Continuously changed genes during postnatal periods in rat visual cortex

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ABSTRACT

The current study aimed to determine genes that changed continuously throughout the postnatal developmental process of the rat visual cortex. Tissue samples were taken at postnatal day 0, day 10 (before eye opening), day 20 (before the critical period) and day 45 (end of development) and subjected to microarray and real-time RT-PCR analyses. A temporal pattern of expression was revealed for 24 genes that continuously increased (18 genes) or decreased (6 genes) as visual cortex development progressed and were common among all age groups. Our data provide a relevant set of genes whose expression levels correlate with visual cortex development and represent a novel group that may affect temporal-specific regulation.

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It has been well-established that the structural and functional capabilities of the brain’s visual cortex are not fully developed at birth, and the visual experience can markedly influence the course of future visual cortex development [2, 4, 7, 27]. Manipulation of visual input during a restricted time, known as the critical period, alters the pattern of synaptic connectivity within the visual cortex. After that time, the visual cortex is less sensitive or wholly desensitized to experience-dependent plasticity. There are different critical periods in visual cortex development in different species. In rodents, this period begins at eye opening, peaks around 4 weeks of age, and thereafter declines over several weeks to months [8, 11, 14]. Although the onset and duration of the critical period could be regulated by changing visual inputs, such as dark-rearing or one eye suturing, the plasticity in the visual cortex is the result of a series of events controlled in an activity-dependent manner in natural situations [2]. It is clear that both the innate mechanism responsible for the formation of functional cortical architecture and the experience-dependent mechanisms facilitating plasticity play important roles during visual cortex development [4, 6, 12].

Gene expression in the visual cortex is crucial for the developmental plasticity since both protein synthesis and proteolytic activity are involved in the ocular dominance plasticity [21, 26]. It has been demonstrated that the influence of neurotransmitters (NMDA, GABA, NE, Ach, serotonin), signal molecules (PKA, ERK, CaMKII, CREB), neurotrophic factors (BDNF, VEGF) and other mediators (such as, disabled-1 and tPA) contribute to the plasticity of the visual cortex [5, 15, 27, 29]. Most of the previous studies on development-associated mediators were performed on the visual cortex specifically during the critical period. Before onset of the critical period (i.e., birth to the third postnatal week in rat), the animal should acclimatize itself to the environment and open its eyes at the 2nd week.

Microarray analyses of global gene expression in activity-dependent development of the primary visual cortex have been reported in normal, dark-reared or monocular deprived mice [18, 20, 28], and in normal (infant vs adult) and monocular enucleated monkeys [17]. By differential display and DNA array techniques, Ossipow et al. found that transcripts of protein kinases and phosphatases were obviously regulated in 1-month and 4-month-old Sprague–Dawley (SD) rats [23]. Prasad et al. [24] examined gene expression levels in the visual cortex of cats at birth, on day 10 and day 30, and compared the results with those obtained from adult cats and dark-reared cats. They found 52 candidate genes that influence visual cortex plasticity. These genes include, but are not limited to, participants in second messenger systems, cell adhesion, transmitter recycling and cytokines.

Most previous studies reported genes that were activity- or plasticity-specific expression in the visual cortex. However, there should be some essential molecules that promote the maturation of the anatomy and molecular architecture in the developing visual cortex. In order to identify the genes associated with the postnatal development of the rat visual cortex, we used genome-wide gene expression analysis to investigate which transcripts’ levels increased or decreased continuously during the postnatal period. Using tissues obtained at day 0, day 10 (before eye opening), day 20 (before the critical period) and day 45 (end of development),

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we performed microarray screening via Affymetrix oligonucleotide arrays to determine the genes that up- or down-regulated continuously at the defined four time points. Data were validated by real-time reverse transcription polymerase chain reactions (real-time RT-PCR). We found 24 genes that might contribute to the developing visual cortex in the rat.

