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Differential gene expression of neonatal and adult DRG neurons correlates with the differential sensitization of TRPV1 responses to nerve growth factor

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Abstract

Cultures of neonatal and adult dorsal root ganglion (DRG) neurons are commonly used in *in vitro* models to study the ion channels and signaling events associated with peripheral sensation under various conditions. Differential responsiveness between neonatal and adult DRG neurons to physiological or pathological stimuli suggests potential differences in their gene expression profiles. We performed a microarray analysis of cultured adult and neonatal rat DRG neurons which revealed distinct gene expression profiles especially of ion channels and signaling molecules at the genomic level. For example, Ca^{2+}-stimulated adenylyl cyclase (AC) isoforms AC3 and AC8, PKCδ and CaMKIIα, the voltage-gated sodium channel β1 and β4, and potassium channels $K_v1.1$, $K_v3.2$, $K_v4.1$, $K_v9.1$, $K_v9.3$, $K_{ir}3.4$, $K_{ir}7.1$, $K_{2P}1.1$/TWIK-1 had significantly higher mRNA expression in adult rat DRG neurons, while Ca^{2+}-inhibited AC5 and AC6, sodium channel $Na_v1.3$ α subunit, potassium channels $K_{ir}6.1$ $K_{2P}10.1$/TREK-2, calcium channel $Ca_v2.2$ α1 subunit, and its auxiliary subunits β1 and β3 were conversely down regulated in adult neurons. Importantly, higher adult neuron expression of ERK1/2, PI3K/P110α, but not of TRPV1 and TrkA, was found and confirmed by PCR and western blot. These latter findings are consistent with the key role of ERK and PI3K signaling in sensitization of TRPV1 by NGF and may explain our previously published observation that adult, but not neonatal, rat DRG neurons are sensitized by NGF.

Keywords

Dorsal root ganglion; Microarray; Ion channels; ERK1/2; PI3K/P110α

Cultures of neonatal or adult DRG neurons are commonly used to study the ion channels and signaling events underlying physiological and pathological conditions such as nociception and hyperalgesia (Hall A.K., 2006; Melli G. and Höke A., 2009). It is commonly assumed that either developmental stage is appropriate for many studies, choice often reflects experimental convenience. It can be demonstrated, however, that adult neurons are functionally different from their neonatal counterparts. For example, burst firing induced by an afterdepolarizing potential (ADP) is often seen in adult rat DRG neurons, but not in

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neonatal (P1) neurons, reflecting a much larger transient (T type) Ca^{2+} current in the adult (Lovinger D.M. and White G., 1989). Adult rat DRG neurons have an enhanced endogenous survival pathway conferred by either higher p73 expression (Walsh G.S., et.al, 2004) or higher constitutive Hsp27 expression (Lewis S.E., et.al, 1999) rendering them invulnerable to stimuli that cause apoptotic death of their neonatal counterparts. Phenotypes of DRG neurons reflect expression of specific sets of ion channels, receptors, signaling and cytoskeletal molecules. The distinct phenotypes of neonatal vs adult neurons confer different responsiveness to identical physiological stimuli, and different regenerative responses to damage or injury. After axotomy, adult sensory neurons survive for at least 4 months, while most neonatal neurons die by 7 days (Lewis S.E., et.al, 1999). Neonatal peripheral inflammation upregulates CGRP in both small and large diameter neurons, while postnatal inflammation increases the number of IB4 binding neurons (Beland B and Fitzgerald M, 2001). Furthermore, partial peripheral nerve injury causes neuropathic pain-like hypersensitivity in adult, but not in neonatal rats, consistent with observations in humans. T-cell infiltration and activation in adult dorsal spinal cord is a major contributor to this latter phenomenon (Costigan M., et.al, 2009). Finally, the neuroimmune response to nerve injury in DRG of adult rats, but not of young rats, also contributes to the higher incidence of neuropathic pain in adults (Vega-Avelaira D., et. al, 2009).

