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# Structure and distribution of a broadly expressed atypical sodium channel

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**Abstract A cDNA clone isolated from a rat dorsal root ganglion library encodes a 195 kDa voltage-gated sodium channel-like protein (SCL-11) with homology to the mouse (87%) and human (72%) atypical Na$^+$ channels and rat partial clone NaG (98%). Two dominant mRNAs of 4.5 and 7 kb are expressed. The transcripts are present in lung, Schwann cells, pituitary and tissues containing smooth muscle cells. No functional channels could be detected on oocyte injection with cRNA, consistent with the absence of structural features necessary for voltage-gated sodium channel activity.**

*Key words:* Dorsal root ganglion; Voltage-gated sodium channel; cDNA cloning

## 1. Introduction

Voltage-gated sodium channels (VGSCs) comprise an α-subunit and two distinct types of β-subunits [1]. Many different mammalian α-subunit cDNAs have been cloned [2–8]. Several distinctive structural properties of the VGSC α-subunits suggest that they are members of the same multigene family. The S4 voltage-sensor segments [9], which contain a repeated motif of a positively charged amino acid residue followed by two hydrophobic residues, are highly conserved among VGSCs [2–8]. The intracellular loop between domains 3 and 4 which plays an important role in inactivation is also highly conserved [2–8]. Ion selectivity reflects a distinctive pore structure [10,11]. cRNAs which encode α-subunits synthesise functional VGSCs in either *Xenopus* oocytes or cell lines (e.g. [3,5,7]).

Atypical sodium channels form a distinct sub-group of VGSC-like proteins [13–15]. We report here the isolation and characterisation of a full-length cDNA from rat dorsal root ganglia (DRG) that encodes a protein with 98, 87 and 72% identity to the partial rat clone NaG, and the mouse (mNa$_v$2.3) and human (hNa$_v$2.1) atypical sodium channels, respectively [13–15]. We present evidence that this protein has a distinct function from VGSCs.

## 2. Materials and methods

### 2.1. Materials

Restriction enzymes and DNA modifying enzymes were obtained from Gibco-BRL and Boehringer Mannheim. Oligonucleotides and nucleotides were obtained from Stratagene, Amersham and Pharmacia. DNA sequencing reagents were obtained from USB Corp.

### 2.2. RNA isolation, cDNA library construction and screening

Approx. $5\times10^5$ plaques of a rat DRG cDNA library were screened with a [$^{32}$P]dATP random-primed rat A0 cDNA probe defined as glial sodium channel-like [7,13,17]. The cDNA inserts of positive clones were excised into pBluescript-SK, and both strands were sequenced with M13 (−40) universal, M13 reverse and sodium channel-like specific primers.

### 2.3. Distribution and expression studies

10–30 μg of total RNA from neonatal rat tissues [16] was separated on 1.2% agarose-formaldehyde gels, and blotted onto Hibond-N (Amersham). The relative amounts of RNA were judged by ethidium bromide staining or by hybridization with a cyclophilin probe. For RT-PCR experiments, 3–5 μg total RNA from neonatal rat tissues and cell lines was treated with DNase I and extracted with acidic phenol. cDNA was synthesized with SuperScript reverse transcriptase using random primers. cDNA was amplified (35 cycles, 94°C, 1 min; 52°C, 1 min; and 72°C, 1 min) simultaneously with SCL11-specific primers, 5′-CCATGACAAGCTCTGCTTTATGT-3′ and 5′-TATCCTTTTAGTAAATGTGTAC-3′, and L-27 primers (5′-ATCGCTCCTC AAACTTGACC-3′ and 5′-AAAGCCGTCATCG-TAAAGAAC-3′). L-27 primers were added to the PCR reaction 7 cycles after the start. PCR products were separated on agarose gels, alkali-transferred onto Neulon membranes and subsequently hybridized with SCL11 and L-27 probes.

240 bp *Pst*I-*Eco*RV fragments (position 4776–5018) bearing part of the SCL11 C-terminal domain was subcloned into pGem4Z linearised with *Sma*I and *Pst*I. The DIG-UTP (for in situ) and [$^{32}$P]UTP (for Northern blots) antisense cRNAs were generated using Sp6 RNA polymerase. Hybridization with Northern blots was performed at 65°C for 24 h in 50% formamide, 5×SSC, 50 μg/ml yeast tRNA, 0.5% SDS and 5×Denhardt's solution. The membranes were washed at 68°C for 1 h in 0.5×SSC, 0.5% SDS, and exposed for 1–10 days. In situ hybridization was carried out as described [18].

Oocyte expression studies were carried out as described [7]. SCL11 in pBS-SK was cut with *Sma*I and *Sal*I and subcloned into the oocyte expression vector pSp64GL. The resulting plasmid was linearised with *Sal*GI and capped cRNA transcribed and injected into *Xenopus* oocytes.

