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GPR88 - a putative signaling molecule predominantly expressed in the striatum: Cellular localization and developmental regulation

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Abstract

GPR88 is a putative G-protein-coupled receptor that is highly and almost exclusively expressed in the striatum. Its function remains unknown. We investigated GPR88 cellular localization and expression levels across development in different functional domains of the striatum in juvenile (P25), adolescent (P40), and adult (P70) rats, by in situ hybridization histochemistry. At all ages, GPR88 mRNA expression was most robust in the sensorimotor (lateral) striatum and was detected in virtually every neuron. Expression was highest in juveniles and decreased thereafter with regionally distinct trajectories. Thus, in the dorsal striatum, there was a progressive decrease from juveniles to adolescents to adults. In contrast, in the nucleus accumbens, the only (modest) decrease occurred between juveniles and adolescents. These findings indicate that GPR88 is expressed in all striatal neurons, but is differentially regulated across development in different striatal regions.

Keywords

GPR88; caudate-putamen; nucleus accumbens; rat; striatum

Introduction

The basal ganglia comprise a group of forebrain nuclei that are interconnected with the cerebral cortex, thalamus and brainstem [1;2]. Basal ganglia circuits mediate diverse brain functions including motor control, sensorimotor integration, attention, reward and cognition. Thus, dysfunction in basal ganglia circuits is implicated in a wide spectrum of disorders, including Parkinson's disease, Huntington's disease, obsessive-compulsive disorder, attention-deficit hyperactivity disorder, and psychostimulant addiction (e.g., [2–7]). The striatum (caudate-putamen, nucleus accumbens) constitutes the main input station of the basal ganglia and is thus critically important for the function of basal ganglia circuits. The striatum is composed of medium-sized GABAergic projection neurons and a small proportion of interneurons (<3% of striatal neurons) [8]. The projection neurons are divided

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into two subtypes, which give rise to the “direct” (striatonigral) and “indirect” (striatopallidal) output pathways [9]. The principal inputs to striatal neurons include excitatory (glutamate) afferents from various cortical and thalamic areas that drive striatal activity, and dopamine inputs from the midbrain that modulate activity flow in striatal output pathways [10].

The pharmacological strategies used in the treatment of disorders involving the striatum often focus on the dopamine (and glutamate) systems. For example, 3,4-dihydroxyphenyl-L-alanine (L-DOPA), a dopamine precursor, and antipsychotic medications targeting the D2 dopamine receptor have been mainstays in the treatment of Parkinson’s disease and schizophrenia, respectively, for decades. However, these treatments have several limitations. For one, dopamine and glutamate systems are present in many brain areas and mediate many brain functions. Systemically administered pharmacological agents targeting dopamine or glutamate signaling will thus also affect brain functions unrelated to basal ganglia circuits and produce unwanted side effects. Moreover, these drugs are often not fully effective. For example, after long-term treatment with L-DOPA or dopamine receptor antagonists, disruptive motor side effects typically emerge, which restricts the usefulness of these drugs [11]. Therefore, considerable emphasis has been placed on identifying alternative mechanisms that regulate basal ganglia systems, with the hope that these will lead to the development of better medications for such disorders.

One approach used to develop more selective agents is to find and evaluate new candidate molecules whose anatomical localization is regionally more restricted. GPR88 is such a molecule; it is almost exclusively localized in the striatum and, within this structure, is preferentially expressed in lateral parts [12;13]. Based on sequence homology, GPR88 is proposed to encode a novel G-protein-coupled receptor [12]. GPR88 expression has been shown in humans and other primates, rats and mice [12–15].

Presently, almost nothing is known on the function of GPR88. A recent study reported that, in GPR88 knockout mice, basal extracellular dopamine levels in the striatum were lower, while amphetamine-induced dopamine release was normal [15]. These mice also displayed increased apomorphine-induced stereotypy and amphetamine-stimulated locomotor activity [15]. These results suggest that GPR88 plays a role in the regulation of dopamine signaling in the striatum. Consistent with an interaction with the dopamine transmission, another study found that dopamine depletion or receptor stimulation produced (moderate) changes in GPR88 expression in striatal neurons [13]. However, manipulation of glutamate input to the striatum or various other drug treatments also altered the expression of GPR88 [13].