We previously reported that nerve growth factor (NGF) sensitizes the responses of the transient receptor potential vanilloid receptor 1 (TRPV1) to heat or capsaicin in isolated adult rat DRG neurons, but unexpectedly fails to do so in neonatal neurons (Zhu, W., et.al, 2004). This does not reflect absence of the relevant receptors, as individual neurons from both developmental stages express TRPV1 and the high affinity NGF receptor, TrkA. Alternatively this dramatic divergence of response suggests changes in the network of signaling molecules linking NGF mediated TrkA activation to TRPV1 sensitization between adult and neonatal DRG neurons. We examined this possibility by conducting a microarray analysis of gene expression profiles of cultured adult or neonatal rat DRG neurons focused on ion channels and signaling molecules with the aim to provide clues as to the mechanistic differences in NGF sensitization of TRPV1. We also confirmed some gene expression changes associated with our previous description of the trkA-to-TRPV1 signaling pathways at both mRNA and protein levels via real-time PCR and Western blotting, respectively.

DRG neuron isolation and culture

DRG neurons from adult (150~200 gm) and neonatal (postnatal day 0~1) rats were dissociated and cultured as described previously (Zhu et al., 2004) with modification. In brief, DRG were removed from all levels of isolated spinal cords and dissociated by combining dispase/collagenase digestion and mechanical disruption through a series of fire-polished glass pipettes. The resulting suspension of single cells was washed with bicarbonate-free Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen) for four times and neurons separated from supernatant containing debris with digressive speed centrifugation. The resulting single cell suspension enriched in DRG neurons was plated in poly-D-lysine-coated dishes, and maintained in DMEM supplemented with 10% fetal bovine serum (FBS) (Hyclone) and 100 units/ml penicillin and 100μg/ml streptomycin at 37° C under 5% CO_2.

Total RNA extraction and cDNA synthesis

After 16-18 hours in culture, DRG neurons were washed twice with PBS and total RNA extracted using RNA Easy kits (Qiagen) by the manufacturer's instructions. The RNA samples were digested with RNase-free DNase at 37°C for 1 hour to remove genomic DNA, followed by phenol-chloroform extraction and alcohol precipitation. Two μg of RNA were

denatured with 1 μM oligo dT (15) at 70°C for 5 minutes, followed by reaction with MMLV reverse transcriptase at 42°C for 1 hour to synthesize the first-strand of cDNA. Following RNase H-mediated second-strand cDNA synthesis, the cDNA was purified and served as a template for subsequent *in vitro* transcription. RNA and cDNA samples were stored at −80°C until use.

Gene microarray experiments

Affymetrix gene microarray experiments were conducted at the Center for Medical Genomics of IU School of Medicine. Four RNA samples each from adult or neonatal cultures were processed with GeneChip® Rat Genome 230 2.0 Arrays of cDNA. *In vitro* transcription was carried out in the presence of T7 RNA polymerase and a biotinylated nucleotide analog/ribonucleotide mix for complementary RNA (cRNA) amplification and biotin labeling. The biotinylated cRNA targets were cleaned up, fragmented, and hybridized to the Rat Genome 230 2.0 chips overnight. Following three stringent washes, the chips were incubated with fluorescent conjugated anti-biotin antibody for several hours, followed by three stringent washes, and scanned with an Affymetrix GeneChip® Scanner 3000 7G. The results were analyzed with Affy5 software.

Real time PCR

The resultant cDNAs were amplified on an ABI PRISM 7900HT (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems). The PCR protocol was as follows: 1× 95°C, 10 min → 45× 95°C, 15 sec → 60°C, 1min → 1× 95°C, 15 sec → 60°C, 15 sec → 95°C, 15 sec (for melting curve analysis) → 72°C, hold. After amplification, the PCR products were analyzed with the ABI PRISM sequence detection software (Applied Biosystems). The primers of interest were designed by Primer3 software. The primer sequences are shown in Table 1. Amplification of TrkA, TRPV1, ERK1/2, PI3K/P110α was normalized to the internal control cDNA (ribosomal housekeeping gene L27) to calculate relative expression using the 2–ΔΔCT method.

Western Blot Analysis

DRG neurons from adult or neonatal rats were lysed with buffer (Zhu and Oxford, 2007) and protein concentrations were determined using a BCA Protein Assay Kit (Pierce). Proteins (30-50 μg) were separated in 6-10% SDS PAGE gel, and transferred to PVDF membranes. After 30 minutes blocking, the membranes were incubated with primary antibody (1:1000 rabbit anti rat ERK1/2, TrkA, and TRPV1 C-terminal antibody; 1:20,000 mouse actin antibody) for 1.5 to 2 hours at room temperature. After 3 TBS-T washes, the membranes are incubated with 1:20,000 goat anti-rabbit antibody conjugated with Alexa Fluor 680 or 1:10,000 goat anti-mouse antibody conjugated with IRdye 800 (Molecular Probes) for 1.5 hours at room temperature. After 3 TBS-T washes, membranes were scanned on an Odyssey plate reader (Licor) at channel 700 or 800.