## 3. Results

A rat DRG cDNA library was screened with a sodium channel-like insert isolated from a subtractive library [17]. The cDNA clone with the longest insert, SCL11, was completely sequenced. The sequence of SCL11 (Fig. 1, line 3) consists of 32 bp of 5′-untranslated region (UTR), a 5106 bp open reading frame that encodes a protein of 1702 amino acids with a calculated molecular mass of 195 kDa, and 1440 bp of 3′-UTR including a non-consensus polyadenylation signal (AAATAA) at position 6508, followed by a poly(A) tail (25 bp). The deduced amino acid sequence of SCL11 shows 49 and 42% identity with the brain type II TTX-sensitive VGSC [2], and the sensory neuron TTX-resistant VGSC SNS, respectively [7] (Fig. 1). A higher level of homology occurs between SCL11 and the atypical sodium channels isolated from the human heart, mouse tumour cell line AT-1 and rat astrocytes [13–15]. The overall degree of amino acid sequence identity to the human channel is 72%, to the mouse channel 87%, and to a partial sequence of the rat gene, 98% [13–15].

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ratii  MARSVLVPPG PDSFRFFTRE SLAAIEQRIA EEKA..KRPK QERKDEDDEN  48
  sns  .MELPFASV- TTN--R--P- ---E--KQ-- AHR-AK-ART KH-GQ--KGE  49
SCL11  ....M-TS-E -KGLVP--A- --EL-KNH-- .......... KKCNE-HE-E  36

ratii  GPKPNSDLEA GKSLPFIYGD IPPEMVSEPL EDLDPYYINK K.TFIVLNKG  97
  sns  K-R-QL--KD CNQ--KF--E L-A-L-G--- -----F-STH R.--M----S  98
SCL11  DL--SR-I-- --K---A--T L-QGT----- --V----YV- RN--M---RN  86

ratii  KAISRFSATS ALYILTPFNP IRKLAIKILV HSLFNVLIMC TILTNCVFMT  147
  sns  RT-------W --WLFS---L --RT---VS- --W-SIF-TI ---V---C--  148
SCL11  RV-F--N-V- I-CT-S-LSS L-RAV--V-- -P-LRL--LI SV--DSIL-C  136
                                         D-I     S1

ratii  MSNPPDWTKN VEYTFTGIYT FESLIKILAR GFCLEDFTFL RNPWNWLDFT  197
  sns  RTDL-E...K ---V--V--- --A------- ----NE--Y- -D-------S  195
SCL11  ---L-E-ILA --N-CL---- --I-V-VI-- -IWAGS-S-- GDL------S  186
                    D-I    S2

ratii  VITFAYVTEF VNLGNVSALR TFRVLRALKT ISVIPGLKTI VGALIQSVKK  247
  sns  ---L---GAA ID-RGI-G-- ---------- V-------V- -----H--R-  245
SCL11  -TL-ELI-RS SP-SSLPMFK -I-T--I--I -PLNH--QS- -VT-V-CL--  236
          D-I   S3                D-I   S4

ratii  LSDVMILTVF CLSVFALIGL QLFMGNLRNK CLQWPPDNST FEINITSFFN  297
  sns  -A--T----- -------V-- ---K---K-- -I........ ..........  277
SCL11  -LGAIA-AL- F-T-SS-F-M G------KH- -VR--QEDG. ..........  275
                 D-I    S5

ratii  NSLDWNGTAF NRTVNMFNWD EYIEDKSHFY FLEGQNDALL CGNSSDAGQC  347
  sns  ....R---DP HKAD-LSS.. ...-MAEYIF IKP-TT-P-- ---G----H-  318
SCL11  -DVMYS--GS Q......... YH-LEREN-- YM--ARY--- ---KT---L-  316

ratii  PEGYICVKAG RNPNYGYTSF DTFSWAFLSL FRLMTQDFWE NLYQLTLRAA  397
  sns  -G--V-L-TP D--DFN---- -S-A------ -------S-- R---Q----S  368
SCL11  ----M---E- S--DN-F--- -N-G--L-AM -------YP- L--HQI-Y-S  366
                                D-I  SS1      D-I SS2

ratii  GKTYMIFFVL VIFLGSFYLI NLILAVVAMA YEEQNQATLE EAEQKEAEFQ  447
  sns  --M--V---- ---------V -------T-- ----S---IA -I-A--KK--  418
SCL11  --I------- IS-WFA--MA S-F-GILT-- --QEK-RAS- -SRDMDSKCH  416
                    D-I    S6

ratii  QMLEQLKKQQ EEAQAAAAAA SAESRDFSGA GGIGVFSESS SVASKLSSKS  497
  sns  EA--V-Q-E- -VLEAL.... .......... .--DTT-LQ- HSG-P-A--N  453
SCL11  -TVKEFEEEH -G-EL..... .......... .......... ..........  431

