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Comparative Gene Expression Analysis Reveals a Characteristic Molecular Profile of the Superior Olivary Complex

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ABSTRACT

The superior olivary complex (SOC) is a very conspicuous structure in the mammalian auditory brainstem. It represents the first binaural processing center and is important for sound localization in the azimuth and in feedback regulation of cochlear function. In order to define molecular determinants of the SOC, which are of potential functional relevance, we have performed a comprehensive analysis of its transcriptome by serial analysis of gene expression in adult rats. Here, we performed a detailed analysis of the SOC's gene expression profile compared to that of two other neural tissues, the striatum and the hippocampus, and with extraocular muscle tissue. This tested the hypothesis that SOC-specific or significantly upregulated transcripts provide candidates for the specific function of auditory neurons. Thirty-three genes were significantly upregulated in the SOC when compared to the two other neural tissues. Thirteen encoded proteins involved in neurotransmission, including action potential propagation, exocytosis, and myelination; five genes are important for the energy metabolism; and five transcripts are unknown or poorly characterized and have yet to be described in the nervous system. The comparison of functional gene classes indicates that the SOC has the highest energy demand of the three neural tissues, yet protein turnover is apparently not increased. This suggests a high energy demand for fueling auditory neurotransmission. Such a demand may have implications on auditory-specific tasks and relate to central auditory processing disorders. Ultimately, these data provide new avenues to foster investigations of auditory function and to advance molecular physiology in the central auditory system.

Key words: superior olivary complex; transcriptome; serial analysis of gene expression; energy metabolism; central auditory processing disorders

The superior olivary complex (SOC) represents the first binaural processing center in the mammalian brain and is important for sound localization in the azimuth and in feedback regulation of cochlear function. To gain further insight into the molecular requirements underlying auditory processing in the brainstem, we recently generated a comprehensive library of the SOC transcriptome from 2-month-old rats (Koehl et al., 2004). In the current study, we perform a detailed comparative analysis between our serial analysis of gene expression (SAGE) library and publicly available SAGE libraries generated from nonauditory neural and muscle tissues. The results of this study help to identify molecular determinants of the SOC, which are of functional relevance in normal audition and may be altered in central auditory processing disorders.

Mammalian Superior Olivary Complex

The mammalian SOC is located in the pontine brainstem rostral to the facial nucleus and between the roots of the abducens nerve and the facial nerve, i.e., cranial

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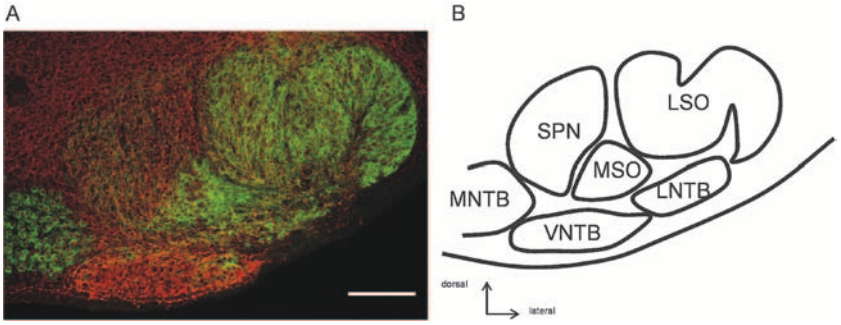

Fig. 1. Anatomy of the superior olivary complex. **A:** Principal nuclei are depicted due to their immunoreactivity against the vesicular glutamate transporter 1 (green) and the microtubule-associated protein 2 (red). Modified from a cover picture appearing in *Cell Tissue Research* in 2005 with kind permission of Springer Science and Business Media. **B:** Schematic drawing of the arrangement of the various nuclei of the superior olivary complex. LNTB, lateral nucleus of the trapezoid body; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; SPN, superior paraolivary nucleus; VNTB, ventral nucleus of the trapezoid body. Scale bar = 200 μm.

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nerves VI and VII. The SOC consists of several interrelated nuclei (Fig. 1). Usually, three principal nuclei are distinguished, the medial superior olive (MSO), the lateral superior olive (LSO), and the medial nucleus of the trapezoid body (MNTB) (Schwartz, 1992; Reuss, 2000). In humans, cells of the MNTB do not coalesce to form a distinct nucleus (Moore, 1987), whereas the MSO is large and prominent (Moore, 2000). The three principal nuclei are surrounded by more loosely organized periolivary groups, the superior paraolivary nucleus, the lateral nucleus of the trapezoid body, and the ventral nucleus of the trapezoid body (Schwartz, 1992). In rodents, the latter two nuclei are occasionally referred to as the ventrolateral periolivary group and the ventromedial periolivary group, respectively (Osen et al., 1984; Thompson and Thompson, 1991). Moreover, in cats and bats, the nomenclature sometimes refers to the superior paraolivary nucleus as the dorsomedial periolivary nucleus (Schwartz, 1992; Grothe and Park, 2000). Although periolivary cell groups vary between species in their size, orientation, shape, and cellular composition, the morphology and the connections to and from the cell groups are similar across a number of species studied (Moore, 1987; Schwartz, 1992).

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The SOC is the first center in the auditory pathway where information from the two ears converges. It receives several ascending projections from both cochlear nuclei (CN). The projections from spherical bushy cells of the anterior ventral cochlear nucleus (AVCN) converge on neurons in the MSO. LSO principal neurons receive direct input from the spherical bushy cells of the ipsilateral AVCN, and indirect input from globular bushy cells of the contralateral AVCN via the ipsilateral MNTB, which acts as a relay station. These projections are tonotopically organized. In addition, there are several other projections into the various nuclei that arise either from the CN or from other cell groups of the SOC itself. Efferent projections from the SOC can be divided into ascending and descending projections (Helfert et al., 1991; Schwartz, 1992; Reuss, 2000). Ascending projections transport information mainly into the inferior colliculus (IC) (Schwartz, 1992; Loftus et al., 2004), where the input is integrated with that from other auditory centers, from motor systems, and from the somatosensory system (Casseday et al., 2002). Descending projections of the olivocochlear bundle make direct synapses with outer hair cells and with peripheral processes of type I spiral ganglion cells at the base of inner hair cells (Warr, 1992) and also project to the CN.

The major functions performed by the SOC neurons are sound localization and feedback control of cochlear mechanisms. The binaural convergence of input into the SOC enables animals to localize a sound source in space. According to the classical duplex theory (Thompson, 1882; Rayleigh, 1907), the major cues for sound localization in the azimuth are interaural time differences (ITDs) and interaural level differences (ILDs). Low-frequency sound sources (up to 2 kHz in mammals) are localized by ITD analysis, whereas substantial ILD is generated only at high frequencies (in humans above 2–3 kHz), where the wavelength is short and the head can create an effective acoustic shadow at the ear furthest to the sound source (Yin, 2002). The first representation of ILD occurs in LSO neurons. These neurons are excited by stimulation of the ipsilateral ear and inhibited by stimulation of the contralateral ear due to the conversion of the contralateral excitation into inhibition by MNTB neurons (Yin, 2002). This inhibitory/excitatory (IE) binaural type of input renders LSO neurons sensitive to ILD. Higher sound amplitudes at the ipsilateral ear, compared to the contralateral ear, are encoded by an increased firing rate in the LSO neurons. In contrast, lower stimulation amplitude at the ipsilateral ear, compared to the contralateral input, slows down the firing rate (Caird and Klinke, 1983).

The first representation of ITDs occurs in MSO neurons. These neurons are excited by signals coming from each ear (EE neurons). They fire maximally when inputs from the two ears arrive simultaneously. MSO neurons, therefore, are considered to be binaural coincidence detectors. In a model originally proposed by Jeffress (1948), a series of axons, with ladder-like branching patterns that give rise to collaterals of different length, the so-called delay lines, form the anatomical substrate for simultaneous arrival of inputs within the coincidence detector, i.e., the MSO array. Simultaneous arrival (= coincidence) occurs when the sum of the acoustic delay and axonal delay on one side equals that on the other side. The difference of arrival of a sound between the two ears (= acoustic delay) is thus transformed into a place code. Extensive confirmation of the Jeffress model came from work in the barn owl (Konishi, 2003). Recent analysis, however, suggests a differ-

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ent means of ITD detection in small mammalian brains (Mcalpine et al., 2001; Brand et al., 2002) in that precisely timed glycinergic inhibition is required for encoding the physiologically relevant range of interaural time differences in the MSO (Brand et al., 2002; Grothe, 2003). Support for different coding strategies was obtained by modeling and statistical analysis of electrophysiological recordings, revealing that head size and sound frequency are important factors for optimal neural population coding of ITD (Harper and Mcalpine, 2004).

