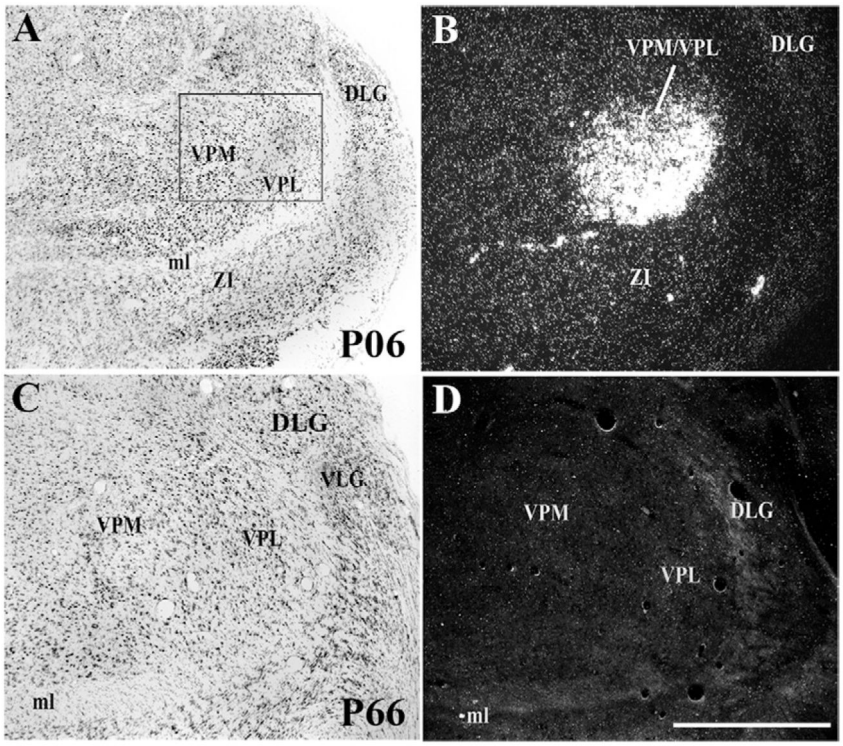

Fig. 8.

Distribution of CART mRNA in the ventral posteromedial (VPM) and ventral posterolateral (VPL) thalamic nuclei of male rats. (A–D) Bright-and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the VPM/VPL of male (M) rats at P06 (A, B) and at P66 (C, D). (A, C) Adjacent sections submitted to Thionin staining for reference. Square in (A) represents the area of interest used for quantification. Abbreviations: DLG, dorsal lateral geniculate nucleus; ml, medial lemniscus; VLG, ventral lateral geniculate nucleus; ZI, zona incerta. Scale bar=400 μm.

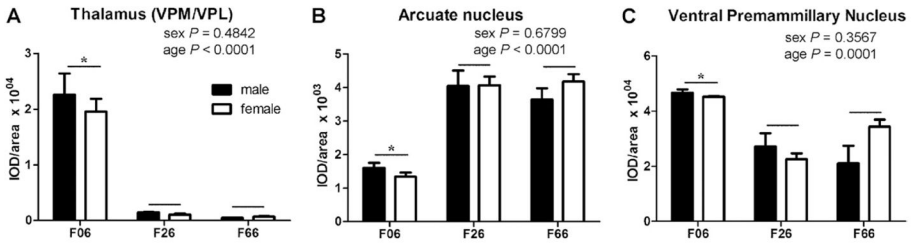

Fig. 9.

Bar graphs showing the quantification analysis of hybridization signal in the ventral posteromedial (VPM) and ventral posterolateral (VPL) thalamic nuclei (A), arcuate nucleus (B), and the ventral premammillary nucleus (C) of male and female rats in different postnatal developmental stages (P06, P26, P66). Quantification was performed using integrated optical density (IOD). Data are expressed as mean±SEM (black bars = male, white bars = female). *Statistically different from P26 and P66 ($P<0.05$). *P*-values for both factors considered in the statistical analysis (sex and ages) are illustrated in each graph. No interaction between factors was observed ($P>0.05$).