All protocols conformed to the ARVO statement for the care and use of animals in research. SD rats were reared under normal conditions (22 ± 2°C and 60 ± 10% relative humidity with a 12-h light–dark cycle) and had free access to food and water throughout the experimental period. All age-matched rats from various groups were sacrificed at progressive time points, including postnatal day 0 (P0, n = 20), day 10 (P10, n = 15), day 20 (P20, n = 15) and day 45 (P45, n = 10). The visual cortex samples were obtained as described previously [23]. Briefly, the animals were deeply anaesthetized with 10% chloral hydrate (400 mg/kg body weight) and ventricularly perfused with an ice-cold phosphate buffer (0.1 M, pH 7.4) for a few minutes. Fresh visual cortex was dissected from the binocular area (all of Area 17 and possibly a small part of Area 18) and immediately placed in cold Trizol (Invitrogen, MD). All samples were stored at −80°C until use.

Microarray assay was performed using pooled samples according to the guidelines for eukaryotic sample and array processing in the Affymetrix protocol. In brief, RNAs were isolated from pooled rat visual cortex using the Trizol reagent and purified by RNeasy micro kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. RNA concentration and quality were assessed via spectrophotometry and agarose gel electrophoresis. Double-stranded cDNA was synthesized from 10 µg total RNA using Affymetrix one-cycle CDNA Synthesis Kit and the cDNA product was purified using the Affymetrix GeneChip Sample Cleanup Module. The resulting double-stranded cDNA acted as a template to produce cRNA using a biotinylated nucleotide analog/ribonucleotide mix (GeneChip IVT Labeling Kit). Following completion, quality control, four test analyses were conducted by using GeneChip® Test3 Arrays (Affymetrix, Part No. 900341) to evaluate target quality and standardize experiments. Labeled cRNA was fragmented and hybridized to Affymetrix Rat Genome 230 2.0 Arrays in four experimental sets at a genome analysis center (Gene Tech Biotechnology Co. Ltd., Shanghai, China). The chip was washed and scanned (GeneArray TM scanner 3000) according to the directions detailed in the Affymetrix GeneChip® expression analysis technical manual.

Upon completion of the scan, the image file was displayed in the Affymetrix GeneChip® Operation Software (GCOS). The signal was extracted from the image. Data normalization, log transformation, statistical analysis and a pattern study were performed with GCOS [17,22]. Probe sets used for quality control and genes that scored as Absent or Marginal on a chip were excluded for the expression of those genes assayed exhibited a change in their expression levels by RT-PCR equally matched to the microarray data (e.g., Akap7, Asam, Casp3, Gpr88 and Stk32c at P45/P0, from a total of 36 compared gene-pairs revealed unpronounced opposite changes. There was a close similarity between RT-PCR and microarray analyses.

Next we investigated the transcripts that up- or down-regulated continuously during successive time intervals. The gene changes were analyzed by comparing those probes present on chips of changed probes were used to randomly select genes for validation by real-time RT-PCR assay. The primers for were used for PCR amplification in all four age categories (in each pair of primer sequences, the forward sequence is listed 1st and the reverse 2nd): (1) Akap7: GGAATGGCGATTGGCAGTAT, CCCCCCGTGCCTGTTCCCATCT; (2) Asam: ACCATAGACCCATTGACCAAAAG, TGGTTTTTCTCGGTTCTCT; (3) Csp3: TGGAAAAAGCCAGCCGTCTGG, CGCCGGCTGGTGAAGTAAGCCAGTACG; (4) Cxcr4: TGGCCACATGGGGTTTGTAATC, CTTCCGTCTGGTGTTAATAC; (5) Egr1: CCAAGGCTGGTCTTCCAGGTTCC, TGGGGCTTGGTTTTATCTAG; (6) Enpp2: TGAAGAATTCTGGCCTCCTGTTG, CTTCCGTCTGGTGTTAATAC; (7) Fabp1: TCAGAAGGGCCAAAGGTCTTGT, CATAACACGCGCCAGCAGCGATC; (8) Gpr88: TAAAAAGCTTTTGGCCTTG, TGCTGTATTCTGGATAATTTGTT; (9) Inpp5p: GGGAAAACACCCGGCGGAC, CGAGCACAAGCAGCAGCTG; (10) Rpsa: ACACCCGTGGACACTTCAATAC, CTGTGTATTACCCCGACGAGATTG; (11) Stk32c: CCTTGTGCGTGGTTTGGCT, CCAACACCCCTCTAGTAATCAGT; (12) Vamp1: CTTTTCTTCAATTCTCAGTCAGAGACAGCAGTGCTAC; (13) beta-actin (rat, for housekeeping gene): CTGGGTATGAACTTTCGTGG, CTAATGACTTTCTTGGTGCT.