The descending pathways originating from the SOC, and running in the olivocochlear bundle, likely play a role in development and frequency-dependent protection of the auditory system from loud sound and in preserving the dynamic range in noisy environments (Liberman, 1991; Liberman and Gao, 1995; Rajan, 1995, 1996; Vetter et al., 1999). Transection of the olivocochlear bundle in neonatal cats, for instance, led to elevated thresholds at the characteristic frequency (CF) of auditory nerve fibers; tuning curves displayed a reduction of the tip-to-tail ratios (the difference between CF and low-frequency tail thresholds) and a decreased sharpness of tuning (Walsh et al., 1998). An additional function of the descending pathways may be the enhancement of speech processing at high levels of ambient noise (Hienz et al., 1998).

Molecular Mechanism of Coding Timing in Auditory Neurons

To preserve and reliably transmit auditory information, auditory neurons display characteristic morphological and physiological properties that ultimately are determined by the complement of expressed genes (Oertel, 1997; Trussell, 1999, 2002). One remarkable feature of auditory brainstem neurons is their ability to follow reliably stimulation frequencies of up to 1,000 Hz with high-frequent trains of phase-locked action potentials (Wu and Kelly, 1993). This is partially due to the very short duration of their excitatory postsynaptic potentials (EPSPs), which in turn is likely caused by the specific expression of specific AMPA receptors and K^+ channels. For example, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of auditory brainstem neurons revealed a high expression of the flop version of the AMPA receptor subunit GluR4 (Geiger et al., 1995). Receptors containing this subunit show a very short desensitization time constant of less than 1 msec and deactivation times of under 0.5 msec (Mosbacher et al., 1994). In addition to brief synaptic currents, short EPSPs are obtained through short membrane time constants, and these in turn are likely provided by outward-rectifying, low-threshold K^+ channels, such as Kv1.1 or Kv1.2, and outward-rectifying, high-threshold K^+ channels, including Kv3.1 and Kv3.3. The relevant genes are abundantly expressed in the auditory brainstem (Perney et al., 1992; Wang et al., 1994; Perney and Kaczmarek, 1997; Grigg et al., 2000; Li et al., 2001). Additional molecular specializations in the SOC include calcium-binding proteins that enable the neurons to cope with the cytoplasmic calcium loading mediated through glutamate receptors during strong synaptic transmission (Lohmann and Friauf, 1994; Vater and Braun, 1994; Berrebi and Spirou, 1998).

Global Gene Expression Analysis

Identified genome sequences from humans (Venter et al., 2001) and various model organisms such as the mouse (Waterston et al., 2002) and the rat (Gibbs et al., 2004), in combination with large collections of expressed sequence tags (ESTs) (Adams et al., 1991, 1995), laid the foundation for comprehensive gene expression analyses. On the nucleic acid level, mainly two techniques are currently applied to analyze the transcriptome (= all expressed genes) within a cell population or tissue. The first technique is the microarray analysis (Schena et al., 1995) and the second is the serial analysis of gene expression (SAGE) (Velculescu et al., 1995).

Microarray analysis is a hybridization-based technique that has the strength to visualize simultaneously the expression of thousands of genes within a few days. Major disadvantages are inherent difficulties in the comparison between experiments performed by different groups or by using different array platforms. Moreover, a microarray analysis represents a closed approach, i.e., only those genes can be detected for which a corresponding probe was spotted on the array (Bucca et al., 2004; van Bakel and Holstege, 2004).

SAGE, in contrast to the microarray analysis, is a sequence-based method that randomly samples short cDNA fragments, the so-called tags, with a length of 14 to 21 base pairs. After sequence acquisition, the tags are counted and mapped back to transcripts. One advantage of SAGE is that it represents an open approach, which is independent of an a priori knowledge of putatively expressed genes. A second advantage is that it easily allows comparisons between different libraries, even if they were generated by different groups (Koehl et al., 2004). Drawbacks of the technique are the higher costs and the duration of several weeks to months for sequencing 30,000–70,000 tags that are required to get an amount of data equivalent to that obtained with microarray experiments (for a detailed discussion of the SAGE methodology, see Anisimov et al., 2002; Trendelenburg et al., 2002; Koehl et al., 2004).

MATERIALS AND METHODS

Data Analysis

The generation of the SAGE library of the SOC of 2-month-old rats was reported previously (Koehl et al., 2004). The other four libraries were taken from rat hippocampus (Datson et al., 2001), rat striatum (Geo accession number GSM29627), rat extraocular muscle (Cheng and Porter, 2002), and extraocular muscle of dark-reared rats (Cheng et al., 2003). Tag-to-gene-mapping was performed using SAGEmap (Lash et al., 2000), based on UniGene release 138 (21 December 2004). Reliability scores for tag annotations were provided by SAGEmap; the score takes into account the different transcript categories in the database (e.g., RefSeq sequences, ESTs with and without polyadenylation signal) and their number matching the respective tag. Annotations with reliability scores $\leq$ 1,000,501 were excluded from further analysis, as these tags match only to ESTs without a polyadenylation signal (T.O. Suzek, NCBI, personal communication). For statistical analysis, libraries were compared pairwise and the P values were computed by the z-test using the SAGEstat program (Ruijter et al., 2002). Only those tags were considered that had a tag count > 4 in either of the two libraries. SAGE data of the SOC are available at GEO (http:www.ncbi.nlm.nih.gov/geo/) under accession numbers GSM24492 and at ID-GRAB (http://www.id-grab.de/sage/).

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To compare gene expression of functional classes, text string searches and Gene Ontology searches were performed using the EASE software (Hosack et al., 2003). To calculate the average tag count of a functional class, the sum of all tags of this class within each library was divided by the number of distinct tags in this class. For energy metabolism, tag counts of the genes encoding proteins involved in glycolysis, the tricarbonic acid cycle, oxidative phosphorylation, and fatty acid metabolism—as determined by EASE—were extracted and analyzed. For protein synthesis, the keywords "ribosom*" and "*translation*" were used in a query of an access database. Likewise, for protein degradation, the keywords "*protease*," "*proteasom*," and "*ubiqutin*" were used. To analyze the expression of genes important for myelination, genes involved in the myelination process as indicated by GeneCards (Spea15, Mbp, Plasmolipin, Cldn11, S100b, Mog, Olig, Ugt8, Omg, Plp, Cnp1, Pmp22, Gfap, Plp2, Gjb1, Mag, Mobp) (Safran et al., 2003) were chosen for analysis. To analyze antioxidant proteins, the respective names of 19 genes were extracted from the gene ontology database (Apc, ApoeE, Cat, Cygb, Gpx1, Gpx3, Gpx4, Mt3, Prdx2, Prdx3, Prdx4, Prdx5, Prdx6, Ptgs1, Ptgs2, Sod1, Sod3, Txnrd1, Txnrd2). Additional searches were performed with the keywords "*receptor*," "*transporter*," "*channel*," "*calcium*," "*synap*," "*syntaxin*," and "*neuron*."

RESULTS

In a previous publication (Koehl et al., 2004), we have compared the results of a SAGE library of the rat SOC (31,035 tags, 10,473 unique tags) with those of a SAGE library of the rat hippocampus (76,790 tags, 28,748 unique tags) (Datson et al., 2001). To narrow down genes that may have an important function in the auditory brainstem, we here present a more elaborate comparative analysis and include three additional SAGE libraries that are publicly available: a library of the striatum (22,090 tags, 9,897 unique tags; N.S. Cai, personal communication; Geo accession number GSM29627), a library of the extraocular muscle [EOM; 54,764 tags, 17,602 unique tags (Cheng and Porter, 2002)], and a library of the extraocular muscle of dark-reared rats [EOM_DR; 31,776 tags, 10,105 unique tags (Cheng et al., 2003)].