ratii  EKELKNRRKK KKQKEQAGEE EKEDAVRKSA SEDSIRKKGF QFSLEGSRLT  547
  sns  AN-RRP-... .......... .....-KSRV --G-T..... ....DDN-SP  476
SCL11  .......... .......... .......... .......... ..........  431

ratii  YEKRFSSPHQ SLLSIRGSLF SPRRNSRASL FNFKGRVKDI GSENDFADDE  597
  sns  QSDPYNQRRM -F-GLSS... GR--A-HG-V -H-RAPSQ-- SFPDGITP-D  523
SCL11  .......... .......... .......QCI WFYEEVLDLL -L-LCIKEME  454

ratii  HSTFEDNDSR RDSLFVPHRH GERRPSNVSQ ASRASRGIPT LPMNGKMHSA  647
  sns  GVFHG-QE-- -G-ILLGRGA -QTG-LPR-. ......PL-Q S-NP-RR-GE  566
SCL11  .......... .......... .......... .......... ..........  454

ratii  VDCNGVVSLV GGPSALTSPV GQLLPEGTTT ETEIRKRRSS SYHVSMDLLE  697
  sns  EGQL--P... ......-GEL TAGA---PAL H-TGQ-S... ..FL-AGY-N  602
SCL11  .......... .......... ........-- QI-MK--SPT -INTTL-I--  476
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ratii  DPSR.QRAMS MASILTNTME ELEESRQKCP PCWYKFANMC LIWDCCKPWL  746
  sns  E-F-A----- VV--M-SVI- -----KL--- --LIS--QKY ---E--PK-R  652
SCL11  -TALGHK... .........- -P-T--KE-- L-----TKT- F--K-SPC-I  514

ratii  KVKHVVNLVV MDPFVDLAIT ICIVLNTLFM AMEHYPMTEQ FSSVLSVGNL  796
  sns  -F-MALFEL- T---AE-T-- ----V--V-- --------DA -DAM-QA--I  702
SCL11  -LNEFADRII TH-LF--FLV ---I--IC-L -L--F--S-E LM-L-AI---  564
                      D-II   S1

ratii  VFTGIFTAEM FLKIIAMDPY YYFQEGWNIF DGFIVSLSLM ELGLANVEGL  846
  sns  ---VF--M-- AF----F--- ----KK---- -CV--TV--L --SASKKGS-  752
SCL11  --I--Y-I-- I------H-- G---IS-H-- -SIL-V-G-T -ML--DI-EI  614
          D-II   S2                      D-II  S3

ratii  SVLRSFRLLR VFKLAKSWPT LNMLIKIIGN SVGALGNLTL VLAIIVFIFA  896
  sns  ----TL---- ---------- --T------- ---------F I---------  802
SCL11  T-FILVP-I. FI--G-YA-P FKN-MR-L-R ALV--KD-V- LVS-FIYFS-  663
             D-II   S4                              D-II   S5

ratii  VVGMQLFGKS Y...KECVCK ISNDCELPRW HMHHFFHSFL IVFRVLCGEW  943
  sns  L--K--LSED -GCR-DG-S. VW-GEK-.-- --CD------ V---I-----  850
SCL11  -F--K---R- -...-D---H VDQ--QRQ-- --SD-L-AYV T---I-----  710
                                         D-II  SS1    SS2

ratii  IETMWDCMEV AGQTMCLTVF MMVMVIGNLV VLNLFLALLL SSFSSDNLAA  993
  sns  --N--V---- SQKSI--IL- LT---L---- -----I---- N---A---T-  900
SCL11  ---L-E---- --EAW-IPFY ---IL----L I-Y--VG.-V ---A-YD.-T  758
                                D-II     S6

ratii  TDDDNEMNNL QIAVGRMQKG IDFVKRKIRE FIQK....AF VRKQKALDEI  1039
  sns  PE--G-V--- ---LA-IQVL GHRAS-A-AS Y-SSHCRFHW PKVETQ-GMK  950
SCL11  -EVSK-AK-- ----AWIKMV -NCVLL--.. .......... LC-E-TVSTE  796

ratii  KPLEDLNNKK DSCISNHTTI EIGKDLNYLK DGNGTTSGIG SSVEKYVVDE  1089
  sns  P-L....... ...T-SEAKN HIAT-AVSAA V--L-KPALS -PK-NHG...  987
SCL11  ATDQTCDPSV KEN--GH-LS -LSNTQTF-R YKDQ.....S -GT--TP-T-  841

ratii  SDYMSFINNP SLTVTVPIAL GESDFENLNT E.EFSSESDM EESKEKLNAT  1138
  sns  ....DF-TD- NVW-S----E ----LDELEE DM-QA-Q-SW Q-EDP-GQQE  1033
SCL11  -ESQ-L-AS- -VSET----S ----I---D. NK-TR-K-AN GSS---MKQS  890