Each gene was tested in three independent experiments and calculated as we described previously [30].

The expression values of 12 transcripts at 4 time points were compared between their real-time RT-PCR and the microarray data (Fig. 1). The direction of change for each of the 12 genes from the four time point samples could be reproduced. Most of those genes assayed exhibited a change in their expression levels by RT-PCR equally matched to the microarray data (e.g., Akap7, Asam, Casp3, Cxcr4, Egri, Enpp2, Fabp7, Inpp5d, Rpsa, Vamp1). Two genes, Gpr88 and Stk32c at P45/P0, from a total of 36 compared gene-pairs revealed unpronounced opposite changes. There was a close similarity between RT-PCR and microarray analyses.

Next we investigated the transcripts that up- or down-regulated continuously during successive time intervals. The gene changes were analyzed by comparing those probes present on chips of
Table 1
List of genes changed continuously in three periods.

<table>
<thead>
<tr>
<th>Probe set ID</th>
<th>Gene Symbol</th>
<th>Representative Public ID</th>
<th>P10/P0</th>
<th>P20/P10</th>
<th>P45/P20</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>136773_at</td>
<td>Ca2</td>
<td>NM_019291</td>
<td>1.6</td>
<td>1.1</td>
<td>1.5</td>
<td>Carbonic anhydrase 2</td>
</tr>
<tr>
<td>136774_at</td>
<td>Gta3</td>
<td>NM_031509</td>
<td>4.8</td>
<td>2.0</td>
<td>1.4</td>
<td>Glutathione S-transferase A3</td>
</tr>
<tr>
<td>1367959_a_at</td>
<td>Scnb1</td>
<td>AF182949</td>
<td>3.1</td>
<td>1.0</td>
<td>1.2</td>
<td>Sodium channel, voltage-gated, type I, beta</td>
</tr>
<tr>
<td>1387010_s_at</td>
<td>Scnb1</td>
<td>NM_017288</td>
<td>3.6</td>
<td>1.0</td>
<td>1.1</td>
<td>Sodium channel, voltage-gated, type I, beta</td>
</tr>
<tr>
<td>1368688_at</td>
<td>Ntr2</td>
<td>NM_022695</td>
<td>3.2</td>
<td>1.9</td>
<td>1.0</td>
<td>Neurotensin receptor 2</td>
</tr>
<tr>
<td>1368943_at</td>
<td>Rnase4</td>
<td>NM_020082</td>
<td>2.9</td>
<td>1.3</td>
<td>1.2</td>
<td>Ribonuclease, RNA A family 4</td>
</tr>
<tr>
<td>1371475_at</td>
<td>Rnase4</td>
<td>BI284831</td>
<td>2.2</td>
<td>1.0</td>
<td>1.1</td>
<td>Ribonuclease, RNA A family 4</td>
</tr>
<tr>
<td>1370228_at</td>
<td>Tf (Scrprb)</td>
<td>AA945178</td>
<td>4.3</td>
<td>2.1</td>
<td>1.7</td>
<td>Transferrin/signal recognition particle receptor, B</td>
</tr>
<tr>
<td>1370434_a_at</td>
<td>Mobp</td>
<td>X89638</td>
<td>8.3</td>
<td>3.9</td>
<td>1.3</td>
<td>Myelin-associated oligodendrocytic basic protein</td>
</tr>
<tr>
<td>1370517_at</td>
<td>Nptx1</td>
<td>UI8772</td>
<td>1.4</td>
<td>1.4</td>
<td>1.1</td>
<td>Neuronal pentraxin 1</td>
</tr>
<tr>
<td>1373510_at</td>
<td>Vamp1</td>
<td>BF281373</td>
<td>1.7</td>
<td>1.1</td>
<td>1.2</td>
<td>Vesicle-associated membrane protein 1</td>
</tr>
<tr>
<td>1375043_at</td>
<td>Fos</td>
<td>BF415939</td>
<td>3.6</td>
<td>1.4</td>
<td>1.1</td>
<td>FBJ murine osteosarcoma viral oncogene homolog</td>
</tr>
<tr>
<td>1379483_at</td>
<td>Blhhb2</td>
<td>AI548256</td>
<td>1.7</td>
<td>1.6</td>
<td>1.3</td>
<td>Basic helix-loop-helix domain containing, class B2</td>
</tr>
<tr>
<td>1383072_at</td>
<td>Pygm</td>
<td>AW919180</td>
<td>1.9</td>
<td>1.2</td>
<td>1.0</td>
<td>Muscle glycogen phosphorylase</td>
</tr>
<tr>
<td>1383887_at</td>
<td>RDG1306991</td>
<td>AA924984</td>
<td>5.9</td>
<td>2.0</td>
<td>1.2</td>
<td>Similar to protein C20orf103 precursor, lysosome-associated membrane glycoprotein</td>
</tr>
</tbody>
</table>