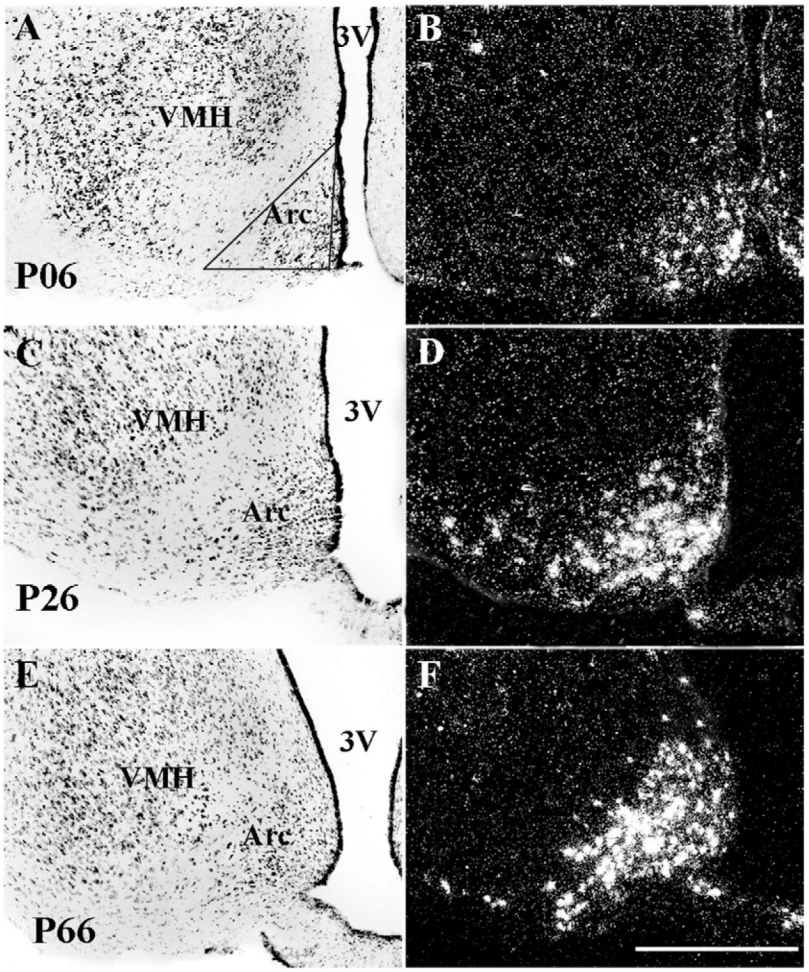

Fig. 10.

Distribution of CART mRNA in the arcuate nucleus (Arc) of male rats. (A–F) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the Arc of male rats at P06 (A, B), at P26 (C, D) and at P66 (E, F). (A, C, E) Adjacent sections submitted to Thionin staining for reference. Triangle in (A) represents the area of interest used for quantification. Abbreviations: 3v, third ventricle; VMH, ventromedial nucleus of the hypothalamus. Scale bar=400 μm.

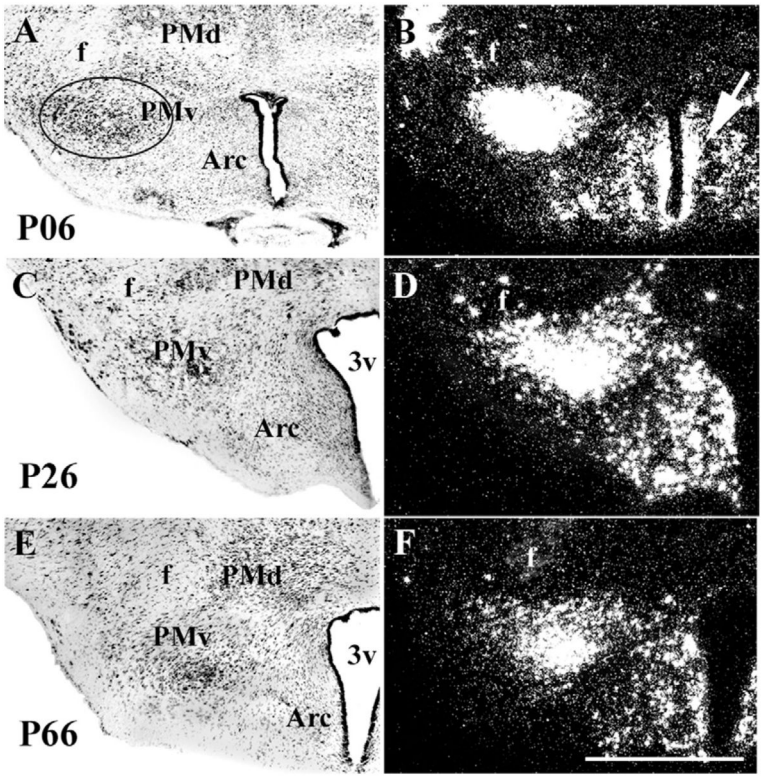

Fig. 11.

Distribution of CART mRNA in the ventral premammillary nucleus (PMv) of male rats. (A–F) Bright- and darkfield photomicrographs showing the distribution of CART mRNA expression (hybridization signal) in the PMv of male rats at P06 (A, B), at P26 (C, D) and at P66 (E, F). (A, C, E) Adjacent sections submitted to Thionin staining for reference. Ellipse in (A) represents the area of interest used for quantification. Abbreviations: 3v, third ventricle; Arc, arcuate nucleus; f, fornix; PMd, dorsal premammillary nucleus. Note the increased CART expression in the ependymal layer of the 3v in rats at P06 (arrow). Scale bar=400 μm.