Beyond its gross distribution in the striatum, little is known on subregional differences in expression, cellular localization [13] and developmental regulation of GPR88. In this study, we investigated the cellular localization of GPR88 mRNA and its association with specific functional domains (as defined by their predominant cortical inputs; [16]) of the rat striatum, in order to determine the striatal targets of potential GPR88-selective pharmacological agents. We further assessed the developmental regulation of GPR88 by comparing expression in juveniles (P25), adolescents (P40), and adults (P70). The cellular localization was established by fluorescence in situ hybridization histochemistry in combination with immunohistochemistry. Expression levels were measured by radioactive in situ hybridization histochemistry.

Materials and methods

Subjects

Cellular localization and expression levels of GPR88 mRNA in the striatum were investigated in juvenile (postnatal day 25, P25), adolescent (P40), and adult (P70) male Sprague-Dawley rats (Harlan, Madison, WI, USA) (n=6 per group). The animals were allowed to habituate for 3 days after arrival before they were killed for tissue processing. All procedures met the NIH guidelines for the care and use of laboratory animals and were approved by the Rosalind Franklin University Animal Care and Use Committee.

Tissue preparation

The rats were killed with CO₂. The brain was rapidly removed, frozen in isopentane cooled on dry ice, and stored at -30 °C until cryostat sectioning. Twelve µm thick coronal sections were thaw-mounted onto glass slides (Superfrost/Plus, Daigler, Wheeling, IL, USA) and dried on a slide warmer. In preparation for the in situ hybridization histochemistry, the sections were fixed in 4% paraformaldehyde/0.9% saline for 10 min at room temperature, incubated in a fresh solution of 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% saline (pH 8.0) for 10 min, dehydrated, defatted for 2 times 5 min in chloroform, rehydrated, and air-dried. The slides were then stored at -30 °C until hybridization.

Double labeling by fluorescence in situ hybridization histochemistry combined with immunohistochemistry

Oligonucleotide probes (GPR88₁, GPR88₂, enkephalin; 48-mers; Invitrogen, Rockville, MD, USA) were labeled using the DIG-ddUTP oligonucleotide 3'-end labeling kit (Roche, Mannheim, Germany). The probes had the following sequence: GPR88₁, complementary to bases 451–498; GPR88₂, bases 613–660, GenBank Accession number AB042407; enkephalin, bases 436–483, M28263. The two GPR88 probes produced the same signal distribution; they were combined to increase the signal strength. No signal was observed with corresponding sense probes.

One hundred µl of hybridization buffer containing a mix of the two digoxigenin-labeled GPR88 probes (10 pmol each), or the digoxigenin-labeled enkephalin probe (10 pmol), was added to each slide. The sections were coverslipped and incubated at 37 °C overnight. After incubation, the slides were first rinsed in four washes of 1X saline citrate, and then washed 3 times 20 min each in 2X saline citrate/50% formamide at 40 °C, followed by 2 washes of 30 min each in 1X saline citrate at room temperature. The sections were then incubated 15 min in 2% H₂O₂ to remove endogenous peroxidase activity, rinsed 5 min in TNT washing buffer (0.1 M TRIS-HCl, 0.15 M sodium chloride, and 0.05% Tween 20), incubated 30 min in TNB blocking buffer (0.1 M TRIS-HCl, 0.15 M sodium chloride, and 0.5% blocking reagent; PerkinElmer, Waltham, MA, USA), and 30 min in anti-digoxigenin antibody conjugated with horseradish peroxidase (1:40, in TNB blocking buffer, Fab fragments; Roche). After 3 times 5 min rinses in TNT washing buffer, 200 µl of fluorophore tyramidine solution (TSA Plus fluorescence system; PerkinElmer) was added to each slide for 10 min, and the slides were then rinsed 3 times 5 min in TNT washing buffer. Slides were incubated overnight in mouse anti-neuronal nuclei (NeuN) antibody (1:500, clone A60; Millipore, Temecula, CA, USA), rinsed 3 times 5 min in TNT buffer, and incubated 2h in Alexa Fluor 594 goat anti-mouse antibody (1:200; Invitrogen, Carlsbad, CA, USA). The sections were finally washed 3 times 5 min in TNT buffer, briefly rinsed in water, air-dried and coverslipped with PVA-DABCO (Sigma-Aldrich; Allentown, PA, USA).