P10, P20 and P45 with an earlier set of probes on each chip (P10 vs P0, P20 vs P10 and P45 vs P20). There were only 26 probes in total, representing 24 genes, that showed either increased or decreased expression throughout the development course and that were common among all age groups (Table 1). Each probe with its corresponding gene name, gene symbol and function (GO term) was retrieved from the NetAffx annotation database (April 2005 version; NetAffx) [19] and from eGOn (explore GeneOntology) [3]. Among these 26 probes, 20 showed increased expression and 6 probes showed decreased expression. Two probes (1367959_a_at and 1387010_s_at) with almost identical changing levels at three successive intervals, represent the same gene, Scnb1, which is essential for the generation and propagation of action potentials in striated muscle and neuronal tissues [1]. Two other probes (1368943_at and 1371475_at) representing another gene, Rnase4 (RNA A family 4), revealed similar changing levels during postnatal development. According to the gene ontology (GO terms) and literature reports, the revealed gene sets include encoding products associated with nervous system development, (metal) ion binding/transport, metabolism, regulation of neuronal synaptic plasticity, regulation of transcription, signal transduction, introduction of apoptosis (see Table 2 for a complete list). In addition, Tctex1 (t-complex testis expressed 1), a component of (axonemal) dynein complex, is down-regulated in visual cortex. There were no exact functional descriptions for the putative gene products associated with two of the probes (i.e. 1383887_at and 1375358_at). However, probe 1383887_at, corresponding to UniGene record number AA924984, represents a protein similar to protein C20orf103 precursor (AAH87680) as it has a lysosome-associated membrane glycoprotein (Lamp-1) (http://www.genome.jp/dbget-bin/www_bget?rnmo:362220) motif. Lamp-1 is an integral membrane protein, specific to lysosomes, and it plays roles in cell activation, mediator release and maintaining the structural integrity of the lysosomal compartment [9,10]. Blast analysis of 1375358_at represented gene sequences (AA998150), yielded a gene product similar to the VPS10 domain receptor protein SORCS2 (Sorcs2). It has reported that Sorcs2 is highly expressed in the developing and mature murine central nervous system, and is

Table 2
The major bio-functionalities of up-/down-regulated genes in rat visual cortex.