For statistical analysis, a Student's *t*-test was used with $p<0.05$ as a criterion for significance. Data are presented as mean ± SEM.

Results and Discussion

Of the 31,099 gene probes contained in the GeneChip® microarray, 20,453 genes are expressed in both neonatal and adult rat DRG neurons. Of these genes, 1915 genes were down-regulated in adult neurons, while 1508 genes were up-regulated in adult neurons compared to neonatal neurons at a 0.05 significance level (Figure 1). A subset of these genes showing mRNA expression changes between adult and neonatal rat neurons is represented

in Table 2 grouped in functional classes. TRPV5 is the only TRP isoform whose expression was significantly up-regulated in adults, and it is of note that TRPV1 mRNA expression was not different between neonatal and adult DRG neurons, consistent with recent reports (Wang H., et al., 2008; Hjerling-Leffler, J., et. al., 2007).

There were no significant differences in mRNA expression of neurotrophic factors including NGF and GDNF between adult and neonatal neurons, however mRNA for NT-3 and the artemin receptor, GFRα3, were significantly down-regulated, while that of BDNF and its receptor TrkB were significantly up-regulated in adult neurons. Importantly, mRNA expression of the high affinity NGF receptor, TrkA, along with p75, TrkC, Ret, GFRα1 and GFRα2, were not different between adult and neonatal neurons. A decrease in the percentage of TrkA positive neurons in adult compared to neonatal DRG has been reported in rat and mouse (Molliver D.C. and Snider W.D., 1997; Benn S.C., et al; 2001). This does not necessarily imply that the total TrkA expression is lower in adults, since adult TrkA positive neurons could have relatively higher TrkA mRNA levels than in neonates. Furthermore, adult rat DRG has more neurons than neonatal counterparts (Popken G.J. and Farel P.B., 1997), complicating numerical comparisons. In the signaling molecule gene subgroup, the mRNA expression of MAPK signaling components downstream of NGF-TrkA was significantly up-regulated in adult neurons including ERK2, p38β, and JNK3. ERK1 was also marginally up-regulated ($p=0.0575$). PI3Kc, a class III PI3K catalytic subunit, and P110α, a class I PI3K catalytic alpha polypeptide, showed significant up-regulation in adult neurons. In contrast, mRNA expression of the PI3K regulatory subunit p85α was down-regulated, while expression of other PI3K subunits (P110β, P85β, P55γ) was not significantly different. The Ca^{2+}-stimulated adenylyl cyclase (AC) isoforms AC3 and AC8 were significantly up-regulated, while Ca^{2+}-inhibited AC5 and AC6 were down-regulated in adult neurons. To our knowledge, this is the first report of differential expression of AC isoforms in neonatal or adult DRG neurons. mRNA expression for the protein kinases PKCδ and CaMKIIα was up-regulated, while the PKA regulatory subunits β1 and γ1 were down-regulated, in adult neurons. The nearly 5-fold increase in CaMKIIα expression is remarkable and interesting, as evidence indicates CaMKII mediated phosphorylation can sensitize TRPV1, although no known pathway from trkA activation involves this enzyme.

Several mRNA expression changes were also observed for ion channel genes. Expression of the sodium channel $Na_v1.3$α subunit and auxiliary β3 subunit was reduced by over 4-fold and 2.4-fold, respectively in adult neurons. This is consistent with a report of $Na_v1.3$ expression in developing neurons, but not in adult neurons (Waxman SG, et. al., 1994) and significant co-expression of β3 with $Na_v1.3$ following axotomy (Takahashi N, et. al., 2003). In contrast, the sodium channel β1 and β4 subunits were dramatically up regulated by 4.4-fold and 12-fold, respectively. The analysis also revealed significant up-regulation of the potassium channels $K_v1.1$, $K_v3.2$, $K_v4.1$, $K_v9.1$, $K_v9.3$, $K_{ir}3.4$, $K_{ir}7.1$, and $K_{2P}1.1$/TWIK-1 in adult rat DRG neurons, while the inwardly rectifying $K_{ir}6.1$ and two-pore $K_{2P}10.1$/TREK-2 channels were down-regulated. Finally, the N-type calcium channel $Ca_v2.2$ α1 subunit, and auxiliary subunits β1 and β3 were modestly, but significantly, down-regulated in adult neurons.