Sections were examined with a fluorescence microscope (Nikon ECLIPSE E400) linked to a high resolution Hamamatsu Orca-ER digital camera (C4742-80). Images were captured

using Stereo Investigator software (MBF Science, Williston, VT, USA). Gene expression in the striatum was evaluated in sections from three rostrocaudal levels (rostral, approximately at +1.6 mm relative to bregma, [17]; middle, +0.4; caudal, -0.8; Fig. 1), in a total of 23 sectors mostly defined by their predominant cortical inputs [16]. Eighteen of these sectors represented the caudate-putamen and 5 the nucleus accumbens.

Radioactive in situ hybridization histochemistry

The GPR88₁ oligonucleotide probe was labeled with [35S]-dATP as described earlier [16]. One hundred μ l of hybridization buffer containing labeled probe ($\sim 3 \times 10^6$ cpm) was added to each slide. The sections were coverslipped and incubated at 37 °C overnight. After incubation, the slides were washed as described above. The sections were air-dried and then apposed to X-ray film (BioMax MR-2, Kodak) for 3 days.

Hybridization signals on film autoradiograms were measured by densitometry (NIH Image; Wayne Rasband, NIMH, Bethesda, MD, USA). The films were captured using a light table (Northern Light, Imaging Research, St. Catharines, Ontario, Canada) and a Sony CCD camera (Imaging Research). The “mean density” value of a region of interest was measured by placing a template over the captured image. Mean densities were corrected for background by subtracting mean density values measured over white matter (corpus callosum). Values from corresponding regions in the two hemispheres were then averaged. The images of film autoradiograms displayed in Figure 1 are computer-generated and contrast-enhanced (linear). Maximal hybridization signal is black.

Statistics

The effect of age on GPR88 mRNA levels in the different striatal sectors was determined by one-factor ANOVA. The developmental trajectories in the caudate-putamen vs. nucleus accumbens were compared by two-factor ANOVA with age as between-subject variable and striatal region as within-subject variable. Newman-Keuls post hoc tests were used to describe differences between individual groups (Statistica, StatSoft, Tulsa, OK, USA). For illustrations of topographies (maps), gene expression in a given region was expressed relative to the maximal expression observed in the P25 group (% of max. P25). The difference in GPR88 expression between P25 and P70 was expressed as the percentage of the maximal change (% of max. Δ).

Results

GPR88 expression in the forebrain

Expression of GPR88 mRNA was very pronounced in the striatum (caudate-putamen, nucleus accumbens) on all rostrocaudal levels (Fig. 1). Robust expression was also present in the olfactory tubercle. In the cortex, modest GPR88 mRNA levels were found in superficial layers (layers 2 and 3), from the cingulate (dorsomedial) to the piriform areas (ventrolateral), while deep layers showed minimal or no expression (Fig. 1). In marked contrast to the striatum, no GPR88 expression was detected in the globus pallidus (Figs. 1 and 2).

GPR88 expression in striatal neurons

At the cellular level, the fluorescent GPR88 mRNA signal had a distinctive granular appearance (Figs. 2 and 3). Both in the caudate-putamen (Fig. 2, middle row) and in the nucleus accumbens (not shown), GPR88 expression was detected in every cell labeled with the neuronal marker NeuN, independent of age. For comparison, approximately 50% of the neurons expressed enkephalin mRNA (i.e., indirect pathway neurons) (Fig. 2, top row). In contrast, in the globus pallidus, NeuN-positive cells did not express detectable levels of

GPR88 mRNA (Fig. 2, bottom row). Therefore, our data indicate that, in the striatum, GPR88 is localized in both direct and indirect pathway neurons, as well as in interneurons. Notably, GPR88 mRNA was detected in large neurons (Fig. 3), presumably cholinergic interneurons. The intensity of the signal in these large neurons was typically in the lower range of signals detected in medium-sized neurons (Fig. 3). Overall, we did not observe a difference in the cellular localization of GPR88 mRNA between the 23 sectors of the striatum.

Regional and developmental variations in GPR88 expression in the striatum

Despite GPR88 expression in all striatal neurons, there were distinct regional variations and developmental changes present on all three rostrocaudal levels examined (Figs. 1 and 4). Independent of age, GPR88 mRNA levels displayed a robust medial-lateral gradient, with highest levels laterally, in the sensorimotor striatum (dorsolateral, ventrolateral sectors) and more moderate levels in medial, associative sectors (Figs. 1 and 4). GPR88 expression was also more moderate in the nucleus accumbens (Figs. 1, 4 and 5).

