Coordinated regulation of gene expression by Brn3a in developing sensory ganglia

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Summary

Mice lacking the POU-domain transcription factor Brn3a exhibit marked defects in sensory axon growth and abnormal sensory apoptosis. We have determined the regulatory targets of Brn3a in the developing trigeminal ganglion using microarray analysis of Brn3a mutant mice. These results show that Brn3 mediates the coordinated expression of neurotransmitter systems, ion channels, structural components of axons and inter- and intracellular signaling systems. Loss of Brn3a also results in the ectopic expression of transcription factors normally detected in earlier developmental stages and in other areas of the nervous system. Target gene expression is normal in heterozygous mice, consistent with prior work showing that autoregulation by Brn3a results in gene dosage compensation. Detailed examination of the expression of several of these downstream genes reveals that the regulatory role of Brn3a in the trigeminal ganglion appears to be conserved in more posterior sensory ganglia but not in the CNS neurons that express this factor.

Supplemental data available online

Key words: Brn3a, POU-domain, Trigeminal ganglion, Microarray, Sensory neuron

Introduction

Studies of the developing vertebrate nervous system have revealed a large number of transcription factors that are expressed in specific populations of neurons or their precursors. In dividing neuroepithelial cells, transcription factors of the bHLH, homeodomain and other classes characterize regions of the neural tube with specific developmental potentials. Later in neurogenesis, other transcription factors are expressed in specific populations of neurons, and may persist in the mature nervous system. Naturally occurring and induced mutations of both the early and late transcription factors have been shown to exert profound effects on neural development.

Transcription factors reside permanently or conditionally in the nucleus, and are presumed to work by interacting with specific cis-acting binding sites in the vicinity of the transcription units they regulate. These ‘target genes’ in turn mediate the effects of the transcription factor on developmental fate decisions, neuronal phenotype and cell survival. However, the downstream targets of these factors cannot necessarily be inferred from their expression patterns, because they are usually not congruent with those of other classes of neural genes, such as neurotransmitters or their receptors. In a few cases, plausible regulatory relationships have been established between neural transcription factors and their targets, but for the vast majority, no clear pathways are known. Using conventional methods applied to individual genes, establishing these transcription factor-target relationships is quite inefficient.

In principle, comparing the transcript pool of neural tissue from a wild type animal to that of an animal under- or over-expressing a given factor should yield a complete set of genes regulated in that cell type. However, because of the tremendous cellular diversity present in most regions of the nervous system, the resulting changes in gene expression in a specific cell type may be obscured by the heterogeneity of the sample. Furthermore, the changes in target gene expression may be regulated indirectly, either as downstream or compensatory effects.

We have been engaged in studies of Brn3a (Pou4f1 – Mouse Genome Informatics), a transcription factor of the POU-domain family which is expressed in terminally differentiating neurons of the sensory peripheral nervous system and caudal CNS. Targeted mutations in mice have shown that Brn3a is necessary for the correct development and/or survival of neurons in the sensory ganglia and some CNS nuclei (McEvilly et al., 1996; Xiang et al., 1996). Sensory neuron death in *Brn3a* knockout mice is preceded by loss of neurotrophin receptor expression (Huang et al., 1999; Ma et al., 2003), and by markedly defective axonal growth (Eng et al., 2001). Despite the success of the knockout approach in demonstrating the importance of Brn3a and related POU factors in neural development, these experiments have yielded little information about what genes these factors regulate, and why they are essential for normal axon growth or neuronal survival.

In the present study, we have used microarrays to compare the patterns of gene expression in the trigeminal ganglia of *Brn3a* knockout and wild-type mice. To maximize the homogeneity of the samples and to minimize secondary effects on gene expression, we have analyzed embryonic ganglia. At the stage chosen for analysis, embryonic day 13.5 (E13.5),

major defects in sensory axon growth are observed in the mutant mice (Eng et al., 2001), but the phase of marked sensory neuron death has not yet commenced (Huang et al., 1999).

Our results demonstrate that Brn3a regulates a coordinated program of gene expression that defines the phenotype of developing trigeminal neurons, including the regulation of neurotransmitters, receptors, ion channels, mediators of axon growth, and other transcription factors. Many of these target genes have known roles in sensory neurons and are strong candidates for mediating the observed effects of Brn3a on axon growth and cell survival. Some of the genes regulated by Brn3a in the trigeminal ganglion are also changed in other sensory ganglia in *Brn3a* knockout mice, but do not appear to be altered in Brn3a-expressing CNS neurons, suggesting that the roles of Brn3a in the sensory system and CNS may be distinct.

Materials and methods

Matings, embryos and immunohistochemistry

To generate tissue for microarray analysis, timed matings of Brn3a heterozygote animals were performed, and the embryos harvested at embryonic day (E)13.5. Only embryos at E13.5±0.5 days, based on the staging system of Theiler (Theiler, 1972), were pooled for microarray analysis. Trigeminal ganglia were removed by blunt dissection and carefully freed of adherent non-neural tissue with fine forceps. Only complete ganglia were used for analysis. Dissected ganglia were placed in RNAse inhibitor solution (RNAlater, Ambion) and frozen until enough tissue was harvested to be pooled. Embryos were genotyped from a sample of tail or hindlimb tissue harvested at the time of ganglion dissection. Genotypes were determined for the native *Brn3a* allele and the neomycin resistance cassette included in the null allele as previously described (Eng et al., 2001). Approximately 10-12 genotyped ganglia were sufficient to provide 5 μg of total RNA for a single analysis, which was extracted using the RNeasy system (Qiagen). The generation of cDNA, production of labeled cRNA, and hybridization to GeneChip arrays were all performed according to standard protocols provided by the manufacturer (Affymetrix).

Non-isotopic in situ hybridization was performed as previously described (Birren et al., 1993). A table of probes used and their sources appears in the Supplemental Data (Table S1, http://dev.biologists.org/supplemental/). Immunofluorescence for Brn3a was performed with rabbit polyclonal antisera as previously described (Fedtsova and Turner, 1995). Immunofluorescence for other antigens was performed with commercially available antibodies, including rabbit anti-calretinin (Swant), rabbit anti-galanin (Peninsula Laboratories), rabbit anti-somatostatin-14 (Peninsula Laboratories), and rabbit anti-tyrosine hydroxylase (Chemicon).

Analysis of expression array data

The primary analysis of microarray data, including determination of the absence/presence of the assayed transcripts, transcript expression levels, and the probability of change in transcript expression between genotypes ('change-p') was performed with Microarray Suite 5.0 (Affymetrix). Two proprietary databases were used to relate microarray results for ESTs to the identity of the expressed transcripts, NetAffx (Affymetrix) and GeneSpring (Silicon Genetics). The results for those transcripts identified in both databases were discordant in less than 1% of cases.

Results

Microarray analysis of gene expression in the developing trigeminal ganglion

To begin to address the complement of genes regulated by Brn3a, we chose to analyze gene expression in the trigeminal ganglia of *Brn3a* wild-type, heterozygous and knockout mice at E13.5. These ganglia do not represent a homogeneous population with respect to their eventual sensory subtype, but at this stage most of the cells in the trigeminal ganglion exhibit Brn3a immunoreactivity (Trieu et al., 2003) and thus it is likely

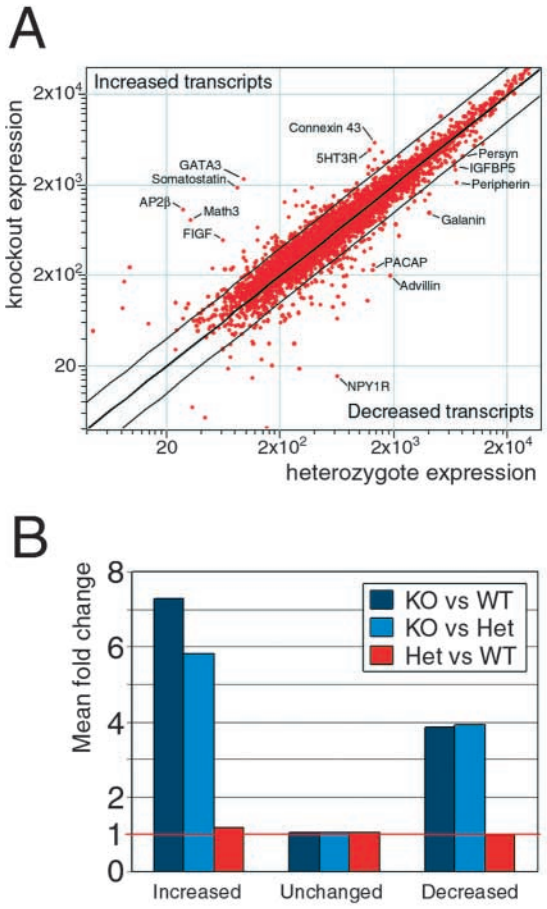

Fig. 1. Expression array analysis of E13.5 trigeminal ganglia. (A) A two-dimensional plot of the hybridization signal for ~4,000 present transcripts, including ESTs, in one of the two experiments comparing *Brn3a* heterozygous and knockout trigeminal ganglia. Values for the expression of the majority of transcripts fall along the central diagonal line representing equal expression in the two genotypes. A few transcripts fall outside the parallel lines indicating a greater than twofold change in expression, and examples of some highly changed transcripts are indicated. (B) To determine whether *Brn3a* heterozygosity results in intermediate levels of target gene expression, two-way comparisons were made between transcript levels in knockout versus wild-type, knockout versus heterozygous and wild-type versus heterozygous ganglia for the 41 increased and 62 decreased transcripts from the U74Av2 array, Experiment 1. Expression levels in wild type and heterozygotes showed a similar mean fold increase or decrease when compared to those in the knockout. However, comparison of expression levels in heterozygotes with those in wild type revealed no significant difference (fold change ~1, red horizontal line) for either the increased or decreased transcripts. As expected, the fold change was also approximately 1 for two-way comparisons between the genotypes for a group of 160 unchanged neural transcripts (0.003<change-p<0.997; see Supplemental Data, http://dev.biologists.org/supplemental/).

that Brn3a regulates genes common to a majority of the neurons present in these ganglia. E13.5 was chosen as the time point for analysis because in mice lacking Brn3a, a clear defect in the growth of axons from the trigeminal ganglion is evident by this stage, with aberrant innervation of peripheral and central targets. Furthermore, the extensive sensory cell death observed in *Brn3a* knockout mice does not take place until E14.5-E16.5 (Huang et al., 1999), so any effects from cell loss should be minimal at this stage.

Although no differences in the phenotype of *Brn3a* wild-type and heterozygous embryos have been identified (Eng et al., 2001), tissue samples from these genotypes were analyzed separately. The comparison of gene expression across all three genotypes was performed to look for subtle differences in heterozygotes, and to provide a partial replication of the results within each experiment. All three genotypes were analyzed in two completely independent experiments.