We previously demonstrated (Zhu et al., 2004) that NGF sensitizes TRPV1 responses in adult, but not neonatal DRG neurons. Thus we sought to capitalize on the microarray evidence to explore the possible underlying molecular events distinguishing the responsiveness at the two developmental stages. We had previously described the key signaling molecules and pathways that connect NGF-trkA activation to TRPV1 sensitization including PI3K, MAPK (ERK1/2), c-Src and PKC (Zhu and Oxford, 2007) and thus focused on their expression levels as they were of documented relevance. Using the microarray data

as a starting point, we sought to confirm changes in ERK1, ERK2, PI3K/P110α, TRPV1 and TrkA at the mRNA and protein levels by real time PCR and Western blotting. One could reasonably predict that the enhanced sensitization in the adult phenotype might reflect an increase in expression of either trkA or TRPV1. However, real time PCR revealed no change of TRPV1 and TrkA mRNA levels between adult and neonatal neurons, consistent with the microarray evidence (Figure 2 A and B). Western blotting further indicated no differences in protein levels of TRPV1 and TrkA between adult and neonatal neurons (Figure 2 C and D).

In contrast, we found significant increases in mRNA expression of ERK1, ERK2 and PI3K/P110α mRNA in adult neurons relative to neonatal neurons by real time PCR. Expression of ERK1, ERK2 and PI3K/P110α were significantly increased by 3.17 ± 0.54, 1.53 ± 0.12 and 2.48 ± 0.50 fold (n=4), respectively (Figure 2 E and F). Compared to neonates, ERK1 and ERK2 protein expression in adult neurons was also significantly increased 1.84 ± 0.22 and 1.77 ± 0.23 fold (n=3), respectively (Figure 2 G and H).

Microarray has been widely employed to reveal gene profiles of DRG and spinal cord after peripheral nerve injury (Xiao HS, et. al., 2002; Costigan M, et. al., 2002, 2009; Szpara ML, et. al., 2007; Vega-Avelaira D., et. al, 2009). Only a few such studies have assessed developmental changes associated with the injury paradigm (Méchaly I, et. al., 2006; Ryu EJ, et.al., 2008). Here we used short-term cultured neonatal and adult rat DRG neurons instead of fresh tissue to study gene profiles, an approach taken for two reasons. (1) Cultures were enriched in DRG neurons with very low non-neuronal gene contamination thus biasing the data toward neuron *specific* gene expression changes associated with the vulnerability to painful stimuli triggered by inflammation or injury. (2) Our previous electrophysiological studies of this developmental switch in sensitization phenotype were manifest in similar short term cultures, thus the resultant gene profiles would provide the relevant comparison. As we found altered expression of more ion channel genes between neonatal and adult neurons than in previous studies of intact DRG, an influence of short-term culture on expression levels cannot be ruled out. Nonetheless, under circumstances identical to those revealing the functionally distinct sensitization phenotype, we detected elevated mRNA expression of specific signaling molecules (ERK1/2 and PI3K) downstream in the NGF-TrkA signaling cascades known to play a critical role in the acute sensitization of TRPV1 by NGF.

Our findings support the notion of a developmental molecular switch occurring during early postnatal stages that underlies the development of sensitization of TRPV1 by NGF in the adult. Interestingly, this switch does not involve changes in the expression of either the initiating receptor, trkA, nor of the ultimate effector, TRPV1. Rather a change in the expression of key intermediate signaling molecules in the pathway(s) connecting the two receptors appears to be involved. Specifically, up-regulation of ERK1/2 and PI3K P110 in the adult DRG is consistent with their previously documented role in NGF-induced sensitization (Zhuang et al., 2004; Zhu and Oxford, 2007). In contrast, lower mRNA expression of PI3K p85α regulatory subunit in adult rat seems not to conform to our previous observation of reduced NGF sensitization of capsaicin responses in p85α null mice (Zhu and Oxford, 2007). This apparent discrepancy might reflect (1) the species difference between rats and mice which has been shown to alter a number of pain responses, (2) p85α was a complete knockout in mice but only decreased in neonatal neurons thus sufficient was available for signaling, or (3) catalytic subunit P110α rather than p85α played a prominent role in NGF sensitization of TRPV1 in adult rat.