Contrasting the overall similar regional patterns between the age groups (e.g., medial-lateral gradient), there were marked differences in GPR88 expression levels across development. GPR88 expression was highest at P25 and then decreased with age (Figs. 4 and 5). Regional analyses revealed a significant decrease in expression ($P < 0.05$) from P25 to P40 in 21, and from P40 to P70 in 17 of the 23 striatal sectors (Figs. 4 and 5). However, unlike in the sectors of the caudate-putamen, the decrease in GPR88 expression in the nucleus accumbens predominantly occurred between P25 and P40 and was limited to the core and the medial shell (Fig. 5). No statistically significant decrease between P25 and P70 was observed in the ventral and lateral shell. Overall these findings demonstrate a robust and progressive decrease from P25 to P70 for the caudate-putamen, intermediate changes in the core of the nucleus accumbens and minor or no changes in the shell (Figs. 4 and 5).

Differential developmental trajectories for GPR88 expression in the caudate-putamen vs. nucleus accumbens

We further determined the developmental regulation of GPR88 expression in caudate-putamen and nucleus accumbens by comparing their developmental trajectories statistically, using pooled values from the respective sectors in these two regions (Fig. 6). This analysis confirmed a differential regulation across age in caudate-putamen vs. nucleus accumbens (main effect of age, $F(2,15)=84.3$, $P < 0.001$; main effect of region, $F(1,15)=0.78$, $P > 0.05$; age $\times$ region interaction, $F(2,15)=6.7$, $P < 0.01$). Post hoc comparisons showed a similar relative reduction in GPR88 levels between P25 and P40 for caudate-putamen and nucleus accumbens (P25 vs. P40, $P < 0.001$; at P40, caudate-putamen vs. nucleus accumbens, $P > 0.05$). However, GPR88 expression further decreased between P40 and P70 in the caudate-putamen ($P < 0.001$), whereas no change occurred in the nucleus accumbens during this period ($P > 0.05$). This differential decrease resulted in a lower relative GPR88 level in the caudate-putamen than in the nucleus accumbens at P70 ($P < 0.01$).

Discussion

In the present study, we investigated the cellular localization and developmental changes in expression of GPR88, a putative novel G-protein-coupled receptor, in the striatum of juvenile, adolescent, and adult rats. We found that GPR88 expression is most robust in juveniles and decreases thereafter towards adult levels. GPR88 is expressed in all striatal neurons, but with distinct regional variations. Our results also indicate a differential developmental regulation for GPR88 expression between regions of the caudate-putamen and the nucleus accumbens. A progressive decrease from P25 to P40 to P70 was observed in

the caudate-putamen, whereas a more moderate decrease occurred in the nucleus accumbens, mostly between P25 and P40.

Regional and cellular localization of GPR88 expression

The expression of GPR88 was evaluated both with qualitative (fluorescence) and quantitative (radioactive) in situ hybridization histochemistry. Consistent with previous studies [12–15], GPR88 expression was very pronounced in the striatum (caudate-putamen, nucleus accumbens) at all ages. Robust expression was also present in the olfactory tubercle. A modest signal was found in superficial layers of the cortical mantle, also consistent with the earlier reports (see above).

Our study provides the first detailed description of the regional distribution of GPR88 mRNA expression throughout the striatum. The expression was mapped in striatal sectors mostly defined by their predominant cortical inputs (see [16]), in order to determine expression levels across the different functional domains. GPR88 is expressed with a distinctive medial-lateral gradient. Expression was most robust in the sensorimotor (lateral) striatum and was more moderate in the associative (medial) and limbic (ventral) striatum. This regional distribution thus matches that of certain G protein-coupled receptors, for example, the CB1 cannabinoid receptor [18;19] and the D2 dopamine receptor [20–22], but also the distribution of some neuropeptides (e.g., substance P [23]). Given that the cell density is similar between these striatal regions, neurons of the sensorimotor striatum appear to express higher levels of GPR88. Pharmacological agents targeting GPR88 would thus be expected to preferentially affect the sensorimotor striatum.