Trigeminal RNA were analyzed using the commercial oligonucleotide-based U74Av2 and U74Bv2 microarrays (Affymetrix). The U74Av2 array represents 12,422 transcripts, including 5993 known genes and 6429 ESTs, and the U74Bv2 array includes an additional 12,411 EST sequences. A significant number of the EST sequences present on both arrays have subsequently been related to identified genes in public and proprietary databases. Of all the transcripts represented on the U74Av2 array, 4885 were detected as 'present' in both experiments in at least one of the three *Brn3a* genotypes, using the manufacturer's standard criteria for array analysis. The transcripts that were reproducibly present on the U74Av2 and U74Bv2 arrays were then further analyzed with respect to their relative expression in the three genotypes.

Two measures were used to compare transcript levels between samples from different genotypes, the change-p value and the fold change in the intensity of the hybridization signal. The change-p value is calculated by proprietary data analysis software (Affymetrix) using the Wilcoxon's signed rank test applied to the hybridization signals for the 16 matched and mismatched oligonucleotide probe pairs representing each transcript in the array. Change-p values <0.003 (increased expression in the arbitrarily designated 'experimental' sample) or >0.997 (decreased in the experimental sample) are considered highly significant. For abundant transcripts, change-p values may be significant even when the fold change in expression is small, because for strong signals even minor relative differences may achieve statistical significance by this method. Because small relative changes in expression are not easily verified, and have uncertain biological significance, a minimum twofold increase or decrease in expression was used as an additional criterion for determining the changed transcripts of interest. More detailed information on the transcripts included and excluded by these criteria are given in Fig. S1, http://dev.biologists.org/supplemental/.

Figure 1A compares the relative expression of all present transcripts in heterozygote and knockout ganglia in one analysis using the U74Av2 array. The vast majority of the expressed transcripts fall between parallel lines designating less than a twofold change in expression. The expression values for significantly changed transcripts are located off the central axis,

Table 1. Increased transcripts in the trigeminal ganglion of *Brn3a* mutant mouse embryos

Description	GenBank	Experiment 1			Experiment 2			Fold
Increased transcripts	acc. no.	Wild type	Heterozygote	Knockout	Wild type	Heterozygote	Knockout	Knockout/ control
Cardiac responsive adriamycin protein	AF041847	4	4	170	11	10	244	34.3
Gata3	X55123	61	61	2524	85	96	2236	33.0
AP-2βa	X78197	23	99	1115	27	28	1037	28.1
Somatostatin	X51468	56	69	1662	63	85	1843	25.7
Calretinin (calbindin 2)	X73985	165	147	1838	57	219	1265	10.5
C-fos induced growth factor FIGF	X99572	17	103	508	38	63	478	9.0
HLH transcription factor Math3	AF036257	98	111	522	120	33	800	7.8
HLH transcription factor Musculin/MyoR	AF087035	195	277	1578	191	274	1685	7.0
Serotonin receptor 3A	M74425	1049	1129	5244	854	1242	4788	4.7
Connexin 43 (2 probe sets)	M63801	994	874	3211	1327	1381	5790	3.9
ART3	Y08027	42	32	154	79	108	214	3.2
Neuroserpin	AJ001700	1088	1316	4134	1564	1590	4559	3.2
Thrombospondin	M62470	55	87	198	79	96	307	3.2
HLH transcription factor NeuroD1	U28068	500	416	1005	378	351	1256	2.8
Brain natriuretic peptide	D16497	163	186	377	154	171	515	2.7
Eyes absent 2	U81603	716	693	1460	405	314	1172	2.7
Galectin 7	AF038562	556	317	877	413	391	1107	2.4
Short stature homeobox protein 2	U66918	1295	1925	3361	1434	1364	3742	2.4
Cytochrome P450, Cyp1b1	X78445	291	261	549	327	397	975	2.3
PG-M core protein	D45889	370	541	1159	478	525	1044	2.3
Tachykinin 1	D17584	95	38	160	152	124	286	2.2
Regulator of G-protein signaling 4	AB004315	78	162	329	162	173	282	2.2
LIM only 4	AF074600	754	789	1397	1224	1205	3132	2.2

This table and Table 2 show expression data for all transcripts from among the 5993 known genes represented on the U74Av2 chip that met the following criteria: (1) Present in at least one genotype in both experiments; (2) change-p value for the comparison of heterozygote to knockout transcript levels showed significantly changed expression (pΔ <0.003 or >0.997) in both experiments; (3) a minimum of a twofold increase or decrease in signal intensity in both experiments. Transcript levels in each experiment were normalized to a mean value of 500. The fold change is calculated as the ratio of knockout/control expression and represents the mean of the individual ratios for the two experiments. A list of genes that exhibited changed expression but did not meet these criteria appears in the Supplemental data, http://dev.biologists.com. Numbers in parentheses following gene names represent the number of probe sets for a given gene when represented by multiple probe sets on the array. These were in every case concordant and results for only one probe set are given.

Table 2. Decreased transcripts in the trigeminal ganglion of Brn3a mutant mouse embryos

Description	GenBank	Experiment 1			Experiment 2			Fold
Decreased transcripts	acc. no.	Wild type	Heterozygote	Knockout	Wild type	Heterozygote	Knockout	Knockout/ control
NPY1 receptor	Z18280	474	242	59	489	635	15	21.4
HoxD1 (Hox-4.9) (2 probe sets)	M87802	723	713	38	837	765	193	11.6
Advillin	AF041448	1455	1407	242	2022	1862	195	7.9
Na^+ channel Scn6a	L36179	211	316	25	220	240	72	6.8
Basonuclin	U88064	450	426	80	796	589	89	6.6
Homeobox transcription factor Hmx1	AF009614	612	542	102	682	728	94	6.6
Phospholipase A2 group VII	U34277	300	321	49	604	457	93	6.0
Runt related transcription factor Runx1	D26532	742	721	126	720	740	149	5.3
PACAP	AB010149	880	697	175	1261	1318	226	5.1
K^+ channel Kcnab2	U65592	1019	827	289	1750	1083	221	4.8
Galanin	L38580	4088	3753	938	3816	4093	952	4.2
Olfactomedin 1 (noelin, pancortin)	D78265	1216	1250	390	1494	1248	386	3.4
Bicarbonate transporter SLC4A4	AF020195	369	375	163	382	288	76	3.4
Insulin-like growth factor binding protein 5 Igfbp5	L12447	9683	10278	2283	7560	6864	3210	3.3
Myotubularin related protein 7	AF073882	370	526	97	686	528	325	3.2
Apolipoprotein E	D00466	1857	1404	405	2029	2694	1086	3.1
FK506 binding protein 1b	AF060872	9309	8866	3041	5809	7165	2071	3.1
Hivep2	Y15907	413	373	128	463	477	167	2.9
Latexin	D88769	1005	1181	402	1816	1596	628	2.7
HLH transcription factor Id1	M31885	1160	1003	366	1259	1210	502	2.7
Synaptotagmin 1	D37792	412	244	111	563	569	242	2.6
Na^+ channel Scn9a	L42338	260	426	140	566	412	178	2.6
C-kit	Y00864	702	484	263	847	795	304	2.5
Elongation factor 1 alpha 2	L26479	3197	3258	1326	3248	3081	1305	2.4
Tyrosine hydroxylase	M69200	3078	3304	1391	3375	4126	1563	2.3
RNA polymerase II 3	D83999	1455	1159	526	997	1218	502	2.3
Synuclein, gamma	AF017255	7459	9458	3209	8466	8145	4084	2.3
Activated leukocyte cell adhesion molecule	L25274	1320	1574	555	1998	1727	981	2.3
Peripherin 1	X15475	3746	4563	2009	6947	6982	2858	2.3
Annexin A2	M14044	9444	11497	4164	9051	12268	5639	2.2
Fgf13	AF020737	926	1137	461	932	1055	463	2.2

Transcripts were selected from among 5993 known genes on the U74aV2 chip according to the criteria described in Table 1. The threshold change-p value for decreased transcripts is pΔ >0.997. The fold change is calculated as the ratio of control/knockout expression.

and the positions of selected mRNAs encoding proteins with known roles in sensory development or function are indicated.

A complete list of the known transcripts that met the criteria for significantly increased expression appears in Table 1, and the significantly decreased genes are shown in Table 2. Most of the known transcripts that were significantly changed in *Brn3a* knockout ganglia encode proteins with established roles in neural function or neural development, including neurotransmitters and their receptors, enzymes of neurotransmitter synthesis, ion channels, specialized components of axons and synapses, mediators of intracellular signal transduction, and transcription factors. The specificity of these changes is underscored by the fact that we observed very few significant changes in transcripts associated with processes other than neurogenesis and neural function, such as factors regulating metabolic pathways, the cell cycle, or apoptosis. A more extensive list of 271 known neural genes, which were expressed in E13.5 trigeminal ganglia but were either unchanged in the *Brn3a* knockout or changed but did not meet the inclusion criteria, appears in Table S2, http://dev.biologists.org/supplemental/.

In addition to the previously known transcripts represented on the U74Av2 array, the U74Av2 and U74Bv2 arrays include oligonucleotide probes for nearly 19,000 ESTs, some of which have subsequently been linked to known genes. Although many significantly changed ESTs on these arrays could not be identified, searches of public and proprietary databases allowed a significant number to be assigned with confidence, and these are summarized in Table 3. Several of the ESTs confirmed results obtained for the known transcripts, including GATA3, AP2β, NeuroD1, Scn9a and Runx1. In addition, analysis of the ESTs contributed a number of novel changed genes, particularly transcription factors and mediators of intracellular transduction pathways.

Target gene expression is unaltered in the trigeminal ganglia of *Brn3a* heterozygotes

Brn3a heterozygous mice are viable and do not exhibit the defects in sensory axon growth or neuronal survival observed in knockouts. In previous studies we have shown that Brn3a attenuates its own expression via an autoregulatory enhancer (Trieu et al., 2003; Trieu et al., 1999). In heterozygotes, increased *Brn3a* expression from the intact allele restores Brn3a transcript levels to approximately 90% of the wild-type level, effectively compensating for reduced gene dosage. If this mechanism in fact normalizes Brn3a regulatory activity in heterozygotes, one would predict that all downstream targets of Brn3a, whether directly or indirectly regulated, would have nearly normal expression levels in heterozygous mice.