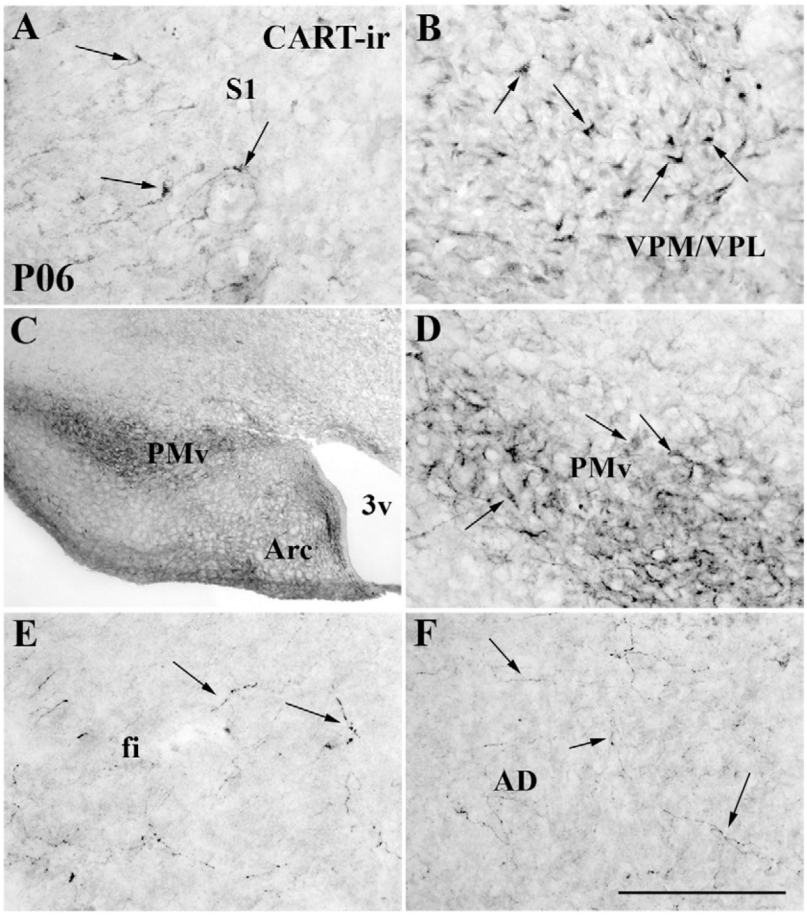

Fig. 12.

Distribution of CART immunoreactivity (CART-ir) in the forebrain of male rats at 6 days of age (P06). (A–D) Brightfield photomicrographs showing the distribution of CART immunoreactive cell bodies (arrows) in the primary somatosensorial cortex (S1, A), in the ventral posteromedial and ventral posterolateral (VPM/VPL) thalamic nuclei (B), and in the ventral premammillary nucleus (PMv, C, D). Note the pyramidal shape of cells in (A) and the multipolar shape of cells in (B) and (D). (D) is a higher magnification of (C). (E, F) Brightfield photomicrographs showing the distribution of CART immunoreactive fibers (arrows) in the fimbria of hippocampus (fi) and in the anterodorsal thalamic nucleus (AD). Abbreviations: 3v, third ventricle; Arc, arcuate nucleus. Scale bar: (A), (B), (D–F)=100 μm; (C)=400 μm.

Table 1

Subjective analysis of CART mRNA expression in the forebrain of male and female rats at different post-natal ages: P06, P26 and P66.++++, very abundant; +++, abundant; ++, moderate; +, low; −, absent

Forebrain regions	P06		P26		P66	
	Male	Female	Male	Female	Male	Female
Somatosensory cortex (S1)	++++	++++	++	++	+	+
Piriform cortex	+++	+++	++	++	+	+
Insular cortex	+++	+++	+	+	+	+
Indusium griseum	++++	++++	++	++	++	++
Dentate gyrus	+++	+++	++	++	+	+
CA1, CA2, CA3 fields	++	++	+	+	+	+
Central nucleus of the amygdala	++	++	++	++	+	+
Nucleus accumbens	++++	++++	++	++	++	++
Ventral posteromedial (VPM)	+++	+++	−	−	−	−
Ventral posterolateral (VPL)	+++	+++	−	−	−	−
Anteroventral periventricular nucleus	+	+	+	+	+	++
Arcuate nucleus	++	++	+++	+++	+++	+++
Ventral premammillar nucleus	++++	++++	+++	+++	++	++
Ependymal layer, posterior 3v	++++	++++	+	+	−	−