Global Comparative Analysis

The comparison between the neural tissues SOC, hippocampus, and striatum revealed that the largest group consisted of library-specific tags. In the SOC library, 45% of the tags were library-specific, that is, they were not observed in the SAGE libraries from striatum and hippocampus (Fig. 2). In the striatum and the hippocampus, 49% and 75%, respectively, of the tags were library-specific (Fig. 2). The second largest group comprised 2,655 tags that were present in all three libraries. Further analysis revealed that these tags mainly represented transcripts associated with basic cellular function, such as translation (i.e., ribosomal proteins, initiation and elongation factors). Interestingly, they were also found in the two libraries obtained from the extraocular muscles (data not shown), strengthening the conclusion that they represented housekeeping genes. The high number of library-specific tags is surprising and may reflect the random sampling character of the SAGE technique, by which low

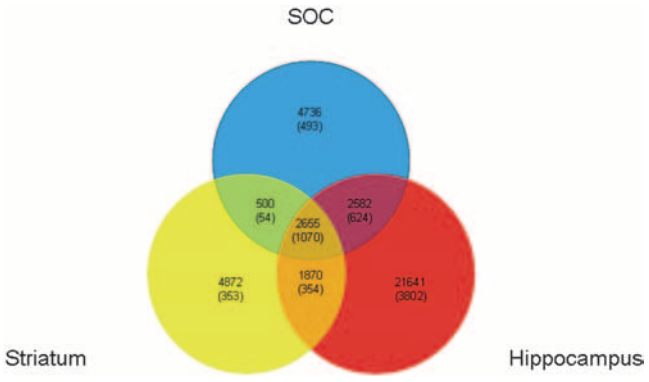

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Fig. 2. Venn diagram illustrating library-specific tags and common tags between SOC, hippocampus, and striatum. The comparison is based on the analysis of the combined tags of all three libraries. The comparison of tags with a count > 1 is shown in parentheses. Libraries were taken from SOC (Koehl et al., 2004), hippocampus (Datson et al., 2001), and striatum (Geo accession number GSM29627).

abundant tags are observed by chance in only one of the libraries analyzed, yet not in the other ones. To check whether this holds true, we applied a more stringent criterion and compared only tags with a count > 1 in any of the three library-specific subsets or in all libraries of an intersection. This reduction drastically decreased the number of tissue-specific tags (Fig. 2). SOC-specific tags were reduced from 4,736 to 493 (10.4%), striatum-specific tags from 4,872 to 353 (7.2%), and hippocampus-specific tags from 21,641 to 3,802 (17.6%). Thus, tissue-specific tags mainly represent low abundant tags. Tags common to all libraries and representing housekeeping genes were only reduced by 60% when all tags with a count of 1 were eliminated.

To apply an alternative, even more stringent criterion for our comparative analysis between the libraries, we analyzed tag abundances for statistically significant differences using the z-test of the SAGEstat program (Ruijter et al., 2002). Only tags with a count > 4 in either of the libraries were used for further comparison. The results are illustrated in Table 1. At a P value < 0.05, 238 and 550 tags were upregulated in the SOC, compared to the striatum and the hippocampus, respectively, whereas 263 and 326 tags were less abundant in the SOC compared to the striatum and hippocampus, respectively. A total of 1,046 and 870 tags were differentially regulated between the SOC and the EOM and EOM_DR, respectively, and 504 tags between hippocampus and striatum (Table 1). Only 237 tags were differentially regulated between the two libraries from the extraocular muscles (Table 1). At $P < 0.001$, 44 and 251 tags were upregulated in the SOC, compared to the striatum and the hippocampus, respectively, whereas 80 and 57 tags were less abundant in the SOC compared to the striatum and hippocampus, respectively. A total of 405 tags were differentially regulated between SOC and EOM, and 256 tags between SOC and EOM_DR (Table 1). Two hundred fifteen tags were differentially regulated between the hippocampus and the striatum. Only 30 tags were differentially regulated between EOM and EOM_DR at this level of significance (Table 1). These data imply the closest similarity between SOC and striatum among the three neural tissues, as shown at both

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TABLE 1. Quantitative assessment of differential gene expression between SAGE libraries

	Overrepresented in				
	$p < 0.05$				
compared to	SOC	Striatum	Hip	EOM	EOM_DR
SOC		263	326	472	411
Stri	238		167	305	324
Hip	550	337		859	583
EOM	574	408	1009		143
EOM_DR	459	362	470	94	
	$p < 0.001$				
compared to	SOC	Striatum	Hip	EOM	EOM_DR
SOC		80	57	134	147
Stri	44		29	87	99
Hip	251	186		382	379
EOM	271	279	233		26
EOM_DR	109	119	96	4	

Libraries were compared pairwise and the P-values were computed by the z-test using the SAGEstat program (Ruijter et al., 2002). Listed are the numbers of tags, which are differentially regulated at a P-value<0.05 (upper part) or at a P-value<0.001 (lower part).

significance levels (501 differentially regulated tags at $P < 0.05$; 122 differentially regulated tags at $P < 0.001$). In contrast, SOC and hippocampus differ most (876 differentially regulated tags at $P < 0.05$; 308 differentially regulated tags at $P < 0.001$). The similarity between striatum and hippocampus was in between, with 504 differentially regulated tags at $P < 0.05$ and 215 differentially regulated tags at $P < 0.001$.

To define transcripts with a potentially important role in SOC function, we next searched for tags that were overrepresented in the SOC compared to both the hippocampus and the striatum. To do so, we applied the stringent P value < 0.001, rationing that the resulting tags represent SOC-specific gene expression. Thirty-three tags were identified (Table 2). Many of them encode proteins that influence neurotransmission, including the synaptic proteins Cplx1, Snap25, and Vamp1, Gpsn2, the Na/K-ATPase subunits Atp1b1 and Atp1a2, the plasma membrane channels Scn1b and Hcn2, the glutamate/glutamine shuttle enzymes Glul and Glud1, and the myelin sheath proteins Mbp, Plp, and Mag. Another large transcript class (Ldhb, AldoC, Atp5a1, Cox4i1, and NDUFA6) encodes proteins of the energy metabolism. Three tags were poorly annotated transcripts (Fndc3 and Riken cDNA clone 1300006L01), and for two tags there was no match in the databases.

Comparison of Specific Functional Protein Classes

Previously, a high-energy metabolism was reported in the SOC, based on glucose uptake measurements (Sokoloff, 1981) and gene expression profiling (Koehl et al., 2004). The comparison of 178 transcripts involved in glycolysis, tricarbonic acid cycle, oxidative phosphorylation, and fatty acid metabolism confirmed that the energy demand in the SOC [average tag count (atc) 15] is higher than in the striatum (atc 12) and the hippocampus (atc 7; Fig. 3A). The energy requirements in muscle, in contrast, apparently exceed that of the neural tissues, as the EOM has an atc of 24 and the EOM_DR of 27 (Fig. 3A). The high energy consumption in the SOC, compared to the other two neural tissues, may reflect the high average firing rate of auditory neurons (Koehl et al., 2004). Another explanation would be a higher rate of protein synthesis and protein degradation caused by the high activity of auditory neurons. In order to address this issue, we compared tag counts for genes encoding proteins for translation and protein degradation across the libraries. In addition, we analyzed tags for heat shock proteins, as these proteins are not only important to protect proteins from stress but also play a pivotal role in folding of newly synthesized proteins (Sollner, 2003; Borges and Ramos, 2005). For protein synthesis, we analyzed transcripts encoding ribosomal proteins and proteins associated with the translation machinery. The functional class of proteins involved in protein degradation was judged by comparing transcriptional expression levels for proteases and for proteins of the proteasome-ubiquitin degradation system (Ciechanover, 2005). Two hundred forty different tags for ribosomal(-interacting) proteins, 76 tags for other proteins involved in translation, 75 tags for heat shock(-interacting) proteins, 71 tags for proteases, 53 tags for the proteasome, and 97 tags for ubiquitin were identified by this approach. The average tag counts indicated that the highest level of protein synthesis occurs in the extraocular muscle (atc 26). Among the neural tissues, the highest value was observed in the striatum (atc 21), being about twice as high as in the SOC (atc 12) and in the hippocampus (atc 10; Fig. 3B). Concerning protein degradation, the highest average tag count was found in the striatum (atc 6), the SOC and EOM_DR each had an atc of 5, and hippocampus and EOM each had an atc of 4 (Fig. 3C). The analysis of transcripts encoding heat shock proteins revealed considerable upregulation in the SOC (atc 11) compared to the other tissues (atc 5 to 6; Fig. 3D). A closer inspection revealed that this was largely due to a single protein, Hspa8, with a normalized tag count of 313 and belonging to the group of the 33 most significantly upregulated genes in the SOC (Fig. 3D, Table 1). Among the three neural tissues, both protein synthesis and protein turnover appeared to be highest in the striatum (atc 21 and atc 6, respectively), followed by the SOC (atc 12 and atc 5, respectively), and being lowest in the hippocampus (atc 10 and atc 4, respectively). The high abundance of heat shock proteins in the SOC may therefore rather relate to their antistress function than to their involvement in the folding of newly synthesized proteins. Together, the data suggest that the increased energy demand of the SOC is not required for increased protein synthesis.