To test this hypothesis, we compared the expression levels of 41 increased, 62 decreased and 160 unchanged transcripts in ganglia from the three *Brn3a* genotypes. For both the increased

Table 3. Significantly changed ESTs in Brn3a mutant mice

Description	GenBank acc. no.	Mean ratio§
Increased transcripts		
Transcription factor Gata3‡	AA717155†	52.1
Transcription factor AP2β‡	AI021690†	18.6
Homeobox protein Iriquois 2	AI851291†	18.4
Homeobox protein Iriquois 1 (2 probe sets)	AA028275†	6.5
LIM only protein Testin 2	AI845609†	6.1
HLH transcription factor NeuroD1‡	AI848674†	3.5
Solute carrier family 1, member 3	AW121315†	3.4
Neuropilin 2	AW046112†	3.1
Protein kinase C-binding protein NEL	AI838010†	2.9
Thymocyte selection-associated HMG box gene	AA688946†	2.9
Bruno-like 4 (2)	AI839880*	2.5
Dual specificity phosphatase 6 Dusp6 (=MKP3)	AI845584*	2.2
Catenin beta interacting protein 1	AI842284†	2.1
Regulator of G-protein signaling 4	AI854153†	2.1
Decreased transcripts		
Regulator of G-protein signaling 10 (2)	AI847399*	13.6
Na^+ channel Scn9a‡	AI593229†	8.6
Ras family GTPase RAP	AA920095†	6.9
Runt-related transcription factor Runx1‡	AI643935†	5.4
Downstream of tyrosine kinase 4 Dok4 (3)	AA982457†	4.8
Protein tyrosine phosphatase H	AI851090†	4.7
Insulin-like growth factor 1 (2)	AA544955†	4.6
Synaptotagmin-like 2	AW124071†	4.1
Doublecortin-like kinase	AI853072†	3.8
G-protein coupled receptor 64	AI132005†	3.6
Src homology 2 containing protein C1 Shc1	AI050321*	3.4
Ataxin-2 binding protein A2bp1 (2)	AW047913†	3.4
Wnt1 responsive Cdc42 homolog Wrch1	AV246963†	3.3
CRB1 isoform II precursor	AW046426†	3.1
Microsomal glutathione S-transferase 3	AI843448*	2.9
Oxysterol binding protein-like 3	AI591488†	2.9
Bicarbonate transporter SLC4A4‡ (2)	AW124701†	2.8
Nerve growth factor receptor p75	AA050723†	2.8
FXYD domain ion transport regulator 7	AI844246*	2.7
Intermediate filament protein Desmuslin (3)	AI852401†	2.7
ELKL motif serine-threonine protein kinase 3	AI430097†	2.6
Actin-binding double zinc finger protein	AI840403†	2.6
Growth factor receptor bound protein 14 Grb14	AW124221†	2.6
N-chimaerin (2)	AI848376†	2.5
Calcium channel, Cacna2d2	AA008996†	2.4
Synaptotagmyn 1‡ (2)	AW125093†	2.4
Axonin, (TAG1, contactin 2)	AW049675†	2.3
Calsyntenin 2	AI842881†	2.3
Neuritin	AI839544†	2.2

Data are given for the relative expression of selected transcripts from amongst approximately 18,000 ESTs represented on the U74Av2 and U74Bv2 arrays.

*Affymetrix U74A chip.

†Affymetrix U74B chip.

‡Also appears as a known transcript on U74Av2 array.

§Calculated for increased and decreased transcripts as described in Table 1 and 2, respectively.

and decreased transcripts, the target gene expression levels in wild-type and heterozygous ganglia showed similar differences from those of the knockout (Fig. 1B). In contrast, the wild-type and heterozygote ganglia did not significantly differ from each other for either class of target genes. These results confirm the complete suppression of a heterozygous phenotype at this stage in the trigeminal ganglia of *Brn3a* knockout mice.

Expression of genes previously reported to be regulated by Brn3a

Numerous genes have been previously reported to be transcriptionally activated by transfected Brn3a in cell culture models of sensory ganglia. These putative Brn3a targets include structural components of axons and synapses, neurotransmitter receptors and oncogenes (Table 4). Almost all of these proposed downstream genes are represented on the U74A and B arrays, and were detected (present call) in E13.5 trigeminal ganglia. However, aside from a modest but statistically significant decrease in the neurofilament NF-H, none of these genes were markedly affected by the loss of Brn3a expression in vivo.

Work in cell line models has also led to the hypothesis that decreased expression of the anti-apoptotic gene *Bcl2* may contribute to the extensive death of sensory neurons in *Brn3a* knockout mice (Smith et al., 1998). In recent work, we have shown that Bcl2 mRNA levels are unaltered in *Brn3a* knockout mice just prior to the onset of cell death (Eng et al., 2003), suggesting that loss of *Bcl2* expression is not a primary defect in *Brn3a* knockout mice. Consistent with this result, two probe sets on the array gave unchanged signals for *Bcl2*. Overall, the results reported here do not support the in vivo regulation of the putative Brn3a targets from previous over-expression studies in transfected cell lines, and illustrate the difficulty of identifying physiological regulatory pathways in these model systems.

Previous studies of *Brn3a* knockout mice have also revealed changes in the expression of several genes in the sensory ganglia. A major focus of these studies has been the neurotrophins and their receptors. We have previously reported microarray assays showing a reduction in TrkA transcripts in *Brn3a* knockout trigeminal ganglia at E13.5 (Ma et al., 2003), and our array analysis is consistent with previous reports that the TrkA neurotrophin receptor is decreased in these mice (Huang et al., 1999; McEvilly et al., 1996). Transcripts for the p75 NGF receptor have been reported to be significantly decreased in mice lacking Brn3a (McEvilly et al., 1996), whilst immunohistochemistry for p75 protein has been reported as unchanged in mid-gestation knockout ganglia (Huang et al., 1999). In the present study, knockout levels of p75 mRNA were approximately 40% of wild type. Transcript levels for BDNF, previously reported to be reduced to undetectable levels at E12.5 in the trigeminal ganglia of *Brn3a* null mice (McEvilly et al., 1996), were found to be unchanged from controls in the present analysis. Loss of expression of the TrkB and TrkC neurotrophin receptors has also been reported in Brn3a null mice, but transcripts for the TrkB and TrkC receptors were not detected (absent call) in any genotype by the probe sets designed for these genes on the U74A array. This is an inconclusive result, which may reflect a problem in array design.

Brn3a regulates the expression of neurotransmitter systems and other transcription factors in multiple sensory ganglia

In addition to the trigeminal ganglion, Brn3a is expressed in neurons of the vestibulocochlear (VIII) ganglion complex, IX/X ganglion complex, and in the dorsal root ganglia (Fig. 2B). In order to verify the gene expression changes noted in the trigeminal array analysis, and to determine whether the trigeminal target genes are regulated elsewhere in the nervous system, we examined the expression of several Brn3a regulatory targets by in situ hybridization and immunohistochemistry in E13.5 embryos.

Table 4. Relative expression of previously reported Brn3a target genes in the trigeminal ganglia of Brn3a mutant embryos

Description	Probe set acc. no.	Reference	Wild type	Heterozygote	Knockout	Knockout × heterozygote change call*	Knockout/ heterozygote ratio
Cell transfection studies							
α-internexin	L27220	Budhram-Mahadeo et al., 1995	9514	10729	13470	I	1.26
Neurofilament, heavy	M35131	Smith et al., 1997	794	807	381	D	0.47
Neurofilament, medium	X05640	Smith et al., 1997	6410	7503	7956	NC	1.06
Neurofilament, light	M55424	Smith et al., 1997	3514	3056	1876	NC	0.61
Synapsin I	AF085809	Morris et al., 1996	10158	11068	13351	I	1.21
SNAP-25	M22012	Lakin et al., 1995	1976	2338	2359	NC	1.01
Bcl2	L31532	Smith et al., 1998	578†	612†	466†	NC	0.76
p53	AB021961	Budhram-Mahadeo et al., 2002	533	578	641	NC	1.11
Nicotinic AchRα3	(§)	Milton et al., 1996	–	–	–	–	–
Knockout studies							
trkA‡	AW124632	Huang et al., 1999	11675	13334	7083	D	0.53
BDNF	X55573	McEvilly et al., 1996	408	327	433	NC	1.33
NGF Receptor p75‡	AA050723	McEvilly et al., 1996	2745	3655	1107	D	0.30
Parvalbumin¶	X59382	Ichikawa et al., 2002b	1412†	1808†	1330†	NC	0.74
Calbindin¶	D26352	Ichikawa et al., 2002b	177	196	302	NC	1.54
Vanilloid-like receptor 1¶	AB021665	Ichikawa et al., 2002a	582	645	490	NC	0.76

All of the listed targets have been proposed as positively regulated target genes of Brn3a, and would thus be predicted to have decreased transcript levels in Brn3a knockout mice. The expression values are derived from the same array experiments as Tables 1-3. Only one experiment is shown, similar results were obtained in both experiments, and complete data appear in Table S1, http://dev.biologists.org/supplemental/.

*Change call values: I, increased, change-p <0.003; NC, no change, 0.003<change-p<0.997; D, decreased, change-p >0.997.

†Absent call. Absent calls in the presence of a strong hybridization signal can result from high background hybridization for a particular set of oligonucleotides on the array. For this reason these results are suggestive of unchaged expression, but are not conclusive.

‡U74B chip EST. All others are known transcripts from the U74Av2 array.

§Not represented on array.

¶Measured in late gestation, and may represent selective cell death.

Among the Brn3a-regulated gene products related to neurotransmitter systems, examination of the 5HT3 receptor mRNA by in situ hybridization confirmed markedly increased expression in the trigeminal ganglion, the IX/X ganglion (Fig. 2C), and the cervical dorsal root ganglion (not shown). Conversely, the regulator of G-protein signaling RGS10 exhibited strong expression in the trigeminal ganglion, IX/X ganglion (Fig. 2D), and dorsal root ganglion (not shown) of control mice, which fell to background levels in *Brn3a* knockout embryos. However, the VIII ganglion did not show increased expression of 5HT3R in *Brn3a* knockout embryos, or endogenous expression of RGS10 in controls.

The expression patterns of the mediator of Ca^{2+} signaling, calretinin, the neuropeptides somatostatin and galanin, and the enzyme of catecholamine synthesis, tyrosine hydroxylase were examined in the trigeminal ganglion by immunohistochemistry. Consistent with cell-autonomous regulation by Brn3a, galanin and tyrosine hydroxylase co-localized with Brn3a protein in the trigeminal neurons of control ganglia (Fig. 2E,F), and the direction and approximate extent of the expression changes in each of these proteins was entirely consistent with the array results (Fig. 2G). We also examined the DRG and spinal cord for changes in the expression of these four proteins (data not shown). Galanin immunoreactivity was markedly decreased in the DRG, but no significant changes in calretinin or tyrosine hydroxylase were evident. Somatostatin immunoreactivity accumulated abnormally in the dorsal root entry zone of *Brn3a* knockout mice, a finding which may reflect either increased expression, or the failure of sensory axons to appropriately enter the CNS in these mutants (Eng et al., 2001), or both. No changes were noted in any of these markers in the Brn3a-expressing neurons of the dorsal spinal cord.

Several transcription factors were also prominent among the most changed transcripts in the array analysis. To verify the array results for the trigeminal ganglion, and examine the expression of these factors in other cranial sensory ganglia and the caudal CNS, we performed in situ hybridization for the increased transcripts GATA3, Irx1, Irx2, AP2b, MyoR, Math3 (Fig. 3A), and NeuroD1 (not shown), and for the decreased transcripts HoxD1 and Runx1 (Fig. 3B), in E13.5 wild-type and *Brn3a* knockout embryos. In each case the direction and magnitude of change in the in situ hybridization signal in the trigeminal ganglion correlated well with the array results.