ratii  S......... .......... .SSEGSTVDI GA........ ..PAEGEQPE  1158
  sns  QLPQVQKCEN HQAARSPASM M---DLAPYL -ESWKRKDSP QV----VDDT  1083
SCL11  -......... .......... .---C----- AIS....... ....-EEEMV  909

ratii  AEPEESLEPE A......... .......... ...CFTEDCV RKFKCCQISI  1184
  sns  SSS-G-.TVD CPDP-EIL-- IPELADDLD- PDD----G-T -RCP--NVNT  1132
SCL11  Y-H-K-KL.. ..HKNGYE-- SSAG-V-R-S R......... ..........  934

ratii  EEG....... .......... .......... .KGKLW..WN LRKTCYKIVE  1206
  sns  .......... .......... .......... S-SPWATG-Q V-----R---  1152
SCL11  .......... .......... .......... .N--I-..R- I----C----  953

ratii  HNWFETFIVF MILLSSGALA FEDIYIEQRK TIKTMLEYAD KVFTYIFILE  1256
  sns  -SW--S--I- ---------- ---N-L-EKP RV-SV---T- R---F--VF-  1202
SCL11  NS---C--GL VT--CT-T-- L-----H--- ---IF---G- MI-A------  1003
                   D-III  S1                      D-III  S2