Next, we analyzed whether the high energy metabolism in the SOC is paralleled by an increased expression of genes encoding antioxidant proteins in order to protect against the increase of oxygen radicals generated via the respiratory chain, for example. Nineteen related gene names were extracted from the gene ontology database and their corresponding tag counts were compared (Fig. 3E). The EOM had the highest average tag count (atc 18), followed by the striatum (atc 16), the SOC and the EOM_DR (atc 14), and finally the hippocampus (atc 11). This order differed from the one observed for the energy metabolism when comparing all libraries or when compar-

TABLE 2. The 33 most significantly upregulated genes in the SOC compared to striatum and hippocampus

Tag sequence	Description	Gene name	SOC	Striatum	Hip
GCTTCATCCA	Myelin basic protein	*Mbp*	1845	824	295
CACATACAAA	Proteolipid protein	*Plp*	805	365	211
CTAAGGAAGT	SPARC-like 1	*Sparcl1*	411	135	213
ACGTCTCAAA	Tubulin, alpha 1	*Tuba1*	408	167	170
GAATAATAAA	Heat shock protein 8	*Hspa8*	313	36	44
TATATTAAAT	Synaptosomal-associated protein 25kDa	*Snap25*	306	18	91
TTCTAGCATA	ATPase, Na+/K+ transporting, beta 1	*Atp1b1*	284	117	100
TGGAAATGAC	Branched chain aminotransferase 1	*Bcat1*	241	86	88
TGATAATGAG	Lactate dehydrogenase B	*Ldhb*	199	27	35
AAATAAATGT	Myelin-associated glycoprotein	*Mag*	196	9	12
AAATAAAGAT	Sodium channel, voltage-gated, type I, beta	*Scn1b*	183	9	9
TTAGAGACCT	Complexin 1	*Cplx1*	173	45	12
TTAATAAATG	Cytochrome c oxidase subunit IV isoform 1	*Cox4i1*	166	41	21
TAGACAAAGG	Transcribed locus		153	14	5
TCTGAGGATG	Aldolase C, fructose-biphosphate	*Aldoc*	150	41	18
TATAGTATGT	Glutamine synthetase 1	*GluI*	147	36	49
TGTACTTGAA	ATPase, Na+K+ transporting, alpha 2	*Atp1a2*	140	9	60
GGAGAACCTT	Neurofilament 3, medium	*Nef3*	137	9	8
CCCTGAGCGG	Transferrin	*Tf*	130	23	13
AATAAAAGTT	Mitochondrial H+-ATP synthase alpha	*Atp5a1*	117	18	29
ATTAACTTGG	Glutamate dehydrogenase 1	*Glud1*	117	32	44
TAAAAAGAAA	N-myc downstream-regulated gene 2	*Ndrg2*	108	5	5
GAAAAATAAA	Ribosomal protein S26	*Rps26*	101	18	16
AATGTACTGA	Hyperpolarization activated K-channel 2	*Hcn2*	88	14	3
GTAATTAGAG	no match		82	9	21
TATTCTCAAC	Vesicle-associated membrane protein 1	*Vamp1*	82	0	10
TTAATAAAAG	T-cell activation protein	*Pgr1*	78	5	23
GGTGTACATA	no match		65	5	7
GAAAATAAAA	Fibronectin type III domain containing 3	*FNDC3*	65	5	1
GAAGAAGAAT	Plakophilin 4	*Pkp4*	65	0	3
CCCATAATCC	Glycoprotein, synaptic 2	*Gpsn2*	62	0	9
TAAAAGATAA	Similar to RIKEN cDNA 1300006L01		55	0	12
TTAATATTTA	NADH dehydrogenase 1 a subcomplex, 6	*NDUFA6*	52	0	10

The SOC library was compared pairwise with striatum or hippocampus and P-values were computed by the z-test using the SAGEstat program (Ruijter et al., 2002). Only those tags are shown which were upregulated in the SOC at a P-value <0.001 compared to both other neural tissues. Tags are listed in descending order of tag count in the SOC. Note that tag counts were normalized to library sizes of 100,000 tags. SOC, superior olivary complex; Hip, hippocampus.

ing only neural tissues. The data suggest no correlation between energy metabolism and antioxidant activity.

Our previous analysis had revealed a high abundance of genes involved in myelination in the SOC (Koehl et al., 2004). This observation is confirmed by the present comparison. According to average tag abundances of 17 transcripts important for myelination, expression of this functional gene class was highest in the SOC (atc 189), at an intermediate level in the striatum (atc 106), and at the lowest level in the hippocampus (atc 37; Fig. 3F). As expected for nonneural tissue, the average tag count in the two libraries of extraocular muscle tissues was very low (EOM, atc 3; EOM_DR, atc 4; Fig. 3F).

Comparison of Neuronal Membrane Proteins

The molecular mechanisms of rapid and reliable neurotransmission are of particular interest in the auditory system, where temporal precision and neuronal integration are of special importance. We therefore extracted receptors, non–receptor-type channels, and transporters and compared their corresponding tag counts. Many proteins within these categories play key roles in neurotransmission. In total, 516 unique tags related to receptors were identified in all five libraries; 114 of them in the SOC. In the category "channels," 143 unique tags were identified, of which 34 were present in the SOC. Finally, 118 unique tags for the category "transporters" were detected and 43 of them were expressed in the SOC. A selection of these proteins is listed in Table 3. T3a

Among the 114 receptor genes identified in the SOC library, 16 encode for neurotransmitter receptor subunits: 1 ionotropic glycine receptor (Glra1), 3 ionotropic (Gabra5, Gabrb1, Gabrg1) and 1 metabotropic GABA (Grp51) receptor, 3 ionotropic glutamate receptors (Grik2, Grik4, GrinA), 1 metabotropic glutamate receptor (Grm4), 3 purinergic receptors ($P2X_1$, $P2X_3$, $P2X_6$), 1 adenosine receptor (Adora2a), 1 cholinergic receptor (Chrm3), 1 bradykinin receptor (Bdkrb1), and 1 histamine receptor (Hrh3). Among them, Glra1, Gabra5, and GrinA showed the highest tag abundances, and Grm4 was restricted to the SOC library.

Within the category "channels," the largest group comprised eight genes that encode K^+ channels [Hcn2, Kv3.1 (Kcnc1), Kv3.2 (Kcnc2), Kv3.3 (Kcnc3), Kv12.2 (Kcnh3), Kir6.2 (Kcnj11), Twik1 (Kcnk1), and Slack (Kcnt1)], followed by six genes for Na^+ channels (Accn2, Accn4, Scn1a, Scn1b, Scn2a1, and Scn4b), and five transcripts for gap junction proteins [connexins 30 (Gjb6), 32 (Gjb1), 36 (Gja9), 40 (Gja5), and 43 (Gja1)]. Two transcripts encoded Ca^{2+} channel subunits [Ca(V)T.1 (Cacna1g), Ca^{2+} chan-

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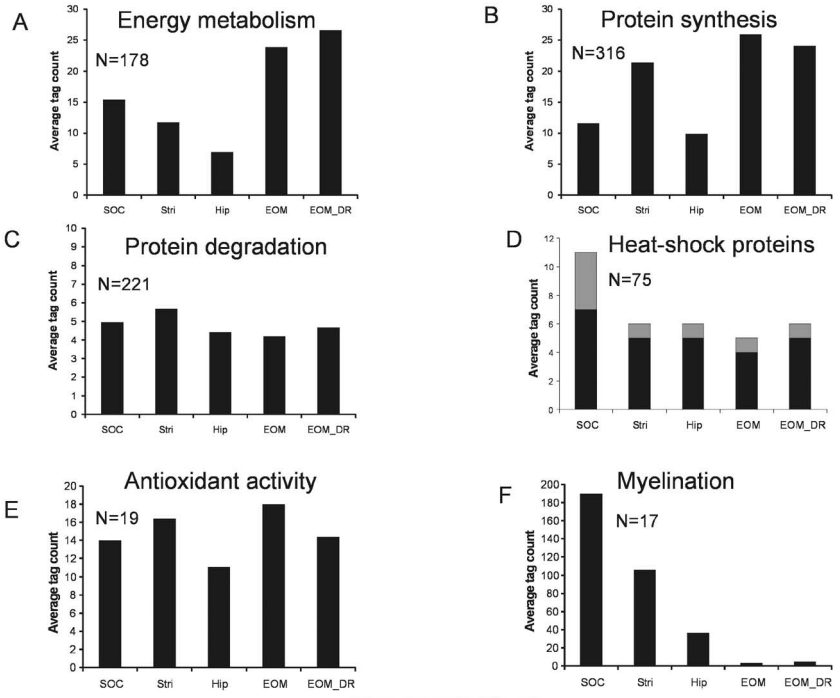

Fig. 3. Mean tag abundance for functional protein classes in five tissues (SOC, superior olivary complex; stria, striatum; hip, hippocampus; EOM, extraocular muscle; EOM_DR, extraocular muscle of dark-reared rats). The five libraries were searched for tags encoding proteins belonging to the following classes: energy metabolism (**A**), protein synthesis (**B**), protein degradation (**C**), heat-shock proteins (**D**), antioxidant activity (**E**), and myelination (**F**). Average tag counts are depicted for each protein class in the five libraries. N indicates the number of different tags analyzed. The gray-filled areas in D depict the contribution of the heat-shock protein Hspa8 to the average tag count.

nel α2/δ3 (Cacna2d3)] or Cl^- channels (Ttyh1, Ttyh2). Among the tags for K^+ channels, Hcn2, Kv3.1, and Kv3.3 showed considerably higher tag abundance in the SOC than in the other tissues, and Slack was only observed in the SOC library. Three other genes with increased expression in the SOC encoded connexins 30 and 32 and the maxi Cl^- channel Ttyh2.