Further examination of these transcripts in the cranial sensory ganglia clearly indicate a role for Brn3a in the coordinated regulation of gene expression in the sensory system. GATA3, Irx1, Irx2, MyoR and NeuroD1 were all expressed in the vestibulocochlear ganglion (VIII) complex in control mice, but were weakly expressed to undetectable in the trigeminal, IX and dorsal root ganglia. In mice lacking Brn3a, the expression of these factors was markedly increased in the trigeminal ganglion and IX/X complex (Fig. 3A). GATA3, MyoR and NeuroD1, but not the Irx transcripts, were also increased in the dorsal root ganglion (not shown). AP2β and Math3 were not detectable in the VIII ganglion of control mice, but showed a similar coordinated increase in expression in the trigeminal and IX ganglia in embryos lacking Brn3a.

The transcription factors HoxD1 and Runx1 showed decreased expression in the array analysis of *Brn3a* knockout mice. In situ hybridization for these transcripts confirmed markedly decreased expression in the trigeminal and IX

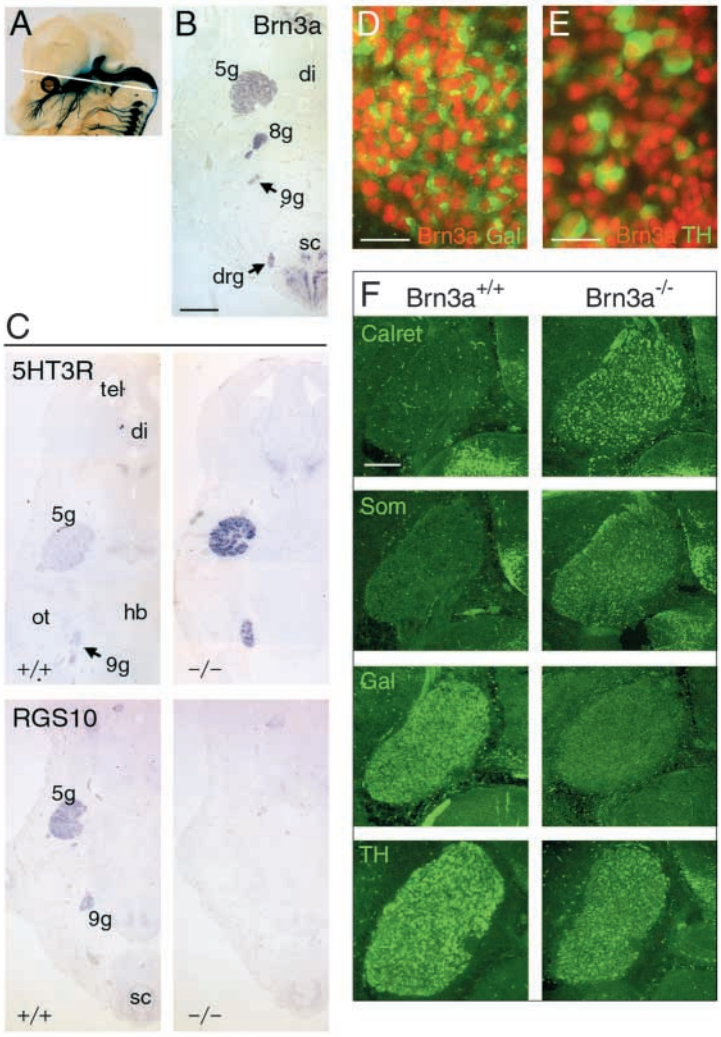

Fig. 2. Brn3a regulates sensory neurotransmitter systems. The cranial sensory ganglia of control (*Brn3a*$^{+/+}$) and knockout (*Brn3a*$^{-/-}$) E13.5 embryos were examined for the expression of components of neurotransmitter systems. (A) The plane of section used in subsequent views is illustrated using an E13.5 embryo stained for the expression of β-galactosidase regulated by a Brn3a sensory enhancer (Eng et al., 2001). (B) In situ hybridization showing the expression of the Brn3a mRNA in the cranial sensory ganglia. (C) In situ hybridization for the 5HT3 receptor, increased in the microarray analysis of *Brn3a* knockout mice, and the mediator of G-protein signaling RGS10, decreased in the microarray. (D-F) Immunohistochemistry for the products of Brn3a target genes in the trigeminal ganglia of E13.5 embryos. (D) Galanin immunoreactivity in the trigeminal ganglion co-localized with Brn3a in a majority of trigeminal neurons. (E) Tyrosine hydroxylase was expressed in a more limited subset of trigeminal neurons, most of which also expresses Brn3a. (F) A comparison of trigeminal ganglia from control mice and *Brn3a* knockouts, showing that, as predicted from the microarray studies, calretinin (Calret) and somatostatin (Som) immunoreactivity is markedly increased in the absence of Brn3a, whilst galanin is reduced to below the threshold of detection, and tyrosine hydroxylase (TH) is also significantly decreased. 5g, trigeminal ganglion; 8g, vestibulocochlear ganglion; 9g, IX/X ganglion complex; di, diencephalon; drg, dorsal root ganglion; hb, hindbrain; ot, otic region; tel, telencephalon; sc, spinal cord. Scale bars: B, 400 μm, D,E, 25 μm; F, 200 μm.

ganglion. Endogenous expression of Runx1 in the VIII ganglion appeared to be less affected.

The regulatory role of Brn3a may be distinct in the CNS

In addition to the sensory ganglia, Brn3a is expressed in specific neurons of the CNS, residing in the habenula, midbrain tectum and tegmentum, hindbrain, dorsal spinal cord and retina. The examination of the hindbrain region and spinal cord by in situ hybridization (Figs 2 and 3) did not indicate any obvious changes in the expression of neurotransmitters or transcription factors in the CNS of *Brn3a* knockout mice. However, in most areas of the CNS, Brn3a-expressing neurons have a scattered distribution, requiring methods of detection with cellular resolution to identify changes in target gene expression. For this reason we examined the CNS of embryos in more detail by immunohistochemistry for the increased gene products calretinin and somatostatin, and for the decreased gene products galanin and tyrosine hydroxylase.

In the midbrain and hindbrain, calretinin and Brn3a are expressed in adjacent but non-overlapping cell populations (Fig. 4A,B), while in the retina, a subset of neurons cells co-express these antigens (Fig. 4D,E). The expression of calretinin was not altered in either of these regions in the absence of Brn3a (Fig. 4C,F). Similarly, somatostatin was not ectopically expressed in the CNS of mice lacking Brn3a (Fig. 4G-K). Galanin and tyrosine hydroxylase were not co-expressed with Brn3a in the CNS as they are in the sensory system (Fig. 4L,M and data not shown), and thus could not be the targets of cell-autonomous regulation by Brn3a. Taken together, the in situ hybridization and immunohistochemical data for the targets of Brn3a regulation in the trigeminal ganglia demonstrate considerable conservation of the regulatory role of Brn3a in sensory neurons at different levels of the neural axis, but suggest a distinct role for Brn3a in the CNS.

Discussion

In this study, we have presented a systematic examination of the changes in gene expression resulting from the loss of a key regulator of sensory neurogenesis. Although the list of significantly changed genes is fairly extensive, the specificity of the developmental effects of Brn3a are underscored by several findings: a majority of the changed transcripts encode proteins with known or hypothesized roles in sensory neuron development or function, a far greater number of neuron-specific genes did not significantly change (see Supplemental Data), and there were almost no significant changes in the expression of ubiquitously expressed or ‘housekeeping’ genes.

Most of the genes with profoundly changed expression can be divided into three functional categories: neurotransmitter systems and ion channels, mediators of axonogenesis/synaptogenesis, and transcriptional regulators. Each of these classes of regulated transcripts may be related to the defects in axon growth and cell survival seen in *Brn3a* mutant mice, but

it is likely that these changes in gene expression synergise to produce the *Brn3a* knockout phenotype, and that no single target gene is sufficient to account for the observed defects.

Beyond explaining the sensory phenotype of *Brn3a* knockout mice, two interesting generalizations may be made which encompass many of the genes with altered expression. First, in the absence of Brn3a, trigeminal development is retarded, in the sense that the expression of numerous markers of a mature sensory phenotype are reduced, and the developmental expression of factors that play a transient role in the early phases of differentiation is abnormally prolonged. Second, several transcription factors are expressed outside their normal axial level in the sensory ganglia, suggesting that Brn3a acts to spatially restrict their expression.

Neurotransmitter systems and channels

The array results clearly demonstrate that Brn3a has a major role in determining the neurotransmitter phenotype of the developing trigeminal ganglia. Expression of the neuropeptides PACAP and galanin and the NPY1 receptor are highly dependent on Brn3a, and the rate-limiting enzyme of catecholamine synthesis, tyrosine hydroxylase, is also significantly reduced in *Brn3a* knockouts. In contrast, the expression of somatostatin and the 5HT3A receptor are