ratii  MLLKWVAYGF QMYFTNAWCW LDFLIVDVSL VSLTANALGY SELGAIKSLR  1306
  sns  ---------- KK-------- ------NI-- T--I-KI-E- -DVAS--A--  1252
SCL11  ---------- KA--S-N-YK ---MV-I-LC L--KGKTRE. ....DLNP-P  1048
                              D-III   S3
```

Fig. 1. The amino acid sequence of SCL11 (third line) aligned with the rat brain type II (first line) and SNS (second line) sodium channels [2,7]. The putative transmembrane segments are underlined and domains are indicated in subscripts. Sequence gaps are indicated by dots. Identical residues are marked with bars. Two alternative exons are included in the composite sequence. The alternative exon in interdomain region 1 is in bold. The alternative exon in D3 is in bold and underlined.

RT-PCR and Northern blot analysis were used to determine the size of the SCL 11 transcript (Fig. 3). Two transcripts of approx. 4.5 and 7 kb were detected in various tissues. An exception is sciatic nerve where only the 7 kb transcript is present. Several other tissues, notably lung, but also pituitary, bladder and vas deferens express SCL11 (Fig. 3A). SCL11 mRNA was identified in PC12 and C6 glioma cell lines, and trigeminal mesensephalic and coeliac ganglia (Fig. 3A). RT-PCR (Fig. 3B), demonstrates the presence of variable levels of SCL11 transcripts in a wide range of tissues and cell lines (Fig. 3B). In situ hybridisation of adult rat DRG sections showed signals from myelinating Schwann cells (Fig. 4), with possible weak signals in some neuronal cell bodies.

SCL11, was subcloned into the oocyte high expression vector pSp64GL and cRNA injected into *Xenopus* oocytes. No voltage-gated channel activity could be detected using incubation and recording conditions that allowed SNS VGSC cRNA transcript activity to be detected routinely [7] in the same batches of oocytes. The mouse SCL11 clone has the EMBL accession number Y09164.

## 4. Discussion

The primary structure of an atypical sodium channel protein from rat DRG has been determined. It has less than 50% identity to functional VGSC α-subunits. We have not been able to express functional voltage-gated channel activity in *Xenopus* oocytes, despite using an oocyte expression vector containing the 5′- and 3′-UTR of the *Xenopus* β-globin gene, which improves expression 50–200-fold [7,19] that routinely results in the synthesis of other functional VGSC (e.g. [7]). Failure of expression could be explained by the necessity for additional subunits, a specialized cellular environment, by cloning artefacts, or the possibility that SCL11 is a pseudogene. These explanations have been noted by Tamkun and colleagues [15], who were also unable to express functional hNa$_v$2.1 and mNa$_v$2.3. There are strong grounds to believe that SCL11, as well as hNa$_v$2.1 and mNa$_v$2.3, may be neither voltage-gated, nor sodium channels.

Many gene families, for example, isoforms of the ATP-gated channels (P2X) share less than 50% overall amino

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ratii  TLRALRPLRA LSRFEGMRVV VNALLGAIPS IMNVLLVCLI FWLIFSIMGV  1356
  sns  ---------- ---------- -D--V----- ---------- ----------  1302
SCL11  SIKF--A--V --Q--R-K-- LR--IKTTLP AVS-F----M I--L--V---  1098
        D-III   S4                                D-III   S5

ratii  NLFAGKFYHC INYTTGEMFD V..SVVNNYS ECQALIESNQ TARWKNVKVN  1404
  sns  -------SK- VDTRNNPFSN -NSTM---K- --HNQ.N-TG HFF-V-----  1351
SCL11  F-------E- -DP-R--R-S -FE..-M-K- Q-EN-V.F-E SMP-E-A-L-  1145

ratii  FDNVGLGYLS LLQVATFKGW MDIMYAAVDS RNVELQPKYE DNLYMYLYFV  1454
  sns  ----AM---A ---------- ---------- GEINS--NW- N---------  1401
SCL11  -----N-F-- -F-----N-- IS--NS-I-- VG-YM--SF- HS-H--T--I  1195
         D-III SS1             D-III SS2

ratii  IFIIFGSFFT LNLFIGVIID NFNQQKKKFG GQDIFMTEEQ KKYYNAMKKL  1504
  sns  V-----G--- ----V----- --------L- ---------- ----------  1451
SCL11  --VV--L-LP -CML-----R ---K--I-Q- -SN--I-VK- --Q-R-L---  1245
         D-III   S6

ratii  GSKKPQKPIP RPANKFQGMV FDFVTKQVFD ISIMILICLN MVTMMVETDD  1554
  sns  ---------- --L--Y--F- --I--R-A-- -I--V----- -I-------E  1501
SCL11  LYADS---AA --R-----FI C-V--HR--N VVVVL---FQ AT-I-IQN-E  1295