Within the category "transporters," the largest group was formed by the Na^+/Cl^--dependent neurotransmitter transporters [Gat1 (Slc6a1), Glyt2 (Slc6a5), creatine transporter 1 (Slc6a8), Xtrp3 (Slc6a20)], followed by the Na^+-coupled neutral amino acid transporters of the Slc38 family (Slc38a2, Slc38a3, and Slc38a4) and the glucose transporters (Slc2a1, Slc2a3, and Slc2a8). Transcripts exclusively observed in the SOC library encoded the glucose transporter Slc2a1 and the amino acid transporter Asc-1 (Slc7a10). Other transcripts that were highly overrepresented in the SOC library encoded the glycine transporter Glyt2, the creatine transporter 1, the Na^+-dependent dicarboxylate transporter Nadc3 (Slc13a3), the monocarboxylate transporter Mct1 (Slc16a1), the phosphate transporter Glvr1 (Slc20a1), the system A amino acid transporter Snat2 (Slc38a2), and the organic anion transporter Oatp-D (Slco1a5).

Miscellaneous Neuronal Proteins

In a last step, we tried to find additional proteins with a potential role in neuronal function, performing several keyword searches. We identified proteins important for neurotransmitter biochemistry, such as the glutamate dehydrogenase Glud1, the glutamine synthetase1 Glul, and the acetylcholine esterase Ache (Table 4). All tags for these proteins were more abundant in the SOC than in the hippocampus or the striatum. Another protein class consisted of calcium-binding proteins. In this group, 5 out of 16 genes were more abundantly expressed in the SOC than in the striatum and the hippocampus: the calcium/calmodulin-dependent protein kinase II gamma (Camk2g), parvalbumin (Pvalb), the S100 calcium-binding protein A1 (S100a1), the calcium-dependent cytosolic phospholipase A2 (Pla2g4a), and the SPARC-related modular calcium-binding protein (Sparcl1). Twenty-four proteins involved in neuroexocytosis were identified by using "synap" or "syntaxin" as keywords. Tags being specifically abundant in the SOC represented transcripts for panthophysin 1 (SypI), syntaxin binding protein 1 (Stxbp1), synaptic glycoprotein 2 (Gpsn2), synaptosomal-associated protein 25 (SNAP25), and synaptotagmin 2 (Syt2). In addition, tags for the vesicular neurotransmitter transporters Viaat (Slc32a1) and Vglut2 (Slc17a6) were specifically present in the SOC library. Finally, proteins containing the term "*neuron*" in their annotation were looked up. Identified transcripts with higher abundance in the SOC than in the other two neural tissues included the neuron-specific vesicle coat protein NAP (Ap3b2), the GABA(A) receptor-associated protein like 1 (GABARapl1), the postsynaptic protein Homer 1, Pacsin 2, Pacsin 3, and the neuronal transmembrane protein Slitrk1; exclusively found in the SOC library was Slitrk2.

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TABLE 3. Comparative quantitative analysis of genes encoding plasma membrane receptors, channels and transporters

Tag sequence	Gene description	Gene name	SOC	Stri	Hip	EOM	EOM_DR
TAGAAAAATG	Monocarboxylic acid transporter Mct1	*Slc16a1*	10	0	0	2	0
TGGCCCAACA	Bradykinin receptor B1	*Bdkrb1*	7	14	78	0	6
TTTATATAAA	Muscarinic cholinergic receptor 3	*Chrm3*	10	5	5	2	0
AGAAAAAAAA	Ciliary neurotrophic factor receptor	*Cntfr*	16	0	4	11	16
AAAGGCAAAG	Lysophosphatidic acid receptor Edg-2	*Edg2*	7	0	3	2	0
TGACAGGAGT	Fibroblast growth factor receptor 2	*Fgfr2*	20	9	1	0	0
ATTGGATTAT	GABA A receptor, alpha 5	*Gabra5*	16	14	10	0	0
CTGCCAAACA	GABA A receptor, subunit beta 1	*Gabrb1*	7	5	8	4	0
AAATTCATTG	GABA A receptor, gamma 1	*Gabrg1*	3	0	0	0	0
CACACAGATA	Glucagon receptor	*Gcgr*	13	0	1	0	0
TCTGTCTTTA	Glycine receptor, alpha 1 subunit	*Glra1*	13	5	4	5	6
AAGCTGGGAG	Ionotropic glutamate receptor kainate 2	*Grik2*	3	5	1	0	0
GATTCTGGGT	Ionotropic glutamate receptor kainate 4	*Grik4*	7	5	0	0	0
TTTCAGGGGA	NMDA receptor associated protein 1	*Grina*	68	32	16	2	6
TATGTACACA	NMDA receptor like 1A	*Grinl1a*	16	9	7	9	6
GTTTTGCAAA	metabotropic glutamate receptor mGlu4	*Grm4*	3	0	0	0	0
GTATCGATTT	metabotropic GABA-B receptor 2	*Gpr51*	10	0	3	0	0
CTCTGCTGCC	Histamine receptor H3	*Hrh3*	7	18	5	0	0
CAAATAAAGA	Lymphotoxin beta receptor	*Ltbr*	16	5	0	5	3
GTAGCCACTA	Purinergic receptor P2rx1	*P2rx1*	3	0	0	0	0
CTGTACTCTC	Purinergic receptor P2rx3	*P2rx3*	3	0	0	0	0
GACACAGTAG	Purinergic receptor P2rx6	*P2rx6*	16	0	0	2	0
TTACTAACAC	Tyrosine kinase receptor 1	*Tie1*	7	0	0	0	6
AAGAGCCCAC	Transient receptor potential channel V6	*Trpv6*	3	0	4	0	0
TGAGGAAAGA	Brain sodium channel BNaC2	*Accn2*	3	0	3	0	0
TTGGGCTGGT	Amiloride-sensitive cation channel 4	*Accn4*	3	0	9	0	9
TAACTTACTT	voltage-dependent Ca-channel Ca(V)T.1	Cacna1g	3	0	0	0	0
GAAGTGAAGA	voltage-dependent Ca-channel α2/δ 3	Cacna2d3	3	0	0	0	0
TGCTCGGGAG	Connexin 43	*Gja1*	39	27	55	5	0
TTAAAAAAAA	Connexin 40	*Gja5*	42	5	20	15	25
TTTGCTGTGA	Connexin 36	*Gja9*	7	5	1	2	0
TCAGTGGGGA	Connexin 32	*Gjb1*	16	0	0	0	0
GTCATTGGAC	Connexin 30	*Gjb6*	10	0	0	0	0
AATGTACTGA	Hyperpolarization activated K-channel 2	*Hcn2*	88	14	3	2	0
AAATAAATTT	K-voltage gated channel Kv3.1	*Kcnc1*	20	0	4	2	0
TTACTAACTG	K-voltage gated channel Kv3.2	*Kcnc2*	7	0	4	0	0
CCCCTCCCCA	K-voltage gated channel Kv3.3	*Kcnc3*	23	0	4	0	0
TTTTTTATAT	K-voltage-gated channel Kv12.2	*Kcnh3*	7	0	3	0	0
GGCAGCTGTC	Inward rectifier K-channel Kir6.2	*Kcnj11*	3	0	1	0	0
GCAGATTGCA	K-channel, subfamily K, Twik 1	*Kcnk1*	23	14	10	0	3
CCTCAGGTCT	Na-activated potassium channel Slack	*Kcnt1*	3	0	0	0	0
TTGGAAAATG	Voltage-gated Na-channeltype 1, α	*Scn1a*	10	5	0	0	0
AAATAAAGAT	Voltage-gated Na-channeltype 1, β	*Scn1b*	182	9	9	46	13
CAGGAAAATG	Voltage-gated Na-channel 2, α 1	*Scn2a1*	3	0	1	0	0
CCCAGCACTT	Voltage-gated Na-channel 4, β	*Scn4b*	16	14	1	13	0
GTGAATTCGA	Tweety homolog 1	*Ttyh1*	72	63	33	0	0
TTTGCTCTCA	Tweety homolog 2	*Ttyh2*	23	5	0	0	0
AGAAGGACCT	Facilitated glucose transporter Glut1	*Slc2a1*	7	0	0	0	0
TTACTGTAGT	Facilitated glucose transporter Glut3	*Slc2a3*	26	14	4	2	0
CAGTCTCGCC	Facilitated glucose transporter Glut8	*Slc2a8*	7	9	0	0	3
CATTTTGTTC	Na-dependent vitamin transporter 6	*Slc5a6*	20	9	0	4	0
AAGATGTGTT	GABA transporter Gat1	*Slc6a1*	33	5	21	0	0
AAGAAAATAT	Glycine transporter Glyt2	*Slc6a5*	13	0	1	0	0
GACATAGCCC	Creatine transporter 1	*Slc6a8*	7	0	0	2	0
TGAATATGTC	Na-/Cl-dependent transporter Xtrp3	*Slc6a20*	39	27	16	49	25
CTTCTGCAGA	y+ system cationic amino acid transporter 1	*Slc7a1*	7	9	0	0	0
TTGTTTATTG	Amino acid transporter, y+ system, Asc-1	*Slc7a10*	23	0	0	0	0
TCCATCCAGG	K/Cl-transporter KCC2	*Slc12a5*	10	18	4	0	0
ATTTCTGATA	Na-dependent dicarboxylate transporter Nadc3	*Slc13a3*	29	0	0	2	0