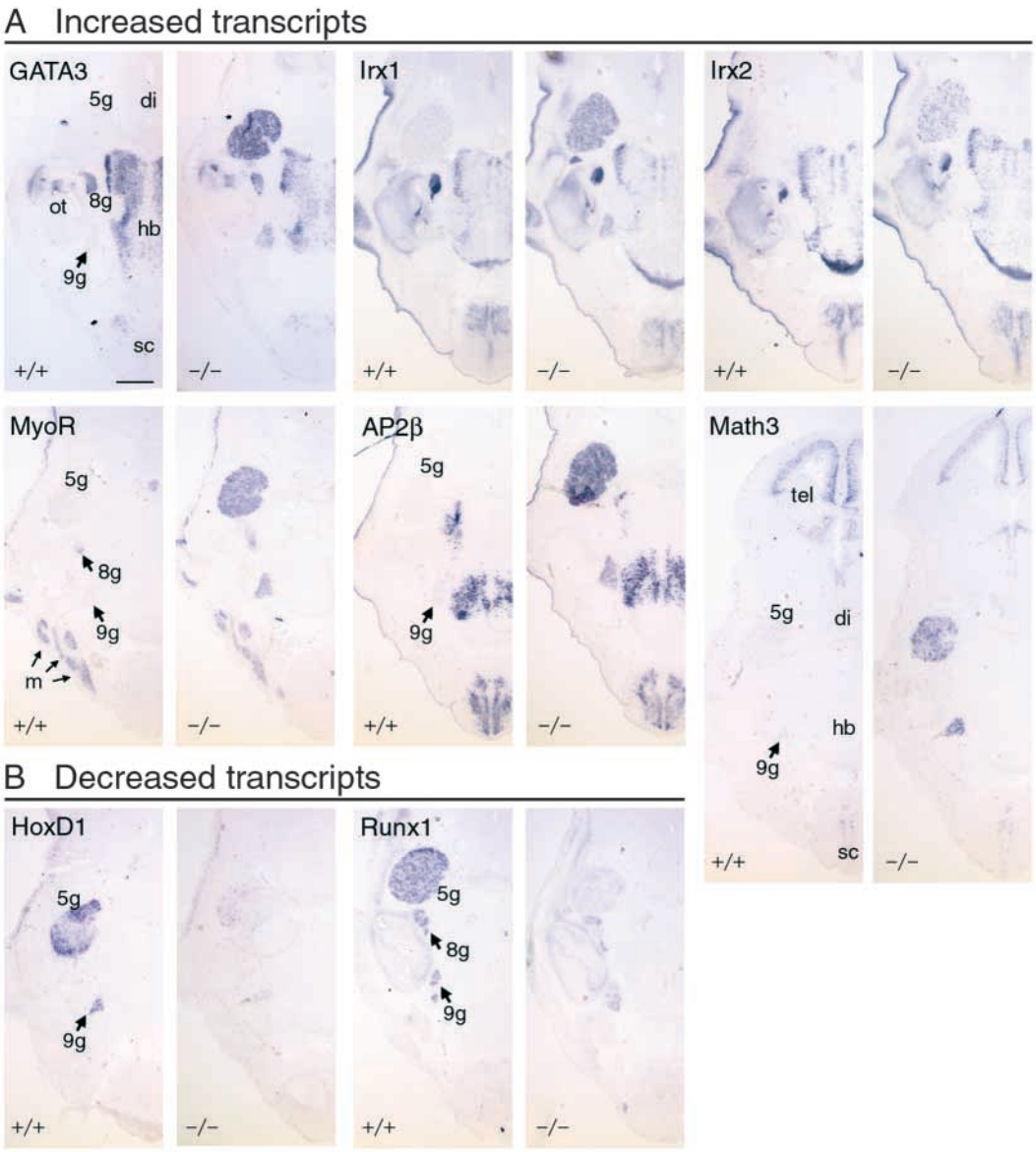

Fig. 3. Coordinated regulation of transcription factor expression in sensory ganglia by Brn3a. (A) In situ hybridization analysis of E13.5 embryos for expression of the transcription factors GATA3, Irx1, Irx2, MyoR, Ap2β and Math3, all of which exhibited increased expression in the microarray analysis in *Brn3a* knockout ganglia. (B) Expression of mRNA for the decreased transcription factors HoxD1 and Runx1. In addition to the cranial sensory ganglia, each of these factors also exhibited previously known patterns of expression in the CNS, and in the case of MyoR, in developing cranial musculature. The plane of section used in all views is shown in Fig. 2A. 5g, trigeminal ganglion; 8g, vestibulocochlear ganglion; 9g, IX/X ganglion complex; di, diencephalon; hb, hindbrain; m, differentiating occipital musculature; ot, otic region; tel, telencephalon; sc, spinal cord.

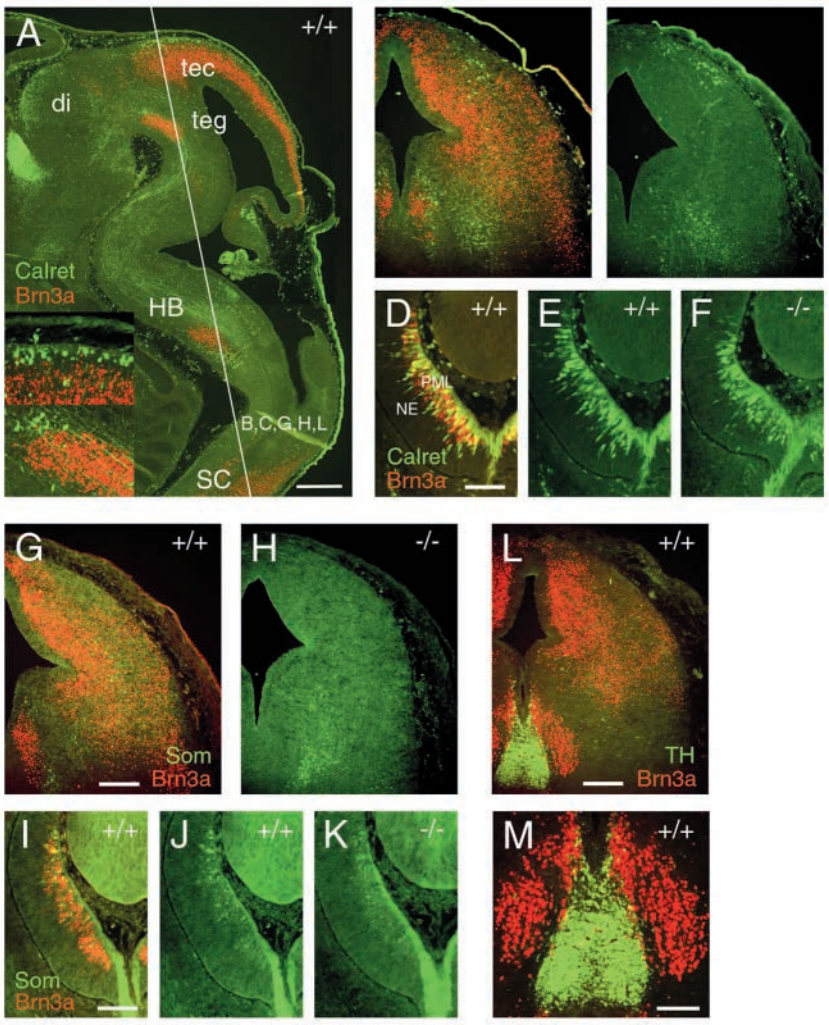

Fig. 4. Cellular expression of Brn3a target genes in the CNS. The brain and retina of E13.5 embryos were examined for alterations in Brn3a target genes identified in the trigeminal ganglion. (A) Calretinin and Brn3a characterize distinct populations of developing neurons in the E13.5 developing thalamus, midbrain, and hindbrain, shown in sagittal section, and are not co-expressed (inset). The diagonal line indicates the plane of section used in the midbrain views (B,C,G,H,L). (B) Control midbrain, showing distinct expression of Brn3a and calretinin. (C) Unchanged expression of calretinin in the *Brn3a* knockout midbrain. (D,E) Control retina, showing calretinin and Brn3a expression in overlapping populations of neurons. (F) *Brn3a* knockout retina showing no apparent increase in calretinin immunoreactivity. (G,H) Distinct patterns of somatostatin and Brn3a immunoreactivity in the midbrain, which are not changed in the *Brn3a* knockout. (I,J) Retinal expression of somatostatin, probably co-localized with Brn3a in a subset of ganglion cells, although the axonal distribution of somatostatin immunoreactivity makes precise cellular co-localization difficult to ascertain. (K) Retinal expression of somatostatin also appears unaltered in the absence of Brn3a. (L,M) Tyrosine hydroxylase and Brn3a identify entirely distinct populations of developing neurons in the ventral tegmental area (VTA) and the tegmentum (nuclei stained red), respectively. Scale bars: A, 400 μm; B,D,G, 100 μm; I, 100 μm; L, 200 μm; M, 50 μm.

markedly increased. Studies in the developing rat have shown that somatostatin is strongly expressed throughout the sensory ganglia soon after neurogenesis, but by mid-gestation its expression is restricted to a relatively small subset of sensory neurons (Katz et al., 1992). Thus the increased expression of somatostatin at E13.5 is very likely to represent a failure in the normal developmental attenuation of this gene, consistent with the idea that *Brn3a* knockout ganglia exhibit a pervasive maturation defect.

Also notable are changes in the expression of sodium channels, including Scn6 and Scn9, which are markedly decreased in *Brn3a* knockout ganglia, and Scn10, which is moderately decreased (Table S2, http://dev.biologists.org.supplemental/). Remarkably, these changes affect only those sodium channels that appear to have specific expression in the sensory nervous system (Goldin, 1999; Waxman et al., 1999), suggesting that Brn3a directly or indirectly coordinates expression of these channels. In contrast, expression levels of most neurotransmitter receptors, such as the GABA and glutamate receptors, and several classes of ion channels with wide expression in the CNS and PNS, are unchanged. Two other markedly changed genes, calretinin and the regulator of G-protein signaling RGS10, have putative roles in the modulation of neurotransmitter signals mediated by Ca^{2+}-dependent and G-protein pathways, respectively. Altered expression of these genes may represent primary changes, or they may occur in an attempt to compensate homeostatically for other changes in neurotransmitter systems.

Changes in expression of genes related to axon growth

Mice lacking Brn3a have marked defects in sensory axon growth, including defasciculation of axon bundles and failure to innervate peripheral and central targets (Eng et al., 2001; Trieu et al., 2003). The transcripts for several proteins known to be involved in axon growth and synaptogenesis were significantly decreased in *Brn3a* null mice. Among the proteins in this category is advillin (pervin), an actin-binding protein with specific expression in sensory and sympathetic ganglia, which increases neurite outgrowth in cultured dorsal root ganglia (Ravenall et al., 2002). Apolipoprotein E knockout mice exhibit anatomical and functional defects in unmyelinated nerve fibers (Fullerton et al., 1998). Although this has been attributed to loss of ApoE expressed in associated glia, our results suggest that the defect may be intrinsic to sensory neurons.

Also decreased in *Brn3a* knockout ganglia were transcripts for the functionally interrelated proteins insulin-like growth

factor 1 (IGF1) and insulin-like growth factor binding protein 5 (IGFBP5). Mice lacking IGF1 have abnormalities in sensory neurons (Gao et al., 1999), and show defective cortical dendritic growth (Cheng et al., 2003). IGFBP5 is a widely expressed protein whose role in vivo has not been clearly defined. However, it is highly expressed in the axon terminals of developing sensory neurons (Cheng et al., 1996), where it is frequently co-localized with IGF1, suggesting that it also has a role in axon growth. Because these proteins are known to interact, relative deficiencies in their expression may have a synergistic effect.

Another group of Brn3a-regulated proteins likely to have a role in axon growth are those involved in cell signaling and intracellular signal transduction. Transcripts with significantly changed expression include N-chimaerin, downstream of tyrosine kinase 4 (Dok4), the low affinity neurotrophin receptor p75, the small GTPases RAP (Ras family) and WRCH1 (Rho family), and Dusp6/MKP3. The expression and potential role of some of these factors in the sensory nervous system has been described; in other cases, the function of related proteins suggest that they may have significant and synergistic effects on axon growth.

Transcription factors

Loss of Brn3a results in profound changes in the expression of several transcriptional regulators of various types, suggesting a web of cross-regulation between genes involved in sensory neurogenesis. The expression of a few transcription factors expressed late in sensory development, such as Runx1, were decreased in the absence of Brn3a, but the majority of the changes were increases, suggesting that Brn3a functions as a repressor of transcription factors that would be temporally or spatially inappropriate in the maturing trigeminal ganglion.