                                     D-IV    S1

ratii  QSQEMTNILY WINLVFIVLF TGECVLKLIS LRHYYFTIGW NIFDFVVVIL  1604
  sns  -GE-K-KV-G R--QF-VAV- -----M-MFA --Q----N-- -V---I----  1551
SCL11  --PQIETAVF -M-SL-TM-- -L--I---TA F-CH---SA- -VH--M--VF  1345
                D-IV         S2                   D-IV    S3

ratii  SIVGMFLAEL IEKY..FVSP TLFRVIRLAR IGRILRLIKG AKGIRTLLFA  1652
  sns  --GSLLFSAI LKSLENYF-- ---------- --------RA ----------  1601
SCL11  --T-LL-PLS -GQ-..--P- S-VQLLL-S- -IHV--PG-- P-VFHD-MLP  1393
                                    D-IV     S4

ratii  LMMSLPALFN IGLLLFLVMF IYAIFGMSNF AYVKREVGID DMFNFETFGN  1702
  sns  ---------- ---------- --S----AS- -N-VD-A--- -----K----  1651
SCL11  --L-----L- ----I----- -------Y-- ----K-A--N -VS------S  1443
                    D-IV     S5                    D-IV

ratii  SMICLFQITT SAGWDGLLAP ILNSGPPDCD PEKDHPGSSV KGDCGNPSVG  1752
  sns  ---------- ---------S- ---T---Y-- -NLPNSNG-. R-N--S-A--  1700
SCL11  --L----V-- FS----M-DA -F--QWS--- -D-IN--TQ- R----S----  1493
       SS1         SS2

ratii  IFFFVSYIII SFLVVVNMYI AVILENFSVA TEESAEPLSE DDFEMFYEVW  1802
  sns  -I--TT---- ---I------ -------N-- ----T----- ---D----T-  1750
SCL11  --Y-----L- -W-II----V VL-M-FL-IP SKRKNRT--- ---RR-FK--  1543
            D-IV     S6

ratii  EKFDPDATQF IEFCKLSDFA AALDPPLLIA KPNKVQLIAM DLPMVSGDRI  1852
  sns  -----E---- -A-SA----- DT-SG--R-P ---QNI--Q- ---L-P--K-  1800
SCL11  NR----R--Y -DST------ -------FM- ----G--V-- ----AA----  1593

ratii  HCLDILFAFT KRVLGESGEM DALRIQMEER FMASNPSKVS YEPITTTLKR  1902
  sns  ---------- -N-------L -S-KTN---K ---T-L--A- ----A---RW  1850
SCL11  ------L--- ---M-KDERV EKILSEI-SG --LA--F-IT ----------  1643

ratii  KQEEVSAIVI QRAYRRYLLK QKVKKVSSIY KKDKGKEDEG TPIKEDIITD  1952
  sns  ---DL--T-- -K---S-M-H RSL.TL-NTL HVPRAE--GV SLPG-GY--.  1898
SCL11  ---A---TI- ----KS-R-R -SD--IQD-P EI-D-R--PN SKG.......  1686
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ratii  KLNENSTPEK TDVTPSTTSP PSYDSVTK.. PEKEKFEKDK SEKEDKGKDI  2000
  sns  FMANSGL-D- SETASA-SF- -------RGL SDRANINPSS -MQNEDEVAA  1948
SCL11  .VHSGQIE-- ASIQTQ.... .....I* 1702

ratii  RESKK* 2005
  sns  K-GNSPGPQ* 1957
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Fig. 1 (*continued*).

acid identity [20]. However, structural motifs and domains or single amino acids which are critical for function are conserved. The atypical sodium channels do not exhibit conservation of key VGSC motifs, such as the S4 voltage sensor [1,21,22]. All VGSCs bear 4 positively charged amino acid residues (Arg or Lys) in the S4 segment of D1, 5 positive charges within the S4 segment of D2, 5 positive charges in the S4 segment of D3 and 8 positive charges in the S4 segment of D4 [2–8]. In contrast, 7 positive residues in the D2, D3 and D4 S4 segments of SCL11 are either substituted or omitted (Arg to Ile$^{618}$, Arg to Pro$^{621}$, Arg is omitted, Lys to Asn$^{1045}$, Arg to Pro$^{1048}$, Arg to Gln$^{1366}$, Arg to Leu$^{1369}$) (Fig. 1). Thus, the S4 segments in D2 and D3 of the SCL11 do not contain a sufficient number of positive charges to be voltage sensors, and show only 30–40% identity to other VGSCs throughout

Fig. 2. (A) An aligned amino acid sequence of ID3 from the SCL11, brain type I, II, II [2,3], brain type 6 [4], glial (Na$_s$) [8], skeletal muscle [5], cardiac [6], sensory neuron (SNS) [7], *Electrophorus electricus* and jellyfish sodium channels [1]. The IFM motif is indicated within a frame. Potential protein kinase C (PKC) phosphorylation sites are marked (●). (B) Alignment of the amino acid sequence of regions encompassing the P domain of the four repeats of the SCL11, mouse atypical sodium channel mNav2.3, human atypical sodium channel hNav2.1 [14,15] brain type I, II, II [2,3], brain type 6 [4], glial (Na$_s$) [8], skeletal muscle [5], cardiac [6], sensory neuron (SNS) [7], *Electrophorus electricus*, *Drosophila para*, *Drosophila* DSC 1 and jellyfish sodium channels, and rabbit brain B1, rabbit cardiac dihydropyridine sensitive and rabbit skeletal muscle dihydropyridine sensitive calcium channels. The residues identified as determinants of ion selectivity are in bold type.

A