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TABLE 3. Comparative quantitative analysis of genes encoding plasma membrane receptors, channels and transporters (continued)

Tag sequence	Gene description	Gene name	SOC	Stri	Hip	EOM	EOM_DR
TAGAAAAATG	Monocarboxylic acid transporter Mct1	*Slc16a1*	10	0	0	2	0
CCAACAAGAA	Monocarboxylic acid transporter Mct6	*Slc16a6*	81	181	171	11	25
TTCATCTGTC	Phosphate transporter Glvr1	*Slc20a1*	42	5	8	2	0
CTGAGCCTTG	Fatty acid transporter Fatp1	*Slc27a1*	36	27	14	4	6
AGGCTTTATG	Zinc transporter Znt1	*Slc30a1*	13	5	3	0	3
TGATTTCAAT	System A amino acid transporter Snat2	*Slc38a2*	46	0	1	5	6
GAGGAAACCA	System N amino acid transporter Snat3	*Slc38a3*	3	0	3	2	0
TGAAAAAAAA	System A amino acid transporter Snat4	*Slc38a4*	52	0	44	95	116
TGAAAGAAAA			7	0	1	4	6
CGTTAAAATA	Organic anion transporter Oatp-D	*Slco1a5*	10	0	1	0	0
ATATAAAGTG	Na-independent organic anion transporter D	*Slco3a1*	13	5	1	4	0

The table lists selected genes that encode plasma membrane proteins (receptors, channels, and transporters). Genes encoding receptors are listed first in alphabetic order, then channels and finally transporters. Note that tag counts were normalized to library sizes of 100,000 tags. SOC, superior olivary complex; Stri, striatum; Hip, hippocampus; EOM, extraocular muscle; EOM_DR, extraocular muscle of dark-reared rats.

DISCUSSION

The recent generation of comprehensive inventories of expressed genes in several organisms established the global gene expression analysis as a promising tool to gain insight into the molecular determinants of physiological and pathophysiological processes. Subsequent comparative global analyses of tissue obtained under different physiological conditions (He et al., 1999; Hermeking et al., 2000; Trendelenburg et al., 2002) or of different tissues (Velculescu et al., 1999; Blackshaw et al., 2001; de Chaldée et al., 2003; Blackshaw et al., 2004) identified and often validated many candidate genes for pathological processes or tissue-specific tasks. Global gene expression approaches allow the identification of potential candidate genes for specific functions without an a priori assumption on what those factors might be. Examples include the identification of the sulfate transporter family member prestin as a cochlear amplifier protein (Zheng et al., 2000), or the identification of metallothionein-II as a major neuroprotective protein in focal cerebral ischemia (Trendelenburg et al., 2002).

In the present study, the comparison of the gene expression profile in the SOC with that of other neural and nonneural tissues led to the identification of various tissue-specific properties. Moreover, it spotted candidate genes for auditory neuron-related specializations and identified many genes that were not previously considered to be important for the SOC. The total quantity of unique transcripts in the SOC exceeded the number of 10,000, thereby precluding a detailed discussion of every identified transcript. Rather, it required a subjective preselection of a subset of transcripts warranting a detailed inspection. As large-scale analysis is performed to generate novel hypotheses, we will allude mainly to genes that had not been studied previously in the SOC. We will briefly speculate on their potential function in the context of known properties of SOC neurons. First, we will focus on proteins involved in shaping neurotransmission. Second, we will discuss the role of high energy metabolism. Finally, we will point out the implication of the data for a genetic dissection of central auditory processing disorders and center-specific tasks. The discussion will thereby focus on the comparison of the SOC with the two other neural tissues (hippocampus and striatum), as such a comparison is of primary neurobiological interest.

Proteins Involved in Propagation of Action Potentials and Synaptic Transmission

SOC neurons compare both binaural differences in time and loudness as part of the mechanism for estimating the direction of a sound source in space. Such temporal cues rely on high-fidelity neurotransmission along the auditory pathway, which is ultimately determined by a distinct molecular repertoire. A comparison with neural structures of nonauditory function, such as the hippocampus and the striatum, should assist in defining genes particularly important to auditory neurons. Indeed, most of the transcripts, which were most significantly upregulated in the SOC compared to the striatum and the hippocampus, encode proteins involved in neurotransmission. They include proteins that are important for action potential generation and propagation. For example, the regulatory voltage-gated Na^+-channel subunit Scn1b increases the functional expression and the current amplitude of heterologously expressed α-subunits that form the ion conducting pore (Makita et al., 1994). Furthermore, the subunit shifts the steady-state activation and inactivation curves toward more negative potentials and accelerates recovery from inactivation (Makita et al., 1994). Its high expression in SOC neurons might contribute to their high-frequency spike rate. Myelination causes saltatory conduction of action potentials and thereby considerably increases conduction velocity.

Several SOC-specific genes are involved in synaptic transmission. Cplx1, Snap25, and Vamp1 encode proteins involved in exocytosis of synaptic vesicles. Vamp1 is localized primarily on synaptic vesicles, whereas Snap25 resides mainly in the plasma membrane (Murthy and De

TABLE 4. Comparative quantitative analysis of genes encoding selected proteins important for neuronal function