The clearest example of the role of Brn3a in restricting the spatial expression of other transcription factors is the ectopic expression of GATA3, Irx1, Irx2, NeuroD1 and MyoR/musculin in *Brn3a* knockout mice. These factors are all expressed in the developing vestibulocochlear ganglion in control embryos, and in the absence of Brn3a are markedly increased in the trigeminal and IX/X ganglia, demonstrating an expansion of the expression domain of these genes in both directions of the rostrocaudal axis. It is likely that some of the downstream changes in gene expression in *Brn3a* knockout ganglia are mediated by these factors, but current knowledge of their role in neural development is not sufficient to predict the effect of their mis-expression in the trigeminal ganglion. GATA3 has a known role in the development of motor neurons originating in rhombomere 4, which innervate the inner ear, and the inner ear itself (Karis et al., 2001). NeuroD1 is also required for normal development of the sensory neurons of the inner ear (Liu et al., 2000), and may have a cross-regulatory relationship with GATA3 (Lawoko-Kerali et al., 2004). Although the role of the Irx genes in sensory development has not been described in mice, the zebrafish protein iro7, a possible paralogue of Irx1, is required for trigeminal placode development in fish (Itoh et al., 2002). The bHLH factor MyoR (musculin) is normally expressed in the developing facial muscles of the first branchial arch, which are innervated by trigeminal neurons, but not in the trigeminal ganglion itself (Lu et al., 2002). Our observation that MyoR is expressed in the developing auditory system is the first report of the sensory expression of this gene, and its role in neurogenesis is unknown.

Although it was not detected in the vestibulocochlear ganglion at this stage, AP2β showed a similar pattern of ectopic expression in the trigeminal and IX cranial ganglia in E13.5 *Brn3a* knockout embryos. AP2β is normally expressed in the embryonic hindbrain and spinal cord, but little is known about its role in neural development. The nervous system of *AP2β* mutant mice, which die from polycystic kidney disease, has no obvious abnormalities (Moser et al., 1997). However, mice lacking the related factor AP2α, which is highly expressed in migrating neural crest and in the developing sensory ganglia, exhibit extensive cranial abnormalities and dysgenesis of the cranial ganglia (Schorle et al., 1996). There is some evidence that AP2β is a weak transcriptional activator, and may oppose gene activation by AP2α (Bosher et al., 1996). Thus the increased expression of AP2β observed here may mimic some aspects of the loss of AP2α.

The increased expression of Math3 and NeuroD1 in *Brn3a* knockout trigeminal ganglia, together with decreased expression of the inhibitor of bHLH function Id1, suggest a marked increase in bHLH activity in the absence of Brn3a. Math3 and NeuroD1 have been characterized in the early development of the trigeminal ganglion (E9.0), where both factors appear to be downstream of the neurogenic HLH factor Ngn1 (Ma et al., 1998). Thus the increased expression of bHLH factors in *Brn3a* knockout mice may reflect the abnormal persistence of genes normally down-regulated as sensory development progresses. Although the loss of NeuroD1 or Math3 alone does not have an obvious effect on neurogenesis in the trigeminal (Tomita et al., 2000), the increased expression of multiple bHLH genes may have a synergistic effect in *Brn3a* knockout mice.

Possible mechanisms of sensory cell death in mice lacking Brn3a

Embryonic day 13.5 was chosen for gene expression analysis because it precedes the extensive loss of sensory neurons observed at later stages in *Brn3a* knockout mice, and consistent with this, we did not observe altered expression of genes usually associated with cell death pathways, such as caspases or bcl2-family genes. Sensory cell death in mice lacking Brn3a occurs after these neurons normally become neurotrophin dependent, and the decreased expression of neurotrophins and their receptors in *Brn3a* knockout mice has been suggested as a cause of this mortality (Huang et al., 1999). We have previously reported that the expression of the TrkA neurotrophin receptor mRNA is moderately decreased in *Brn3a* knockout mice (Ma et al., 2003). This observation, and the decreased expression of the p75 low affinity NGF receptor shown here, are consistent with previous results (Huang et al., 1999; McEvilly et al., 1996). However, because the TrkA receptor is generally regarded as anti-apoptotic, and the p75 receptor as pro-apoptotic in sensory neurons (Huang and Reichardt, 2001), it is not obvious what net effect a moderate decrease in both receptors would have on cell survival. Given the severity of the axon growth defects in *Brn3a* knockout mice, another possibility is that excessive neural death occurs because of a failure to obtain target-derived neurotrophins, but this hypothesis has not been tested directly.

Tissue specificity of gene regulation

In the present study we have defined a set of genes regulated by Brn3a in sensory ganglia. This represents one of the first comprehensive descriptions of the in vivo regulatory targets for any factor regulating vertebrate neurogenesis. Like many developmental regulators, Brn3a is expressed in a highly specific, yet diverse set of neurons, including those of the retina, diencephalon, midbrain, spinal cord and sensory system, leading to the question of whether Brn3a regulates a common set of targets in these distinct locations. In the present study we have found little evidence that the targets of Brn3a regulation in the trigeminal ganglia are also regulated in the CNS or in the retina. A recent analysis of the regulatory targets of the closely related POU-factor Brn3b in the retina revealed few changed transcripts in common with the present study, despite the fact that the retinal ganglion cells in *Brn3b* knockout mice show a secondary loss of Brn3a (Mu et al., 2004); it also did not detect changes in the retinal target genes in sensory ganglia.

Even within the peripheral sensory system, Brn3a targets appear to be distinctly regulated in the vestibulocochlear ganglion when compared to the coordinated changes in expression in the trigeminal, IX, and dorsal root ganglia. The lack of change in trigeminal target genes in the vestibulocochlear ganglion cannot be attributed to functional redundancy of Brn3 genes. Although Brn3b is also expressed in the vestibulocochlear system, the loss of Brn3a expression in the vestibulocochlear ganglion also leads to diminished expression of Brn3b, and results in significant defects in cochlear innervation (Huang et al., 2001). Thus it appears probable that Brn3a will have at least a partially distinct set of regulatory targets in the auditory system.

The genes downstream from Brn3a in the sensory ganglia are very likely to include targets that are regulated directly, and regulated indirectly by the several other transcription factors that change expression in the absence of Brn3a. One of the surprising features of the current study is the large number of markedly increased transcripts in the knockout ganglia, implying direct or indirect transcriptional repression by Brn3a. Although nearly all prior studies of the transcriptional activity of Brn3a have proceeded from the assumption that it is a positive regulator of gene expression, we have recently shown that Brn3a is a direct repressor of its own expression in the trigeminal ganglion in vivo (Trieu et al., 2003). The recent study of the target genes of Brn3b in the retina showed mainly decreased expression of downstream transcripts (Mu et al., 2004), but this study was conducted with a retina-specific cDNA array, which would be unlikely to include strongly increased transcripts which have low levels of expression in the normal retina. Thus it is plausible that Brn3a, and perhaps other factors in this class, exert their direct effects by transcriptional repression.

Identifying the regulatory targets of neural transcription factors is an essential component of understanding developmental pathways in the nervous system. Here we have demonstrated an extensive program of gene regulation mediated by one such factor. Future studies of this kind will be greatly facilitated by the availability of more complete gene expression arrays based on genomic sequences rather than cDNA libraries. Additional data about the location of the transcription units in the mouse genome, and better information about the DNA recognition properties of the various transcription factor classes, will help to distinguish direct from secondary targets. In addition, the confirmation of direct regulation by chromatin immunoprecipitation may be facilitated by combining this method with array technology or other high throughput methods (Ren et al., 2002). These anticipated technical advances should in principle allow the identification of a complete set of regulatory targets for any transcription factor in any tissue.

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Table S1. In situ hybridization probes

Probe	Investigator	Reference
AP2b	Reinhard Buettner	(Moser et al., 1995)
Irx1, Irx2	Peter Gruss	(Zulch et al., 2001)
Gata3	Alar Karis	(Pata et al., 1999)
5HT3R	Allan Basbaum	(Zeitz et al., 2002)
Runx1	Quifu Ma	Unpublished
Math3, NeuroD1	Ryoichiro Kageyama	(Inoue et al., 2002)
HoxD1	Denis Duboule	(Zakany et al., 2001)
RGS10	Stephen Gold	(Gold et al., 1997)
MyoR	Eric Olson	(Lu et al., 2002)

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Table S2. Moderately changed and unchanged transcripts in the E13.5 Brn3a knockout trigeminal ganglia which have known neural function or belong to families of neurally expressed genes