Given the importance of NGF-trkA signaling in the early development, survival, and repair of nociceptive sensory neurons (Fitzgerald, 2005), but the apparent absence of such

functions in adult nociceptors, we posit that this developmental switch serves to repurpose existing trkA receptors from their critical developmental role to a sensitization role in the adult. To the extent that this latter function involves ERK1/2 and PI3K signaling, their upregulation in adult neurons seems a likely component of the switch. Precedence for a trkA signaling switch was suggested by Liu and Snider (2001) where evidence was provided for a role for ERK1/2 and PI3K in embryonic axonal growth, but the absence of such a role for regenerative axon growth of adult DRG neurons. Despite the appreciation of the developmental importance of NGF-trkA signaling in nociceptors, less is known about the actual signaling pathways underlying the aforementioned developmental functions of NGF-trkA signaling. Further study of the ontology of signaling pathways mediating the various functions (i.e., growth, survival, phenotype maintenance, and sensitization) of NGF-trkA signaling in sensory neurons may reveal potential targets to selectively promote or inhibit these functions mediated by this important neurotrophin. Furthermore, other growth factors such as cytokines or GFLs may also exhibit similar signaling switches that remain to be explored.

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Research Highlights

- A microarray analysis of cultured adult or neonatal rat DRG neurons was conducted
- Signaling molecules and ion channels had higher mRNA levels in adult rat DRG neurons.
- ERK1/2 and PI3K/P110α had higher mRNA and protein levels in adult rat DRG neurons.
- Up-regulation of signaling molecules might explain sensitizing TRPV1 by NGF in adult.

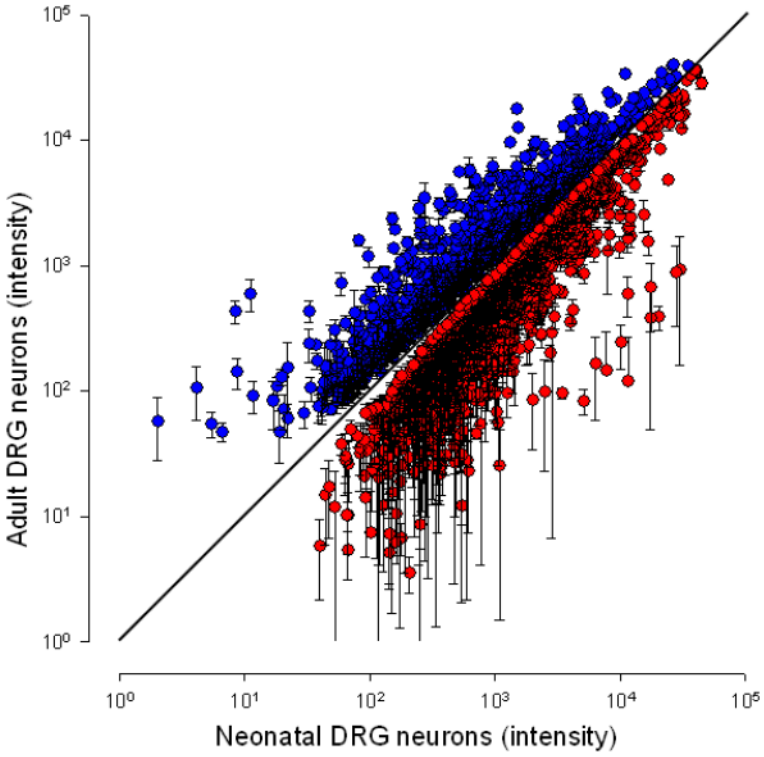

Figure 1.
1915 genes mRNA expressed lower and 1508 genes mRNA expressed higher in cultured adult rat DRG neuron. Four RNA samples from rat adult or neonatal day 0-1 pups DRG neuron culture were processed with GeneChip® Rat Genome 230 2.0 Array. After comparison by affy5 software, 1915 genes mRNA expressed lower (red circle), and 1508 genes mRNA expressed higher (blue circle) in adult rat DRG neuron cultures group at p=0.05 level.