<table>
<thead>
<tr>
<th>Bio-functional categories</th>
<th>Genes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nervous system development</td>
<td>Mlobp, Fos, Bhhb2, Nptx1, S100b, Ntr2, Ms, Dock4-predicted, Dpysls3, Sorcs2</td>
</tr>
<tr>
<td>(Metal) ion binding/transport</td>
<td>Scan1b, Ntr2, Tf (Scrprb), Nptx1, S100b, Gja7, Nr4a1, Adhfe1, RNase4</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Gsta3, Pygm, S100b, Adhfe1, Ca2, Apod, Dpysls3</td>
</tr>
<tr>
<td>Regulation of neuronal synaptic plasticity</td>
<td>Blhhb2, S100b, Arc</td>
</tr>
<tr>
<td>Regulation of transcription</td>
<td>Rnas4, Fos, Bhhb2, Nr4a1</td>
</tr>
<tr>
<td>Protein kinase activity</td>
<td>S100b, Dock4-predicted, Met</td>
</tr>
<tr>
<td>Transferase activity</td>
<td>Gsta3, Pygm, Met</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>Ntr2, Nr4a1, Met, Dpysls3, Dock4-predicted</td>
</tr>
<tr>
<td>Vesicle-mediated transport</td>
<td>Vamp1, Dpysls3</td>
</tr>
<tr>
<td>Synaptic transmission</td>
<td>Scnb1, Nptx1</td>
</tr>
<tr>
<td>Induction of apoptosis</td>
<td>S100b, Nr4a1</td>
</tr>
<tr>
<td>Actin binding</td>
<td>Rnas4, Arc</td>
</tr>
</tbody>
</table>

* Genes in bold are down-regulated in visual cortex during postnatal periods.
regulated by synaptic activity [25]. Together with another two Sorcs genes (Sorcs1 and Sorcs3), Sorcs2 functions in the developing and mature central nervous system [16].

In the current study, we examined genes expressed at all four specific time points, i.e., day 0 (at birth), day 10 (before eye opening), day 20 (before the critical period) and day 45 (end of development), in order to identify essential genes that might be associated with the entire postnatal developmental processes of rat visual cortex. Among a total of 31,042 probe sets assayed, there were only 26 probes (24 genes) that maintained either increased (20 probes) or decreased expression (6 probes) throughout the development course. By playing essential roles in visual cortex, these genes are not expressed in an age-specific manner. Our present data are unique from most of the previous studies involving global gene expression in the visual cortex, as they focused on identifying genes related to developmental plasticity during a specific period [13, 17, 18, 20, 28]. For example, Prasad et al. [24] used an array with 18,371 non-redundant cDNAs (human probes) and reported that more than seven hundred cDNA clones were expressed in cat postnatal and dark-reared visual cortex. These genes fit into three major clusters, i.e., second messenger systems, cell adhesion and transmitter recycling/cytokines, and consisted of 52 plasticity candidate genes. Another study, by Lachance and Chaudhuri, compared gene expression in visual cortex of infant (days 23 and 27), adult and monocular enucleated monkeys, and identified 108 transcripts that appeared to be differentially expressed in normal infants and adults [17]. Also, Majdan et al. used monocular deprivation and dark-rearing of mice to investigate expression differences at day 18, 24, 46 and 104. The results of their study revealed that members of a common gene set, including 11 transcripts, were under the transcriptional regulation of MEK1/2; furthermore, these genes were common to all four age groups and were all down-regulated in cortex deprived of visually driven activity. In our study presented here, we were interested in those genes that are continuously increasing or decreasing their activities during normal rat postnatal development. Independent of dramatical functional/structural changes that occurred during this period, there were 24 genes up- or down-regulated from birth to adult. These genes are known to function in nervous system development, (metal) ion binding/transport, metabolism, regulation of neuronal synaptic plasticity, signal transduction, introduction of apoptosis, etc. (Table 2). Further study on these genes may disclose specific key roles involving regulation of visual cortex development.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2009.07.004.

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