At the cellular level, GPR88 mRNA expression was restricted to NeuN-positive cells in all age groups, thus demonstrating exclusively neuronal expression, consistent with a recent study by Massart and collaborators that used a new GPR88 polyclonal antibody to localize GPR88 protein [13]. GPR88 mRNA expression in all striatal neurons indicates that GPR88 is localized in both projection neuron types as well as interneurons. This is in agreement with the main observation in the above immunohistochemical study that showed robust GPR88 expression in striatopallidal and striatonigral projection neurons [13]. However, our findings differ in part from this study regarding labeling of interneurons. Although Massart et al. [13] also found a (weak) immuno-signal in parvalbumin-positive interneurons (the most numerous type), they reported lack of a signal in interneurons double-labeled for somatostatin, calretinin and choline acetyltransferase (cholinergic interneurons). The reasons for this discrepancy are presently unclear. The lack of an immuno-signal in some interneurons may indicate a lack of GPR88 mRNA translation to GPR88 protein in these interneurons. Alternatively, this difference may reflect a greater sensitivity of the present in situ hybridization histochemistry. For example, we did consistently find a GPR88 signal in large neurons, which had the size of cholinergic interneurons [8], and the GPR88 mRNA levels in these neurons tended to be in the lowest range of labeling in medium-sized neurons. Thus, our findings, together with the lack of GPR88 labeling in the globus pallidus (negative control), argue for GPR88 expression in striatal projection neurons and interneurons.

Developmental regulation of GPR88 expression

Our study is the first to describe the developmental regulation of GPR88 expression in the striatum. In almost all striatal regions, the expression decreases from juveniles to adolescents to adults. This pattern is reminiscent of developmental changes for other G-protein-coupled receptors. For example, D1 and D2 receptors are over-expressed before puberty and are then pruned back to adult levels [24–26]. A similar pattern was observed for adenosine A2A receptors [22]. While not all of these receptors show identical early changes,

the regulation of GPR88 resembles that of D1 and D2 receptor expression between adolescence and adulthood. Similar to D1 and D2 receptors [24;26], GPR88 expression is also differentially regulated between caudate-putamen and nucleus accumbens during this developmental period. In fact, such differential developmental trajectories in anatomically related, but functionally different, brain regions are not uncommon for metabotropic receptors. For example, we have shown that CB1 cannabinoid receptors in the cortex, which are also downregulated from prepuberty to adulthood, are differentially regulated in limbic/associative versus sensorimotor cortical areas [27].

In summary, these findings indicate that, similar to a number of G-protein-coupled receptors, GPR88 undergoes pruning across postnatal developmental, but that the exact trajectory is brain region-specific. Based on these findings, the effects of GPR88-selective pharmacological agents would be expected to be, at least to some degree, region- and age-dependent.

Is GPR88 a G-protein-coupled receptor?

GPR88 was initially proposed to be an orphan G-protein-coupled receptor based on its predicted primary protein sequence, which suggests seven transmembrane domains, as indicated by hydrophobicity analysis [12]. According to this study, the amino acid sequence of GPR88 shows significant homology with β -3 adrenergic and 5HT1D receptors [12]. Based on an analysis of the chemical structure of the putative transmembrane domains, a more recent study clustered GPR88 with metabotropic glutamate and GABA-B receptors [28]. The genomic organization of the human and mouse GPR88 gene was also found to be very similar to that of a number of G-protein-coupled receptors, including several muscarinic, histamine (H1), and dopamine (D1, D5) receptors [12]. Our finding of a developmental regulation similar to that of other G-protein-coupled receptors (early over-expression, followed by pruning; see above) thus extends the list of similarities between GPR88 and such receptors.

A recent ultrastructural study supports an association of GPR88 protein with postsynaptic sites [13]. This study showed that GPR88 immunolabeling is concentrated along the somatodendritic surface of striatal projection neurons, with a pronounced preference for dendrites and dendritic spines. Within dendrites, GPR88 protein was localized in the postsynaptic densities of mostly asymmetrical synapses contacted by terminals immunoreactive for the vesicular glutamate transporter (VGluT) 1, but not VGluT2 or tyrosine hydroxylase [13]. These findings indicate that GPR88 is preferentially associated with synapses receiving glutamate input from the cortex, but not glutamate input from the thalamus (VGluT2), GABA or dopamine inputs.

However, other characteristics of GPR88 are unusual for a functional G-protein-coupled receptor. In particular, the predicted GPR88 product lacks the tripeptide motif DRY at the intracellular boundary of the transmembrane domain 3 [12], which seems to be critical for receptor activation [29;30]. Also lacking are cysteine residues that are necessary to form disulfide bonds between extracellular loops, as is often seen in biogenic amine receptors [30]. Therefore, GPR88 may be a novel kind of G-protein-coupled receptor. Alternatively, given the apparent futility of a more than 10-year search for an endogenous ligand despite the profound interest in putative orphan G-protein-coupled receptors as potential drug targets, GPR88 may be another type [31] of membrane-bound molecule associated with synaptic signaling.