Tag sequence	Gene description	Gene name	SOC	Stri	Hip	EOM	EOM_DR
TTTTGGGCTG	Acetylcholinesterase	*Ache*	7	0	1	0	3
TTTTAAACTG			7	0	5	2	0
TCACTAAAGC	Glul Glutamine synthetase 1	*Glul*	13	5	7	7	3
TATAGTATGT			147	36	49	16	3
CTGCTGTAAT	Glud1 Glutamate dehydrogenase 1	*Glud1*	13	5	5	0	9
ATTAACTTGG			117	32	44	13	19
CATCCTTGAT	Creatine kinase, brain	*Ckb*	283	154	73	7	3
ATCCCTGCGC	Creatine kinase, muscle	*Ckm*	0	0	0	212	173
AAATAATTTT	L-arginine:glycine amidinotransferase	*Gatm*	20	0	0	0	0
AGAGAAAAGG	N-myc downstream-regulated gene 1	*Ndrg1*	23	4	0	0	0
ATCAATAAAT	Doublecortin and Ca/calmodulin-dependent protein kinase-like 1	*Ania4*	3	0	8	0	0
TTACTCTCTC			3	5	3	2	3
TCTGCCCCGA	Calcium binding protein 1	*Cabp1*	10	14	8	0	0
ACCCTCTAAA	Ca/calmodulin-dependent protein kinase II beta	*Camk2b*	3	14	23	5	0
TTTGTGTCTG	Ca/calmodulin-dependent protein kinase II beta	*Camk2b*	20	23	56	53	79
ACGACATAGA	Ca/calmodulin-dependent protein kinase II gamma	*Camk2g*	23	0	17	2	9
ATAAGCACTA	Ca/calmodulin-dependent protein kinase IV	*Camk4*	3	9	1	0	0
CTCACTGTCA	Ca/calmodulin-dependent protein kinase kinase 1α	*Camkk1*	7	14	12	0	0
GTTGACCTGC	Ca-regulated heat stable protein 1	*Carhsp1*	3	9	1	0	0
GCCCCTTTCA			3	0	0	4	3
CGAAATAAAT	Ca-dependent cytosolic phospholipase A2	*Pla2g4a*	7	0	0	0	0
CCCCATCTCA	Parvalbumin	*Pvalb*	59	5	3	168	170
CTACACCATA	calcium and DAG-regulated GEF I	*Rasgrp2*	3	23	1	0	0
CACCAAAGGC	S100 calcium binding protein A1	*S100a1*	10	5	5	18	28
TGCACAGTGC	S100 calcium-binding protein A4	*S100a4*	3	14	3	13	19
GTAAGGTGGA	SPARC-related modular calcium binding protein 1	*Smoc1*	3	0	0	0	0
CCCATAATCC	Glycoprotein, synaptic 2	*Gpsn2*	62	0	9	4	31
GCAAAGTGTC	pantophysin 1	*Sypl*	52	9	22	20	35
TGACCATAAC	Vesicular glutamate transporter Vglut2	*Slc17a6*	23	0	3	0	0
CCCTAGACAT	Vesicular inhibitory amino acid transporter Viaat	*Slc32a1*	16	0	0	0	0
TATATTAAAT	Synaptosomal-associated protein 25	*Snap25*	306	18	91	0	0
ACAGTAGCAT			3	5	1	0	0
TCTGCCCTCT	Synaptosomal-associated protein 91	*Snap91*	26	27	55	0	0
TCCTGTCCCC	Syntaxin 1B2	*Stx1b2*	7	3	0	0	2
GAGTCCTTCC	Syntaxin 4A	*Stx4a*	3	1	0	9	4
CTGCAGCCTG	Syntaxin 5a	*Stx5a*	7	7	36	3	4
TCTGCTAAAA	Syntaxin 7	*Stx7*	3	7	5	0	0
CTGTCACTTA	Syntaxin 12	*Stx12*	3	3	5	0	2
CCTACTGTTT	Syntaxin 18	*STX18*	7	1	5	0	0
TGGGGTTTCC	Syntaxin binding protein 1	*Stxbp1*	52	22	14	0	2
GTCAAACCAG	Synaptic vesicle glycoprotein 2 a	*Sv2a*	3	5	7	0	0
AGTTGGAAAC	Synaptic vesicle glycoprotein 2 b	*Sv2b*	33	27	36	0	0
CTCTGTGTGG	Synaptic vesicle glycoprotein 2c	*Sv2c*	13	14	0	0	0
TAAATGATAC	Synaptobrevin-like 1	*Sybl1*	7	0	0	0	0
CAAAGCTTTT			3	0	0	0	0
CTAGGCATAT	Synapsin I	*Syn1*	7	0	10	0	0
CCGCTATAAC	Synapsin II	*Syn2*	3	9	7	0	0
CTGGAGGTGT			13	59	27	0	0
CCCTGGTCCC	Synaptogyrin 1	*Syngr1*	7	5	4	0	0
GAATTTTTAC	Synaptojanin 1	*Synj1*	7	5	5	0	0
GTTAGGAGCT	Synaptophysin	*Syp*	3	14	17	0	0
GACACTCAAT	Synaptotagmin 1	*Syt1*	7	68	116	0	0
TGGCCCTCTG	Synaptotagmin XI	*Syt11*	3	18	4	0	0
AAAATAAACT	Synaptotagmin 2	*Syt2*	7	0	0	0	0
GTGTAAGGGA	Synaptotagmin 4	*Syt4*	3	18	5	0	0
TATTCTCAAC	Vesicle-associated membrane protein 1	*Vamp1*	82	0	10	2	0
CCCCCAATTC	Vesicle-associated membrane protein 2	*Vamp2*	16	41	34		
CTGGTGAAGG	Neuron-specific vesicle coat protein NAP	*Ap3b2*	13	9	4	0	0
ACATTTCAAT	GABA(A) receptor-associated neuron protein like 1	*GABARapl1*	36	9	8	2	3
ACAGTTCCAG	Homer, neuronal immediate early gene 1	*Homer*	10	3	5	4	0
CTAGGCAAGC	Homer, neuronal immediate early gene 3	*Homer3*	3	27	3	0	6
GAATCCAACT	Similar to neuronal protein 15.6	*Ndufb11*	7	5	9	110	53
GAGAGCTAAC	Neuronatin	*Nnat*	3	5	22	0	0
CTGCACAGAG	Neuron-glia-CAM-related cell adhesion molecule	*Nrcam*	3	5	7	2	0
CCGAGGAGTC	Neuron specific gene family member 1	*Nsg1*	7	14	7	0	0
AGAACTTACC	kinase substrate in neurons 2	*Pacsin2*	7	0	3	2	0
ATGGGAAGGG	kinase substrate in neurons 3	*Pacsin3*	7	5	1	7	0
AATGTATTGT	neuronal transmembrane protein Slitrk1	*Slitrk1*	16	9	5	4	6
CAGAAGGAAG	neuronal transmembrane protein Slitrk2	*Slitrk2*	3	0	0	0	0

The table lists the genes that encode selected neuronal proteins of interest for neurotransmission. Note that tag counts were normalized to library sizes of 100,000 tags. SOC, superior olivary complex; Stri, striatum; Hip, hippocampus; EOM, extraocular muscle; EOM_DR, extraocular muscle of dark-reared rats.

Camilli, 2003). Both proteins are part of the SNARE complex, which also contains syntaxin 1, mainly localized in the plasma membrane (Murthy and De Camilli, 2003). The three SNARE proteins are essential for neurotransmitter release and the formation of a complex among these proteins is the minimal molecular requirement for membrane fusion (Weber et al., 1998; Hilfiker et al., 1999). The interaction between the complementary syntaxin and Vamp transmembrane regions that reside in opposing membranes prior to fusion is likely facilitated by the cytosolic protein complexin (Hu et al., 2002). In contrast to transcripts involved in myelination, we observed no general upregulation of transcripts important for exocytosis. This is surprising in view of the capacity of auditory neurons to follow stimuli of up to several hundred Hz (Wu and Kelly, 1993). Apparently, only few distinct protein species, such as those of the SNARE complex, have to be abundant to ensure sustained exocytosis at such a high frequency.

Three other SOC-abundant transcripts encode the glutamine synthetase 1 (Glul), the glutamate dehydrogenase 1 (Glud1), and the Na^+-dependent neutral amino acid transporter Snat2. These proteins participate in the glutamate-glutamine cycle, which replenishes neurons with glutamate (Mackenzie and Erickson, 2004). After presynaptic release, glutamate is taken up into astrocytes, where it is converted to glutamine by glutamine synthetase. Glutamine is then transferred to the neurons via Na^+-dependent neutral amino acid transporters, where it serves as substrate for the glutamate dehydrogenase to synthesize glutamate. Whereas these three proteins play an essential role in excitatory neurotransmission, the high abundance of Atp1a2 is important for inhibitory neurotransmission. Atp1a2 knockout mice show a high intracellular Cl^- concentration and, consequently, depolarizing actions of inhibitory neurotransmitters in respiratory brainstem neurons (Ikeda et al., 2004). Atp1a2 likely influences the function of the neuron-specific K^+-Cl^- cotransporter KCC2, as both proteins can be coimmunoprecipitated (Ikeda et al., 2004). KCC2 transport activity is both essential and sufficient to render inhibitory neurotransmitters hyperpolarizing (Rivera et al., 1999; Balakrishnan et al., 2003; Lee et al., 2005). The high presence of Atp1a2 transcripts in the SOC is therefore likely related to the fundamental role of inhibitory inputs in sound localization in SOC neurons (Brand et al., 2002; Grothe, 2003).

In addition to the above-mentioned transcripts with highly significant abundance in the SOC, several other receptors, channels, and transporters of the plasma membrane were identified. Despite the fact that they revealed a low or even no differential regulation on the transcriptional level, some of them may play an important role in SOC function. Two interesting candidates are the metabotropic glutamate receptor subunit Grm4 and the metabotropic $GABA_B$ receptor Gpr51. Electrophysiological experiments demonstrated a role of metabotropic glutamate receptors in synaptic depression in the SOC (Barnes-Davies and Forsythe, 1995; Takahashi et al., 1996). Agonists of mGluRs suppressed a high-voltage-type Ca^{2+} conductance in the axonal terminals of the calyx of Held, which led to reduced transmitter release. Results obtained through pharmacological experiments and an expression analysis pointed mainly to mGluR subtypes 4 or 7 (Barnes-Davies and Forsythe, 1995; Takahashi et al., 1996).

Thus, Grm4 represents an attractive candidate for presynaptic depression. The gene is also expressed in the neurons of the AVCN (D. Friedland, personal communication), which give rise to the calyx of Held. By contrast, the metabotropic $GABA_B$ receptor Gpr51 might enhance synaptic efficacy at high frequency. In the avian magnocellular nucleus (the equivalent to the mammalian VCN), the $GABA_B$ receptor agonist baclofen delayed the onset of depression at high frequencies, leading to a fivefold increase in postsynaptic currents (Brenowitz et al., 1998).

An intriguing observation was the identification of two members of a novel large conductance Cl^- channel family, Ttyh1 and Ttyh2 (Suzuki and Mizuno, 2004). Ttyh2 expression was markedly increased in the SOC compared to the striatum and the hippocampus. The fact that Ttyh1 causes large swelling-induced Cl^- currents suggests a role in cell volume regulation (Suzuki and Mizuno, 2004). The role of these Cl^- channels in neurons, however, may be different as the intracellular chloride concentration is tightly regulated due to its importance in inhibitory neurotransmission (Ben-Ari, 2002).