```
SCL11      RNFNKQKIKQ.GGSNIFITVKQKKQYRALKKLLYADSQKPAARPRNKFQGFIC
mNav2.3    ----------.---------------------------V---TP---------LF
hNav2.1    D----H---L.------------R----R----M-E---R-VP--L--L---LF
Br I       D---Q--K-F.--QD--M-EE---Y-N-M---GSKKP---IP--G-----MVF
Br II      D---Q--K-F.--QD--M-EE---Y-N-M---GSKKP---IP--A-----MVF
Br III     D---Q--K-F.--QD--M-EE---Y-N-M---GSKKP---IP--A-----MVF
Br 6       D---Q--K-F.--QD--M-EE---Y-N-M---GSKKP---IP--L--I--IVF
Nas        D---Q--K-L.--QD--M-EE---Y-N-M---GSKKP---IP--G--I--CIF
SkM 1      D---Q--K-F.--KD--M-EE---Y-N-M---GSKKP---IP--Q--I--MVY
Cardiac    D---Q--K-L.--QD--M-EE---Y-N-M---GSKKP---IP--L--Y----F
SNS        D---Q--K-L.--QD--M-EE---Y-N-M---GSKKP---IP--L--Y---VF
EEL        D---R--Q-L.--EDL-M-EE---Y-N-M---GSKKAA-CIP--S-VV--VVY
Jellyfish  D---RL-QQYED-VG--L-PG-RNWVNT--SAAGPKKTRRLT--KS-WRATLF
```

B

| | Domain 1 | Domain 2 | Domain 3 | Domain 4 |
|---|---|---|---|---|
| SCL11 | RLMTQ**D**YPELLYH | RILCG**E**WIETLWE | QVATF**N**GWISIMN | QVTTF**S**GWDGMLD |
| mNav2.3 | ------------- | ------------- | ------------- | ------------- |
| hNav2.1 | ---A-----V--- | -------V----D | ----------T--- | --AI-**A**-------- |
| Br I | ------FW-N--Q | -V--------M-D | -----**K**--MD--Y | -I--S**A**----L-A |
| Br II | ------FW-N--Q | -V--------M-D | -----**K**--MD--Y | -I--S**A**----L-A |
| Br III | -------W-N--Q | -V--------M-D | -----**K**--MD--Y | -I--S**A**----L-A |
| Br 6 | -------W-N--Q | -V--------M-D | -----**K**--MD--Y | -I--S**A**----L-L |
| NAs | -------W-N--Q | -V--------M-D | -----**K**--MD--Y | -I--S**A**----L-A |
| SkM 1 | -------W-N-FQ | ----------M-D | -----**K**--MD--Y | EI--S**A**----L-N |
| Cardiac | ------CW-R--Q | ----------M-D | -----**K**--MD--Y | -I--S**A**----L-S |
| SNS | ------SW-R--Q | ---------NM-V | -----**K**--MD--Y | -I--S**A**----L-S |
| EEl | ---L---W-N--Q | -A--------M-D | --S--**K**--MD--Y | EI--S**A**----L-L |
| para | ------FW-D--Q | -V-------SM-D | -----**K**---Q--- | -MS-S**A**----V-- |
| DSC 1 | Q-I-L--W-NV-N | ---------PL-D | -----**E**--NEV-A | -LM-S**A**--NDV-E |
| Jellyfish | QVC-L--W-SV-N | -----**K**---PQ-D | -T--L**E**--FEV-Q | RIS-A**A**--N-V-E |
| Brain BI | QCI-M**E**GWTD-LY | Q--T--DWNEVMY | T-S-G**E**--PQVLK | RSA-G**E**A-HNIML |
| Cardiac | QCI-M**E**GWTDVLY | Q--T--DWNSVMY | T-S--**E**--PELLY | RCA-G**E**A-QDIML |
| Skeletel | QCI-M**E**GWTDVLY | QV-T--DWNSVMY | T-S--**E**--PQLLY | RCA-G**E**A-QEI-L |

the normally well conserved S4 segments (Fig. 1). Similarly, interdomain region 3 (ID3) is important for fast inactivation of VGSCs [1], and slow inactivation involves residues in the S5 and S6 segments, and the P domain. The ID 3 of SCL11 is markedly distinct from the consensus ID3 sequence (Fig. 2A), although the IFM motif that is essential for fast inactivation is partially recognisable (Fig. 2A).