Conclusions

GPR88 may be an attractive target for pharmacological interventions in pathologies involving the striatum due to its restricted localization to this brain region. Here, we provided a description of the cellular localization of GPR88, indicating that GPR88 mRNA is expressed in neurons of both direct and indirect striatal output pathways, as well as in interneurons. Furthermore, we show that the developmental regulation of GPR88 expression is similar to that of G-protein-coupled receptors. The functional consequences of this GPR88 regulation over the postnatal development remain to be determined.

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Figure 1.

Distribution of GPR88 expression in the striatum. Illustrations of film autoradiograms depict the distribution of GPR88 mRNA in coronal sections from the rostral, middle and caudal striatum in juvenile (P25) and adult (P70) rats. The maximal hybridization signal is black. CPU, caudate-putamen; Cx, cortex; GP, globus pallidus; NAc, nucleus accumbens; OT, olfactory tubercle.

Figure 2. Cellular localization of GPR88 mRNA in the striatum. Examples of neurons labeled for NeuN (neuronal marker; fluorescence immunohistochemistry, red) (left column) and for enkephalin (ENK) mRNA or GPR88 mRNA (fluorescence in situ hybridization histochemistry, green) (center column) in the caudal caudate-putamen and in the globus pallidus are shown for adult rats (P70). Double-labeled neurons (merge, yellow) are depicted in the right column. GPR88 mRNA is expressed in all striatal neurons (middle row), but is not expressed in the globus pallidus (bottom row). White arrowheads indicate examples of NeuN-positive/ENK-negative cells (top row). Scale bars = 25 μ m.

Figure 3. Localization of GPR88 mRNA in medium-sized and large neurons of the striatum. Lower power photomicrographs (top row) depict neurons labeled for NeuN (red; left column), or GPR88 mRNA (green; center column), and double-labeled neurons (merge, yellow; right column) in the caudate-putamen in adults (P70). Higher power photomicrographs (middle and bottom rows) display the neurons in the boxed areas in the top images. Examples of neurons with a relatively low (middle row) or high (bottom row) GPR88 mRNA signal are shown. The white arrowhead indicates a large neuron, presumably a cholinergic interneuron. Scale bar = 10 μ m.

GPR88 mRNA expression

Figure 4.

Topography of GPR88 expression in the striatum at different postnatal ages (left) and differences in expression between P25 and P70 animals (boxed; right). Maps depict the distribution of GPR88 expression in the rostral, middle and caudal striatum in juvenile (P25), adolescent (P40) and adult (P70) rats. For P25, P40 and P70 groups, the data are expressed relative to the maximal value observed in the P25 group (% of max. P25). The differences in GPR88 expression between P25 and P70 rats (Δ P25-70) are expressed as the percentage of the maximal change (% of max. Δ). Sectors with significant differences ($P < 0.05$) are coded as indicated. Sectors without a significant decrease are in white.

Caudate-putamen: c, central; d, dorsal; dc, dorsal central; dl, dorsolateral; dm, dorsomedial; m, medial; v, ventral; vc, ventral central; vl, ventrolateral. Nucleus accumbens: mC, medial core; IC, lateral core; mS, medial shell; vS, ventral shell; IS, lateral shell.

Figure 5.

Expression of GPR88 across development in select areas of the striatum and nucleus accumbens. Mean density values (mean±SEM) for GPR88 mRNA levels in juvenile (P25), adolescent (P40) and adult (P70) rats are depicted for 4 sectors from the middle striatum (top) and the 5 sectors of the nucleus accumbens (bottom). Caudate-putamen: d, dorsal; dl, dorsolateral; vl, ventrolateral; c, central. Nucleus accumbens: mC, medial core; IC, lateral core; mS, medial shell; vS, ventral shell; IS, lateral shell. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vs. preceding age group or as indicated.

Figure 6. Differential regulation of GPR88 expression in the caudate-putamen and nucleus accumbens across development. Normalized mean density values (relative to P25; mean $\pm$ SEM) for GPR88 mRNA levels in the whole caudate-putamen (all sectors pooled, filled circles) and the whole nucleus accumbens (open circles) at P25, P40 and P70 are shown. *** $P < 0.001$, vs. preceding age group; ++ $P < 0.01$, caudate-putamen vs. nucleus accumbens.