Description	GenBank	Experiment 1				Experiment 2				Mean
Moderately increased transcripts	acc. no.	**WT**	**HT**	**KO**	**pΔ**	**WT**	**HT**	**KO**	**pΔ**	**ratio**
neogenin	Y09535	587	562	804	0.002	582	477	902	0.000	1.6
neuronal intermediate filament	L27220	10179	13291	15404	0.478	11230	9927	13965	0.000	1.3
neuronatin	X83569	11403	10435	21142	0.000	8829	9851	12101	0.000	1.6
neuregulin 1	AB025413	373	327	487	0.083	240	288	534	0.000	1.7
amyloid precursor protein	U82624	9184	10751	13079	0.000	8591	10084	14402	0.000	1.4
survival motor neuron	U77714	1152	1012	1058	0.500	827	859	1037	0.000	1.1
alpha-internexin (NF-66)	L27220	11982	12092	11318	0.500	9514	10729	13470	0.001	1.1
secretogranin II	X68837	139	99	136	0.002	87	187	250	0.001	1.5
astrotactin 1	U48797	482	392	605	0.006	491	466	836	0.002	1.6
synaptotagmin 4	U10355	136	221	225	0.375	572	411	507	0.002	1.1
Nedd4a	U96635	3858	3309	4038	0.002	4570	4424	5645	0.003	1.2
complexin 2	D38613	1177	700	1718	0.000	797	778	1159	0.019	1.7
K^+ channel, Isk-related family 1	AJ131398	773	1020	1244	0.426	566	717	1160	0.000	1.6
reticulocalbin 2	AF049125	354	326	349	0.052	874	653	839	0.003	1.1
				ko/ht				**ko/ht**		**Mean**
Moderately decreased transcripts		**WT**	**HT**	**KO**	**pΔ**	**WT**	**HT**	**KO**	**pΔ**	**ratio**
neurofilament protein (NF-L)	M55424	1982	2425	1765	0.999	3514	3056	1876	0.687	1.4
ciliary neurotrophic factor receptor	AF068615	489	536	420	0.999	233	205	109	0.984	1.6
brain beta spectrin	M74773	4094	4558	3511	0.999	5002	4947	4362	0.976	1.2
dynactin 1	U60312	6242	6228	4013	0.999	4395	4930	3533	0.979	1.4
K^+ voltage-gated channel, shaker-related beta1	AF033003	198	147	89	0.996	263	279	164	1.000	1.8
K^+ voltage-gated channel, Q2	AB000497	2802	2727	1487	0.985	2111	2381	1399	1.000	1.7
Ca^{2+}-dependent activator protein for secretion	D86214	1169	835	848	0.792	2093	1766	1194	1.000	1.4
neuropilin	D50086	5306	6070	3037	1.000	6366	6266	3975	1.000	1.7
PFTAIRE protein kinase 1	AF033655	744	858	360	1.000	1080	940	634	1.000	1.9
flotillin 1	U90435	1432	1892	1148	0.981	1914	2126	1330	1.000	1.5
hippocalcin-like 1	AF085192	3675	3483	3016	0.800	4245	5227	3159	1.000	1.3
epimorphin	D10475	2446	2284	2225	0.969	1914	2122	1374	0.998	1.3
glutamate receptor, ionotropic, AMPA2 (alpha2)	X57498	1238	1267	959	0.996	1318	1448	966	1.000	1.4
K^+ voltage-gated channel, shaker-related 6	M96688	207	256	118	1.000	217	236	162	0.999	1.7
protease, serine, 12 neurotrypsin, (motopsin)	D89871	747	842	432	1.000	938	951	670	1.000	1.6
synaptotagmin 11	AB026808	1349	1804	1293	0.999	1302	1332	950	1.000	1.3
ELKL motif kinase	X70764	2819	2837	1380	1.000	2252	2431	1679	1.000	1.7
protein tyrosine phosphatase, receptor type, R	D31898	4782	5625	3871	1.000	5698	5835	4149	1.000	1.4
metaxin 2	AF053550	1106	1217	788	1.000	1889	1515	1251	1.000	1.4
Guanine nucleotide binding protein, gamma 3 subunit	AF069953	10751	11533	6924	1.000	9032	10604	7399	1.000	1.5
neurochondrin-1	AB017608	1538	1319	1145	0.500	1555	1525	1228	0.998	1.3
S100 Ca^{2+} binding protein A10 (calpactin)	M16465	8112	8783	6472	1.000	6430	7232	5491	1.000	1.3
neural proliferation, differentiation and control gene 1	X67209	2494	2415	2351	0.500	1964	2448	1801	1.000	1.1
insulin-like growth factor binding protein 4	X76066	660	535	402	0.275	639	683	556	0.999	1.3
acetylcholinesterase	X56518	708	895	789	0.165	915	893	780	0.999	1.1
Acrogranin precursor	D16195	618	617	611	0.500	703	769	647	0.999	1.1
homer, neuronal immediate early gene, 2	AF093259	757	834	515	1.000	471	493	507	1.000	1.2
fibroblast growth factor receptor 1	U22324	549	564	427	0.993	546	474	255	0.998	1.7
Eph receptor A6	U58332	283	202	150	0.653	231	298	132	0.999	1.8
sialyltransferase 7 E	AB030836	241	277	128	1.000	436	379	211	1.000	2.0
contactin 1	X14943	826	763	578	0.994	1409	1068	654	1.000	1.6
utrophin	Y12229	233	191	142	0.999	344	317	183	0.999	1.6
alpha3 subunit of L-type Ca^{2+} channel	X94404	3435	3098	2097	1.000	3016	2637	1622	1.000	1.7
glial cell line derived neurotrophic factor family receptor alpha 3	AB008833	1143	1507	738	0.984	1454	1490	849	1.000	1.8
syntaxin 1A (brain)	D45208	970	1188	825	0.861	1157	1107	681	1.000	1.5
Sema4f (semaphorin W)	AB022316	2806	2593	1815	0.719	2479	2338	1444	1.000	1.6
protein tyrosine phosphatase, receptor type, G	L09562	457	544	304	1.000	750	699	432	1.000	1.7
long form AMPA receptor subunit	L32372	99	88	87	0.948	379	280	140	1.000	1.7
Na^+ channel Scn10	L42342	931	1174	706	0.999	1754	1584	749	0.999	1.9
				ko/ht				**ko/ht**		**Mean**
Unchanged transcripts		**WT**	**HT**	**KO**	**pΔ**	**WT**	**HT**	**KO**	**pΔ**	**Ratio**
neuregulin 1	AB025413	432	556	604	0.127	381	325	509	0.004	1.3
secretory granule neuroendocrine protein 1	X15830	567	526	477	0.978	1097	589	756	0.006	0.9
synapsin I	AF085809	10983	12514	9491	0.500	10158	11068	13351	0.001	1.0
Nedd9	AF009366	144	143	193	0.075	124	148	102	0.011	1.1
piccolo	Y19186	247	337	422	0.500	279	321	542	0.012	1.6
transforming growth factor, beta 3	M32745	547	528	757	0.027	442	387	550	0.019	1.4
calumenin	U81829	783	796	1120	0.500	845	751	986	0.020	1.3
creatine kinase, brain	X04591	8716	10366	9254	0.500	5613	6415	8772	0.031	1.2
activin receptor IIB	M84120	2211	1678	1724	0.001	1219	1122	1056	0.036	0.9
peptidylprolyl isomerase B, cyclophilin	X58990	1289	1753	2120	0.041	1581	1682	2205	0.052	1.4
insulin-like growth factor 2, binding protein 1	AF061569	932	929	761	0.666	575	493	613	0.083	1.0
brain derived neurotrophic factor	X55573	240	183	255	0.500	408	327	433	0.095	1.2
transformation related protein 53 (p53)	AB021961	247	325	324	0.072	334	338	396	0.102	1.2
dystroglycan 1	U43512	1871	1724	1767	0.078	1301	1156	1741	0.135	1.2

microtubule-associated protein tau	M18775	7669	9582	9007	0.500	7994	8485	9590	0.148	1.1
radixin	X60672	1203	1089	1533	0.026	1908	1789	2168	0.275	1.3
huntingtin interacting protein 2	AB011081	377	323	323	0.500	547	459	476	0.313	0.9
growth hormone	X02891	590	638	694	0.500	604	772	621	0.404	1.0
lysophospholipid receptor B3	AF108021	790	953	1212	0.233	605	742	876	0.404	1.3
huntingtin interacting protein 2	AB011081	1360	1773	1373	0.744	1147	1061	1194	0.433	1.0
leptin receptor	AJ011565	359	612	578	0.500	330	499	650	0.441	1.4
nuclear receptor 2, F 1	X74134	900	918	990	0.086	1834	1927	1767	0.441	1.0
S100 Ca^{2+} binding protein A13	X99921	696	849	540	0.948	629	568	572	0.455	0.8
Eph receptor B2	L25890	244	323	313	0.056	281	186	293	0.463	1.2
serotonin receptor 5HT5	Z18278	627	673	808	0.500	468	467	478	0.478	1.1
neurofibromatosis 2	X74671	1368	1135	1255	0.500	1700	1866	1647	0.500	1.0
fascin homolog 1	L33726	10995	10407	11170	0.500	4996	7176	6646	0.500	1.1
chromogranin B	X51429	601	657	600	0.914	562	682	656	0.500	1.0
benzodiazepine receptor, peripheral	D21207	2239	2456	2715	0.500	1661	1550	1731	0.500	1.1
spinocerebellar ataxia 2 homolog	AF041472	715	693	814	0.500	836	861	869	0.500	1.1
chloride channel, nucleotide-sensitive, 1A	U53455	1660	1316	1230	0.205	1121	1293	1444	0.500	1.0
opioid receptor, sigma 1	AF004927	1231	1164	1095	0.500	868	994	925	0.500	1.0
follistatin-like	M91380	727	809	1097	0.024	735	829	889	0.500	1.3
calnexin	L18888	1271	1238	1282	0.500	1171	781	706	0.500	0.9
diazepam binding inhibitor	X61431	4130	3735	5047	0.006	4334	5255	5615	0.500	1.2
chloride channel 3	X78874	366	443	389	0.500	562	488	558	0.500	1.0
insulin-like growth factor 2 receptor	U04710	744	764	746	0.500	781	699	725	0.500	1.0
nuclear protein 15.6	Y08702	1815	1599	1576	0.500	1864	1857	1831	0.500	1.0
synaptosomal-associated protein, 23kDa	U73143	135	92	152	0.397	133	86	149	0.500	1.4
Niemann Pick type C1	AF003348	756	795	775	0.500	563	561	681	0.500	1.1
S100 Ca^{2+} binding protein A11 (calizzarin)	U41341	1153	1031	1142	0.500	1339	1612	1805	0.500	1.1
insulin-like growth factor binding protein 2	X81580	1639	1312	1149	0.911	1168	1184	1084	0.500	0.9
spinocerebellar ataxia 10 homolog	X61506	6345	6090	5113	0.500	5458	5306	5014	0.500	0.9
chloride channel 4-2	Z49916	615	735	567	0.972	884	925	851	0.500	0.9
synaptosomal-associated protein, 25 kDa	M22012	1622	1875	2008	0.500	1976	2338	2359	0.500	1.1
neural cell adhesion molecule 1	X15052	9356	9637	10969	0.500	7575	8431	9197	0.500	1.2
glucose phosphate isomerase 1 complex	M14220	920	1001	1018	0.500	1197	1218	955	0.500	0.9
platelet-activating factor acetylhydrolase 1b, alpha1	U57746	2392	2616	2205	0.738	1927	1944	2175	0.500	1.0
calpain, small subunit 1	AF058298	3138	2726	1828	0.500	2065	2576	2278	0.500	0.8
fibrillin 1	L29454	438	460	404	0.500	460	559	665	0.500	1.1
epsin 2	AF057286	2493	1916	2232	0.500	1810	1879	1753	0.500	1.0
K^+ channel, subfamily K, member 1 2	AF033017	199	336	233	0.500	162	55	211	0.500	1.4
microtubule-associated protein tau	M18776	5063	5771	4866	0.666	4279	4606	4741	0.500	1.0
calcitonin gene-related peptide-receptor component	AF028242	443	371	389	0.500	427	451	477	0.500	1.0
transformation related protein 53	AB021961	604	465	552	0.263	533	578	641	0.500	1.1
collapsin response mediator protein 1	AB006714	4008	4981	5596	0.390	5085	6167	6633	0.500	1.2
secretogranin III	U02982	482	574	456	0.853	1222	898	892	0.500	0.9
bone morphogenetic protein 1	L24755	554	595	784	0.390	559	506	480	0.500	1.1
purinergic receptor P2X, ligand-gated ion channel 4	AF089751	81	140	78	0.500	157	154	149	0.500	0.8
retinoblastoma 1	M26391	217	203	282	0.778	577	383	490	0.500	1.2
syntaxin binding protein 1	D45903	4039	4488	3882	0.500	4266	4040	3605	0.500	0.9
LERK-2	U07602	1055	1055	922	0.673	854	973	959	0.500	1.0
Eph receptor B4	U06834	2255	2283	2218	0.500	2566	2604	2532	0.500	1.0
K^+ voltage-gated channel, Q 2	AB000503	292	285	272	0.500	315	199	229	0.500	0.9
G protein-coupled receptor 19	U46923	409	348	379	0.175	349	350	414	0.500	1.1
activin A receptor, type II-like 1	Z31664	483	391	396	0.493	431	432	398	0.500	0.9
synaptosomal-associated protein, 91 kDa	M83985	600	408	342	0.954	1240	1101	745	0.500	0.7
protein tyrosine phosphatase, receptor type, A	M36033	349	330	377	0.500	530	427	530	0.500	1.1
S100 Ca^{2+} binding protein A13	X99921	960	1038	1054	0.500	595	628	749	0.500	1.1
radical fringe gene homolog	AF015770	1496	1842	1666	0.500	1667	1791	1677	0.500	1.0
presenilin 1	L42177	1551	1467	1435	0.500	1462	1298	1618	0.500	1.1
insulin-like growth factor I receptor	AF056187	2406	2470	2980	0.500	2131	2078	2411	0.500	1.2
doublecortin	AB011678	4753	5892	6581	0.500	5428	5796	6696	0.500	1.2
transforming growth factor, beta receptor III	AF039601	301	293	325	0.500	221	278	288	0.500	1.1
Eph receptor A8	U72207	387	392	350	0.500	285	288	260	0.500	0.9
ephrin A2	U14941	417	542	532	0.933	466	431	474	0.500	1.1
disabled homolog 1	Y08380	99	239	312	0.001	131	233	299	0.500	1.7
purinergic receptor P2X, ligand-gated ion channel, 1	X84896	566	493	940	0.485	341	383	309	0.500	1.3
glutamate receptor, ionotropic, kainate 5	D10011	665	657	889	0.011	459	537	416	0.500	1.1
epsin 1	AF057285	6069	4897	4902	0.127	4210	3999	4176	0.500	1.0
Eph receptor A4	X65138	188	245	237	0.397	124	178	177	0.500	1.1
middle-MW neurofilament protein, NF-160	X05640	6338	8102	7379	0.500	6410	7503	7956	0.500	1.1
unc13 homolog 1	AF115848	209	125	273	0.500	398	347	261	0.500	1.2
secretin	X73580	8378	6493	11303	0.000	2884	3387	3010	0.500	1.2
synaptotagmin 3	D45858	635	739	655	0.500	534	521	520	0.500	1.0
glutamate receptor, ionotropic, kainate 1	X66118	41	55	74	0.500	120	147	126	0.500	1.2
GABA-A receptor, gamma 2	M62374	446	389	417	0.500	819	753	710	0.500	1.0
beta-2-adrenergic receptor.	X15643	139	165	190	0.500	153	183	179	0.500	1.2
putative neuronal cell adhesion molecule	AF026465	757	561	814	0.006	766	798	977	0.500	1.2
ephrin B3	AF025288	724	987	871	0.493	432	568	606	0.500	1.1
FMS-like tyrosine kinase 3	X59398	796	680	607	0.500	448	639	633	0.500	1.0
plexin 2	D86949	332	513	372	0.500	265	253	249	0.500	0.9