Another distinguishing feature of VGSCs is their selectivity for $Na^+$ [10,11]. Two clusters within the P domains of D3 and D4 are the main determinants of ion selectivity [12]. Substitution of $Lys^{1422}$ and/or $Ala^{1714}$ by glutamic acid in brain type II sodium channels causes increased permeability to calcium [12]. $Lys^{1422}$ is substituted for asparagine at the analogous positions in the SCL11 as well as $hNa_v2.1$ and $mNa_v2.3$ proteins (Fig. 2B). All cloned VGSCs contain $Ala^{1714}$ at equivalent positions, whereas the SCL11 and $mNa_v2.3$ clones contain a serine (Fig. 2B).

VGSC isoforms are differentially distributed among tissues. Brain type I, II, III, NaCh6 and $Na_S$ are expressed in cortex, cerebellum, brain stem, spinal cord and DRG, whereas their transcripts are present at very low levels in muscle cells [2–4,8]. The opposite is true for the muscle VGSC isoforms [5,6]. SCL11 mRNA shows an atypical pattern of expression being present at a high levels in lung, sciatic nerve, pituitary, and bladder and vas deferens (Fig. 3A) and at a low level in brain. Furthermore, using RT-PCR, SCL11 mRNA was shown to be as abundant in spleen and liver as in cortex, cerebellum and hippocampus (Fig. 3B), and the expression level in kidney is comparable to that in heart, skeletal muscle and intestine

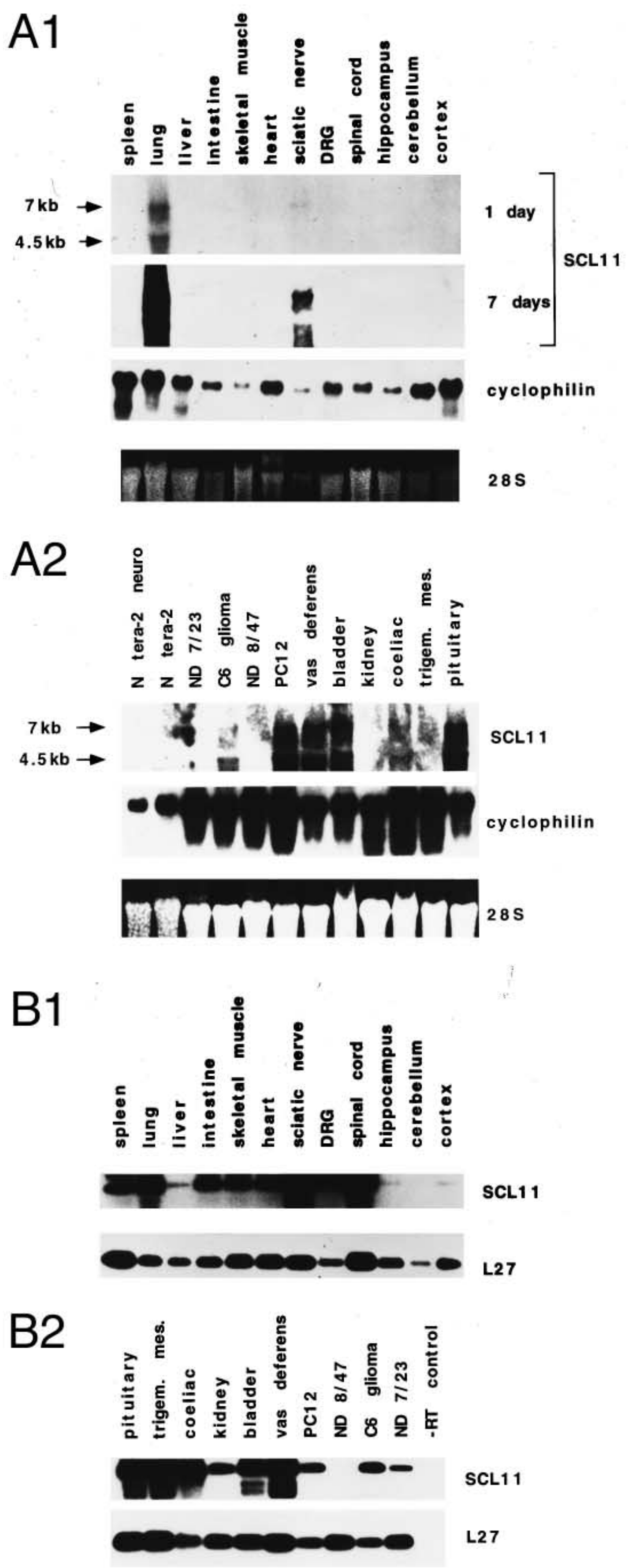

Fig. 3. (A) Northern blot analysis of SCL11 distribution in neonatal rat tissues and cell lines. The estimated size of the SCL11 mRNAs were 4.5 and 7 kb. (B) RT-PCR of random primed cDNA from various new-born rat tissues and cell lines using SCL11-specific primers (upper blot picture) and L-27 ribosomal protein primers (lower blot picture). Control PCR reactions without cDNA were also performed.

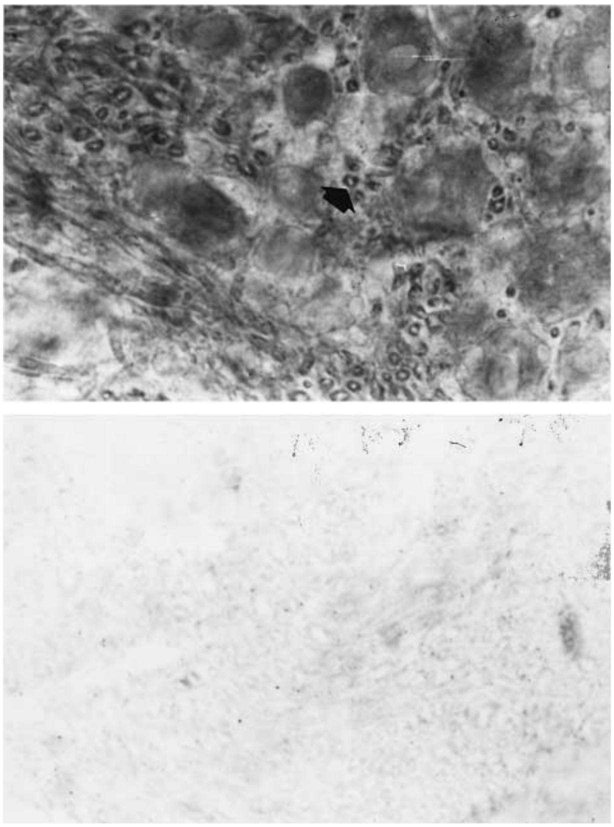

Fig. 4. In situ hybridisation with sections of adult rat neonatal DRG was performed [9] using digoxygenin-labelled cRNA from unique SCL11 sequence. (A) Antisense probe. (B) Sense probe control. Positive signals from Schwann cells are arrowed.

(Fig. 3B). Transcripts of cloned functional VGSCs have not been found by Northern blot or/and RT-PCR in kidney, spleen, lung and liver [4–8].

Could the SCL11 protein be the rat homologue of the mouse and human atypical sodium channels [13–15]? Although mammalian transcripts usually share more than 85–90% amino acid sequence identity for the same protein isoform, SCL11 may be distinct from $hNa_v2.1$ and $mNa_v2.3$ as some proteins encoded by different genes also show 85–90% identity. Thus, brain type II and III VGSCs share approx. 85% overall identity [2,3]. It is therefore difficult to draw any conclusions about the exact relationship of the SCL11, $hNa_v2.1$ and $mNa_v2.3$ proteins.

In summary, the distribution of expression of SCL11 in both neuronal and non-neuronal tissues and the structural features of this protein and other atypical sodium channels suggest that they are neither voltage-gated channels nor do they have motifs that confer sodium selectivity. There is no evidence that they are associated with extracellular rather than intracellular membrane systems. The possible roles of SCL11 and its homologues thus range from their presence as pseudogenes, to a functional role as non-voltage-gated channels of unknown ion selectivity that may contribute to persistent currents.

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