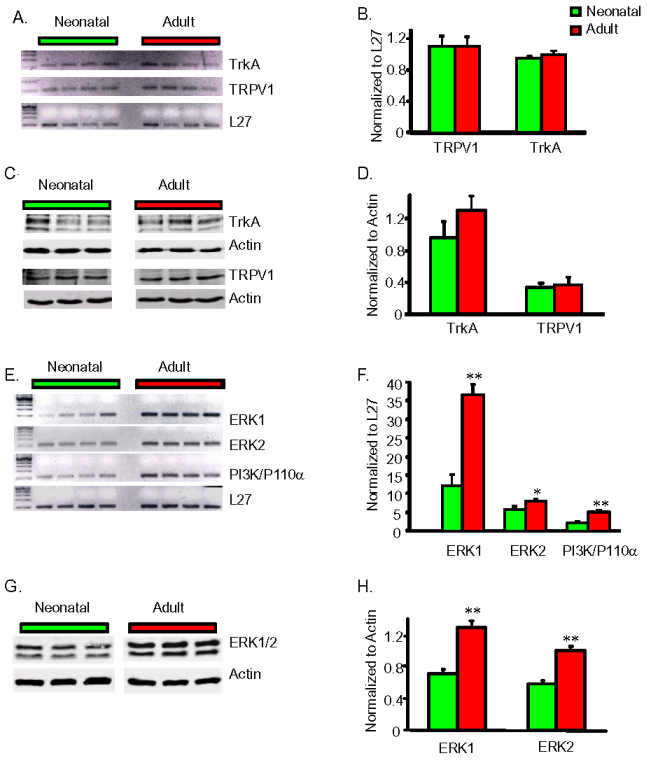

Figure 2.

Expression of ERK1, ERK2 and PI3K/P110α, but not TRPV1 and TrkA, are significantly higher in adult verse neonatal rat DRG neurons. A, B). Agarose gel electrophoresis of RT-PCR products confirmed that TRPV1 or trkA mRNA expression show no significant difference in adult verse neonatal group. C, D). Western blotting of trkA and TRPV1 protein expression in adult and neonatal rat DRG neurons showed that neither trkA nor TRPV1 protein expression showed significant difference between adult and neonatal rat DRG neurons ($p=0.44$ for trkA, $p=0.80$ for TRPV1, n=3). E, F). Agarose gel electrophoresis of PCR products of ERK1, ERK2, PI3K/P110α, and internal control L27 from adult verse neonatal rat DRG neurons showed that, ERK1, ERK2 and PI3K/P110α mRNA expression were significant higher in adult verse neonatal rat DRG neurons. ERK1, ERK2 and PI3K/P110α mRNA expression was increased 3.17 ± 0.54 fold (n=4, $p<0.01$), 1.53 ± 0.12 fold (n=4, $p<0.05$) and 2.48 ± 0.50 fold (n=4, $p<0.005$), respectively, in the adult verse neonatal group. G, H). Western blotting of ERK1 and ERK2 expression in adult verse neonatal rat DRG neurons. ERK1 and ERK2 protein expression was significantly higher in adult rat DRG neurons (both $p<0.01$, n=3). Compared to neonatal, ERK1 and ERK2 protein expression increased 1.84 ± 0.22 fold and 1.77 ± 0.23 fold, respectively.

Table 1

Primer sequences designed to investigate mRNA expression of interested genes by real time-PCR.

Gene	GeneBank Accession Numbers	Primer sequences (5′-3′)	Product size (base pair)
TRPV1	NM_031982	Sense: AGGACCCAGGCAACTGTG Antisense: ATCCCTCAGAAGGGGAACC	113
TrkA	NM_021589	Sense: GACGAAACACCTTTTGGGGT Antisense: TGATCCCAAATTTGCTCCTC	119
ERK1	NM_017347	Sense: GCTGAAGGAGCTGATCTTCC Antisense: AGACCAGGTCCAAAAGGACA	101
ERK2	NM_053842	Sense: AGCTGGACGACTTACCTAAGGA Antisense: CAGTCCTCTGAGCCCTTGTC	112
PI3K/P110α	NM_133399	Sense: TTTGGCTATAAACGGGAACG Antisense: CTGCCGAATTGCTAGGTACG	129
L27	NM_002514	Sense: ACAACCACCTCATGCCAACA Antisense: CTGGCCTTGCGTTTCAGT	103

Table 2

A set of genes showed expression changes between adult and neonatal rat DRG neurons.