ELK1	X87257	332	290	260	0.500	293	222	214	0.500	0.8
prosaposin	U57999	4321	3356	5256	0.119	2897	3333	3697	0.500	1.3
K^+ large conductance Ca^{2+} activated channel, M alpha1	U09383	350	377	341	0.778	396	436	397	0.500	1.0
dopamine receptor 2	X55674	210	234	95	0.545	140	148	143	0.500	0.7
Ca^{2+} channel, voltage-dependent, L type, alpha 1C	U17869	490	454	414	0.500	428	521	619	0.500	1.1
frizzled 8	U43321	684	644	594	0.646	563	499	504	0.500	0.9
alpha-2 adrenergic receptor	M97516	594	677	747	0.109	536	601	732	0.500	1.2
melanocortin 5 receptor	U08354	478	235	414	0.070	322	362	300	0.500	1.0
GABA-A receptor, subunit beta 3	U14420	207	236	87	0.891	300	322	237	0.500	0.6
amiloride-sensitive cation channel 1, neuronal	Y14634	779	863	892	0.500	585	709	641	0.500	1.0
LERK-3	U92885	2340	2158	2514	0.080	1985	1731	1584	0.500	1.0
cholecystokinin B receptor	AF019371	907	984	1407	0.080	811	833	903	0.500	1.3
somatostatin receptor type 4	U26176	1204	1201	1359	0.500	814	837	817	0.500	1.1
galanin receptor type 3	AF042783	652	666	819	0.287	512	642	565	0.500	1.1
cholinergic receptor, nicotinic, alpha polypeptide 6	AJ245706	126	202	129	0.500	104	94	103	0.500	0.9
neuregulin 1	AF059485	990	832	715	0.320	556	454	541	0.500	0.9
growth hormone releasing hormone receptor	L07379	255	280	310	0.300	227	311	278	0.500	1.1
preproinsulin I	X04725	3990	3154	3099	0.500	1835	1912	1914	0.500	0.9
GABA-A receptor, subunit gamma 3	X59300	154	95	120	0.500	155	124	159	0.500	1.1
K^+ voltage-gated channel, S 2	AF008574	145	148	122	0.500	134	121	162	0.500	1.1
purinergic receptor P2X, ligand-gated ion channel, 7	AJ009823	519	466	675	0.478	383	488	527	0.500	1.3
syntaxin 7	AF056323	2659	2595	2397	0.500	2959	3290	2817	0.500	0.9
dopa decarboxylase	AF071068	120	123	151	0.500	159	137	179	0.500	1.2
neuron specific gene family member 2	U17259	1751	1610	1392	0.500	1793	2136	2143	0.500	1.0
hippocampus abundant gene transcript 1	D88315	475	408	458	0.500	681	624	700	0.500	1.1
receptor-like tyrosine kinase	L21707	313	244	292	0.500	371	325	399	0.500	1.1
protein tyrosine phosphatase, receptor type, S	X82288	2720	2526	3022	0.031	1913	2433	2469	0.500	1.1
voltage-dependent anion channel 2	U30838	304	421	421	0.750	1372	959	870	0.500	1.0
sorting nexin 3	AF062482	4274	3594	3785	0.500	4133	5023	4412	0.500	1.0
voltage-dependent anion channel 3	U30839	987	980	1117	0.500	1822	1768	1589	0.500	1.0
Nedd1	D10712	701	673	575	0.500	700	513	512	0.500	0.8
ephrin B2	U30244	808	472	741	0.998	1510	1057	1387	0.500	1.1
synapsin II	AF096867	764	916	823	0.589	717	738	804	0.500	1.0
brain protein I3	X61454	2942	2775	2220	0.500	2160	2196	1686	0.530	0.8
calpain 5	Y10656	655	592	726	0.500	470	641	626	0.530	1.1
transgelin 3	AB031291	2379	3600	2647	0.687	2886	3062	2593	0.537	0.9
opioid receptor-like	AF043276	451	392	377	0.500	402	428	314	0.545	0.8
synaptophysin	X95818	377	413	361	0.500	390	361	270	0.610	0.8
IGF-II	X71922	10816	11258	10599	0.500	6793	7885	7726	0.625	1.0
vanilloid receptor 1 (capsaicin receptor)	AB021665	560	793	706	0.515	582	645	490	0.713	0.9
bone morphogenetic protein 4	X56848	568	665	507	0.500	409	415	386	0.738	0.9
cyclic nucleotide-gated K^+ 3	AJ225124	1662	1574	1632	0.500	1400	1515	1138	0.738	0.9
parathyroid hormone receptor	X78936	465	334	344	0.500	480	483	377	0.750	0.8
plexin 3	D86950	2198	1795	1653	0.500	1652	1782	1568	0.756	0.9
GDNF receptor alpha 1	AF014117	425	365	330	0.738	368	382	357	0.756	0.9
insulin-like growth factor 1	X04480	3103	2668	3699	0.500	1367	1601	1282	0.778	1.1
dishevelled	U10115	1463	1899	1587	0.500	1519	1227	1348	0.784	1.0
ceroid lipofuscinosis, neuronal 3, juvenile	U68064	588	610	544	0.603	475	512	378	0.784	0.8
brain protein 14	X61450	394	425	386	0.500	598	610	484	0.805	0.9
serotonin receptor 5HT4	Y09588	523	521	497	0.404	304	340	342	0.839	1.0
K^+ inwardly-rectifying channel, J 12	X80417	227	247	160	0.948	405	428	352	0.844	0.8
cathepsin D	X68378	1748	1810	1851	0.500	1749	1971	1437	0.853	0.9
Friedreich ataxia	U95736	108	69	56	0.500	157	183	116	0.853	0.7
receptor-associated protein of the synapse, 43 kDa	X15788	514	494	357	0.522	483	504	410	0.861	0.8
synaptobrevin like 1	X96737	96	156	146	0.500	425	272	224	0.877	0.9
vascular endothelial growth factor B	U43836	713	821	668	0.537	649	602	549	0.884	0.9
seizure related gene 6	D29763	261	168	217	0.761	305	360	314	0.888	1.0
serotonin receptor 5HT1D	X94908	259	285	259	0.500	378	350	154	0.917	0.7
semaphorin 4B	X85992	213	148	175	0.500	154	181	145	0.920	0.9
TYRO3 protein tyrosine kinase 3	AB000828	424	385	341	0.500	391	367	327	0.933	0.9
dynactin 3	AF098508	2330	2605	2405	0.830	3044	3415	3067	0.942	1.0
K^+ inwardly-rectifying channel, J 8	D88159	79	81	60	0.830	106	90	101	0.942	0.9
semaphorin 4D	U69535	833	1128	506	0.731	1151	618	562	0.944	0.6
activin receptor IIA	M65287	302	287	197	0.574	308	279	245	0.946	0.8
G-protein-coupled receptor 50	AF065145	2894	2501	2990	0.119	1651	1932	1389	0.950	0.9
platelet derived growth factor, alpha	M29464	692	733	743	0.500	681	705	561	0.957	0.9
dystrobrevin	Z79787	818	743	715	0.500	651	625	469	0.973	0.8
glutamate receptor NMDA1 (zeta 1)	D10028	141	137	97	0.500	98	122	99	0.974	0.8
platelet-activating factoracetylhydrolase, 1b, beta1	U95116	1693	2237	1594	0.946	1973	1781	1514	0.976	0.8
cerebellar degeneration-related 2	U88588	301	331	232	0.778	628	577	458	0.978	0.7
Ca^{2+} channel, N alpha 1B	U04999	876	1042	1134	0.500	795	854	635	0.979	1.0
fibroblast growth factor 18	AB004639	286	245	101	0.991	280	263	92	0.988	0.4
discoidin domain receptor family, member 1	L57509	1222	1218	1190	0.500	1233	1197	985	0.989	0.9
RAS-like protein expressed in neuron	U71202	42	119	13	0.954	56	114	37	0.990	0.3
Ca^{2+}-dependent activator protein for secretion	D86214	3109	2686	2154	0.984	4564	3924	3290	0.995	0.8

Wt, wild type: KO, knockout; HT, herterozygote; Δp value, change-p value.

The results are derived from known transcripts represented on the U74Av2 array. All listed transcripts were assayed as present in at least one genotype in both experiments.

Genes listed here as moderately increased or decreased either met change-p criteria in only one of two experiments, or met change-p criteria in both experiments but did not meet the twofold change criterion for inclusion in the lists of changed transcripts that appear in the text.