Energy Metabolism

Five of the 33 transcripts in the SOC that were most significantly upregulated compared to striatum and hippocampus encode proteins involved in energy metabolism (Ldhb, AldoC, Atp5a1, Cox4i1, and Ndufa3). The high energy metabolism in the SOC was confirmed by the analysis of genes important for glycolysis, the tricarbonic acid cycle, oxidative phosphorylation, and fatty acid metabolism. The average tag count of these transcripts was 15 in the SOC, 12 in the striatum, and 7 in the hippocampus. In addition, we observed an increased expression of several genes of the creatine metabolism in the SOC. They included transcripts encoding the plasma membrane creatine transporter, the enzyme L-arginine:glycine amidinotransferase, which catalyzes the first rate-limiting step in creatine synthesis (Wyss and Kaddurah-Daouk, 2000), and the brain creatine kinase. These findings on the transcriptional level are in excellent agreement with a previous analysis of energy metabolism in the brain. Radioactive deoxyglucose uptake measurements in various rat brain regions placed the energy demand in the striatum with a glucose uptake of 110 ± 4 μmoles/100 g per minute between the ones observed in the SOC (133 ± 7 μmoles glucose uptake/100 g per minute) and the hippocampus (79 ± 3 μmoles glucose uptake/100 g per minute). The high energy demand of the SOC is also indicated by the high abundance of proteins involved in the glutamate-glutamine cycle, which was shown to be proportional to neuronal glucose oxidation (Patel et al., 2004, 2005).

What might be the cause of the high energy demand in the SOC? One explanation is the high activity of auditory neurons (Trussell, 1999). Synaptic transmission is associated with an intense energy demand. A thorough calculation of the energy demands of cortical neurons with a spike rate of 4 Hz suggested that 81% of the total ATP consumption is used for action potential propagation and postsynaptic ion fluxes, whereas the basal energy consumption for the maintenance of resting potentials accounted for just 13% (Attwell and Laughlin, 2001). In line with this calculation, energy consumption is halved in deeply anesthetized brains (Laughlin and Sejnowski, 2003). Further support for the high energy demand associated with synaptic transmission comes from studies on mitochondria. The loss of mitochondria in axonal termi-

nals led to aberrant synaptic transmission in the visual system of *Drosophila melanogaster* (Stowers et al., 2002). In the hippocampus, postsynaptically localized mitochondria appear to be the limiting factors that determine the ability of dendrites to support synapses and to make new synapses in response to potentiating stimuli (Li et al., 2004). Furthermore, synaptic activity modulated the mitochondrial movements in these neurons (Li et al., 2004).

If an extraordinarily high energy supply is indeed mandatory for auditory neurons, appropriate distribution of mitochondria in postsynaptic areas is required. In hippocampal neurons, activity-dependent Ca^{2+} influx results in reduced mitochondrial motility likely to achieve a higher steady-state concentration of mitochondria in active dendritic regions. A surprising facet of neurotransmission in the auditory brainstem is the abundance of AMPA receptors with a high Ca^{2+} permeability (Trussell, 1999), despite the potential cytotoxicity of the heavy Ca^{2+} load in highly active auditory neurons. Several roles have been proposed for this Ca^{2+} influx, such as activation of Ca^{2+}-sensitive ion channels and activation of second-messenger systems to allow for rapid control of the Ca^{2+} concentration in the synaptic cleft (Trussell, 1999). In view of the metabolic constraints on neurotransmission, the high Ca^{2+} permeability of auditory AMPA receptors may alternatively be required for a decreased mitochondrial motility, thus ensuring persistent energy supply. The juxtaposition of sites of generation with sites of consumption will facilitate energy transfer, as ATP diffusion is relatively slow (Ames, 2000). In addition, raised $[Ca^{2+}]_i$ increases the activity of three mitochondrial dehydrogenases and the rate of ATP generation (Ames, 2000). The lack of such mechanisms in inhibitory neurotransmission can be explained by the lower energy demand. Cl^- outward transport has only to surmount the chemical gradient, whereas Na^+ extrusion has to surmount the electrical and the chemical gradients.

Another cause for the observed high energy metabolism in the SOC may be a rapid protein turnover, which is paralleled by a high rate of protein synthesis. Protein synthesis is generally considered to be the most energy-demanding process among the various housekeeping functions of cells (Ames, 2000). In hippocampal neurons, increased neuronal activity led to an accelerated turnover of proteins of the postsynaptic density (Ehlers, 2003). Nevertheless, we observed no increased expression of transcripts encoding proteases and proteins important for protein synthesis in the SOC. These data suggest that the increased energy demand in the SOC is likely not linked to increased protein synthesis and protein degradation.

Genetic Dissection of Central Auditory Processing Disorders and Functional Tasks of Auditory Centers

Many of the SOC-upregulated transcripts likely play an important role for proper function, as discussed above. This implies that mutations therein most probably result in impaired hearing. About 2–3% of children and 10–20% of older adults suffer from central auditory processing disorders (CAPDs) (Gates et al., 1990; Chermak and Musiek, 1997). Individuals with CAPD experience difficulties in comprehending spoken language in competing speech or noise backgrounds. This is likely due to deficits in sound localization and lateralization, reduced temporal resolution of acoustic signals, and deficits in auditory pattern recognition (Chermak and Musiek, 1997). Based on the observed heterogeneity of peripheral deafness (Steel and Kros, 2001), CAPDs are likely caused by a pleiotropy of factors as well. This confounds the identification of the underlying gene defects by classical linkage studies (Morton, 2002). Therefore, a more promising approach employs candidate gene-directed association studies in families with CAPD (Ballabio, 1993; Heller, 2002). In principle, all transcripts specifically upregulated in the auditory system represent excellent candidates for CAPD. Large-scale gene expression analyses in the auditory system, therefore, present an important resource to dissect the molecular basis of CAPD.

Interestingly, mutations in two SOC-upregulated transcripts, Gjb1 and Ndrg1, affect conduction in the central auditory pathway (Nicholson and Corbett, 1996; Kalaydjieva et al., 1998). Gjb1 is a gap junction protein and Ndrg1 is a cytoplasmic protein abundantly expressed in Schwann cells and astrocytes (Wakisaka et al., 2003; Okuda et al., 2004). Its precise role in the maintenance of myelin sheaths is as yet unknown. Both examples point to genes, participating in generation and maintenance of myelin sheaths, as candidates for CAPD. Another large group of candidates for CAPD are genes involved in energy metabolism. Due to the paucity of energy reserves in the brain (Ames, 2000), any decrease in the capacity of neurons to generate energy will rapidly impair function. Marginal but sustained energy deficiency results in a reduced function of the Na^+/K^+-ATPase (Naritomi et al., 1988). Indeed, several mitochondrial diseases are associated with deafness (Wallace, 1999). They include mutations in MTDN6, encoding a protein of the respiratory chain complex I (Ugalde et al., 2003), and TIMM8A, a protein of the translocase of inner mitochondrial membranes (Jin et al., 1996). Interestingly, the repair capacity of oxidative DNA damage in mitochondria decreases during aging, leading to increased number of mutations in mitochondrial genes (Imam et al., 2005). This may contribute to the increased prevalence of CAPD in elderly people.

Another important impact of large-scale gene expression data on auditory neuroscience will be the identification of center-specific promoters. The complexity of the auditory system makes it difficult to identify the precise role of individual processing centers in progressive transformation of acoustic information and the functional consequences of those transformations (Pollak et al., 2003). Spatially restricted genetic ablation of individual genes will assist in defining the function of distinct auditory centers (Callaway, 2005). In addition, several genetic methods exist for a reversible neuronal inactivation on a slow time scale, including overexpression of K^+ channels (Johns et al., 1999; Nadeau et al., 2000) or membrane-tethered toxins to block ion channel functions (Ibanez-Tallon et al., 2004). The application of these methods will present novel approaches to the study of plasticity in the auditory system. Indeed, preliminary results obtained by comparing SAGE libraries of the SOC, the MNTB, the LSO, and three regions of the CN imply considerable differences in gene expression between these structures (data not shown). Their confirmation by independent methods, e.g., in situ hybridization or immunohistochemistry, will reveal whether center-specific promoters exist in the auditory pathway.

In summary, the global gene expression analysis of the SOC revealed various candidate genes for auditory-specific functions. Follow-up studies on these genes will likely provide insights into the molecular basis underlying SOC function. Moreover, the analysis of forthcoming large-scale data from other auditory areas will delineate avenues for targeted and improved molecular and genetic research on the auditory brainstem, which is as yet lacking behind that of many other brain regions.

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