Gene	GeneBank Accession Numbers	Fold change	P-value
TRP channels			
Trpv1	NM_031982	1.45	0.33722
Trpv5	**NM_053787**	**1.43**	**0.03286** *
Trpv6	NM_053686	−2.09	0.11876
Trpc1	NM_053558	−1.30	0.11718
Trpc2	NM_022638	1.35	0.17199
Trpc3	NM_021771	−1.43	0.31677
Trpm8	NM_134371	−1.47	0.36737
Neurotrophic Factors and receptors			
NGF β	BM388972	−1.50	0.39984
TrkA	NM_021589	−1.34	0.22272
P75	NM_012610	1.08	0.43299
BDNF	**NM_012513**	**3.22**	**0.01646** *
TrkB	**NM_012731**	**1.41**	**0.01322** *
NT-3	**NM_031073**	**−4.80**	**0.02407** *
TrkC	NM_019248	1.59	0.12459
GDNF	NM_019139	1.55	0.37196
Ret	NM_012643	1.07	0.65386
GFRα1	NM_012959	1.18	0.42976
GFRα2	NM_012750	−1.20	0.88288
GFRα3	**NM_053398**	**−2.81**	**0.01279** *
Signaling molecules			
ERK1/MAPK3	NM_017347	1.36	0.0575
ERK2/MAPK1	**NM_053842**	**1.36**	**0.02092** *
JNK3/MAPK10	**NM_012806**	**1.70**	**0.02922** *
ERK6/p38β/MAPK12	**NM_021746**	**2.42**	**0.01083** *
PI3K/pik3c3	**NM_022958**	**1.67**	**0.00674** **
P110α/pik3ca	**NM_133399**	**2.05**	**0.04076** *
P110β/pik3cb	NM_053481	1.23	0.27749
P85α/pik3r1	**NM_013005**	**−1.89**	**0.01331** *
P85β/pik3r2	NM_022185	−1.46	0.07989
P55γ/pik3r3	NM_022213	1.60	0.27383
AC3	**NM_130779**	**1.88**	**0.01388**
AC5	**NM_022600**	**−2.32**	**0.01236**
AC6	**NM_012821**	**−1.94**	**7.90E-04**
AC8	**NM_017142**	**2.05**	**0.01687**

Gene	GeneBank Accession Numbers	Fold change	P-value
PKA non-catalytic subunit β1	**NM_031976**	**−1.31**	**0.00366**
PKA non-catalytic subunit γ1	**NM_013010**	**−1.11**	**2.70E-04**
PKCδ	**NM_133307**	**1.48**	**0.04305**
CaMKIIα	**NM_012920**	**4.79**	**0.00657**
Ion channels			
Nav1.3/scn3a	NM_013119	−4.58	0.01017
β1/scn1b	NM_01728	4.40	0.00519
β3/scnb3	NM_139097	−2.44	0.03789
β4/scnb4	NM_001008880	11.96	0.01016
Kv1.1/Kcna1	NM_173095	5.24	0.01241
Kv3.2/Kcnc2	NM_139217	3.59	0.02751
Kv4.1/Kcnd1	XM_217601.3	2.39	0.04325
Kv9.1/Kcns1	NM_053954	6.19	0.04281
Kv9.3/Kcns3	NM_031778	4.84	0.01976
Kir3.4/Kcnj5	NM_017297	2.02	0.01799
Kir6.1/Kcnj8	NM_017099	−7.00	0.02392
Kir7.1/Kcnj13	NM_053608	1.51	0.03075
K_{2P}1.1/TWIK-1/Kcnk1	NM_021688	2.80	0.02288
K_{2P}10.1/TREK-2/Kcnk10	NM_023096	−1.90	0.0404
Ca_v2.2/Cacna1b	NM_147141	−1.72	0.01963
$Ca_v\beta_1$	NM_017346	−1.30	0.03958
$Ca_v\gamma_3$	NM_080691	−1.58	0.00253

*P<0.05

**P<0.01 for non-ion channels genes which are bolded for highlight