A Novel Cation Channel in Rat Cortical Nerve Terminals, Activated by Decreases in Extracellular Calcium

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Ca^{2+} entry is a critical signal at the synapse where it triggers exocytosis, plasticity, and gene expression. Due to the small volume of and restricted access to the synaptic cleft extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$) has been predicted to fall significantly during synaptic transmission. Recordings in intact cortex and single synapses have demonstrated falls of one third in $[Ca^{2+}]_o$ or $[Ba^{2+}]_o$ following moderate activity It has been proposed that mechanisms to reduce the effect of the fall of $[Ca^{2+}]_o$ at the synaptic cleft have a key role in sustaining neurotransmission during periods of high activity Recently, using patch clamp techniques, we demonstrated that decreasing $[Ca^{2+}]_o$ activates a voltage-gated non-specific cation channel (NSCC) in small cortical nerve terminals. We have expanded our previous work in three areas:

- We have investigated whether physiological decreases in $[Ca^{2+}]_o$ and action-potential-like stimulation can activate significant amounts of this current.
- Detection of changes in $[Ca^{2+}]_o$ occurred via an extracellular sensor that was also modulated by Mg^{2+}, Gd^{3+}, and spermidine and regulated the NSCC via a second messenger. The identity of the Ca^{2+} sensor at the nerve terminal is not known but two candidates are the Ca^{2+} receptor (CaR) and the metabotropic glutamate receptor (mGluR). CaRs in the thyroid, parathyroid, and kidney sense Ca^{2+} in the millimolar range and regulate serum $[Ca^{2+}]$. Remarkably $[Ca^{2+}]_o$ has a similar efficacy to glutamate at some mGluRs, also acting in the millimolar range. The CaR and mGluRs are G-protein-coupled receptors which share up to 20% sequence identity and both have been localized immunocytochemically to nerve terminals. We have investigated the $[Ca^{2+}]_o$ sensing machinery and the NSCC in the nerve terminal to compare them to previously identified entities.
- To characterize and so permit comparison of the NSCC with other cation channels we have used fluctuation analysis to estimate the single channel conductance of the NSCC in nerve terminals.

We predict that the NSCC may act to counter the fall in release probability produced by physiological decreases in $[Ca^{2+}]_o$ at the synaptic cleft, by favoring Ca^{2+} delivery, either by broadening presynaptic action potentials or by raising intracellular Ca^{2+} via Na^+-Ca^{2+} exchange.

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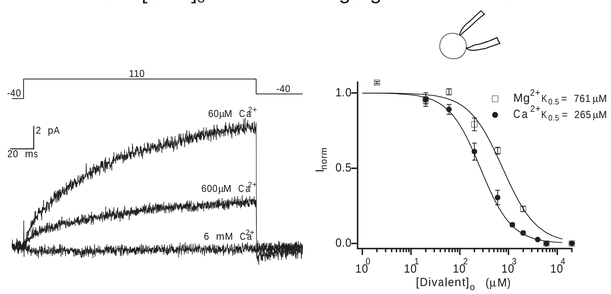
Decreases in $[Ca^{2+}]_o$ reveal a voltage-gated ion current



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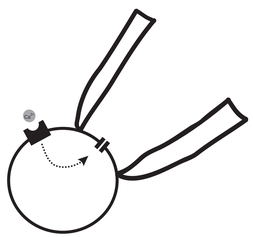
Extracellular Ca^{2+} acts via a second messenger



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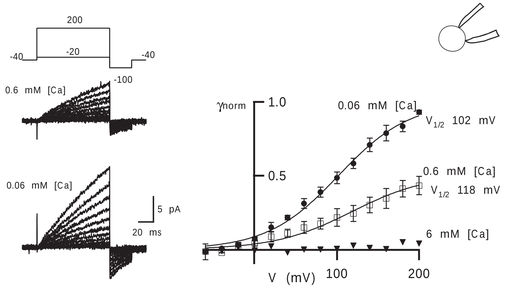
$[Ca^{2+}]_o$ modulates maximal conductance not $V_{1/2}$



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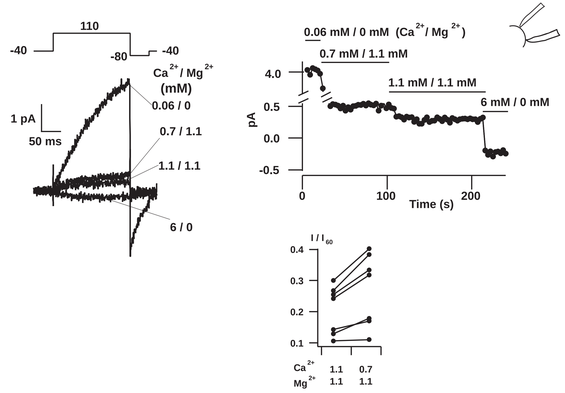
Physiological decreases in $[Ca^{2+}]_o$ activate the NSCC



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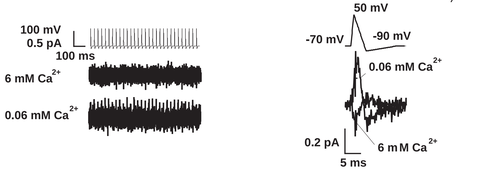
Action potential-like waveforms open the NSCC in low $[Ca^{2+}]_o$



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Comparison between the CaR and the mGluR

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CaR:   30  GDILGGLFPIHFGVAAKDQDLKSRPESVECIRYNFRGFRWLQAMIFAIEEINSDPSLLPNMTLGYRIFDTCNTVSKALEATLSFVAQNKIDSLNLDEFCNCSEHIPST-----IAVVGA 144
           GDI LGGLFPHH       + + K+  E  x      +G   L+AM+FA++ IN+ P LLPN+TLG RI DTC+  + ALE +L+FV +  I+   + C        T     + V+GA
MGluR: 47  GDITLGGLFPVH----GRGSEGKACGELKK-----EKGIHRLEAMLFALDRINNDPDLLPNITLGARILDTCSRDTHALEQSLTFV-RALIEKDGTEVRCGSGGPPITKPERVVGVIGA 156

CaR:   145 TGSGVSTAVANLLGLFYIPQVSYASSSRLLSNKNQYKSFLRTIPNDEHQATAMADIIEYFRWNWVGTIAADDDYGRPGIEKFREEAEER-DICIDFS-ELISQVSDKXXXXXXXXXXNS 262
           +GS VS  VAN+L LF IPQ+SYAS++  LS+ ++Y  F R +P+D +QA AM DI+  +WN+V T+A++  YG  G+E F +++ E   +CI  S ++  +                 S
MGluR: 157 SGSSVSIMVANILRLFKIPQISYASTAPDLSDNSRYDFFSRVVPSDTYQAQAMVDIVRALKWNYVSTLASEGSYGESGVEAFIQKSRENGGVCIAQSVKIPREPKTGEFDKIIKRLLETS 276

CaR:   263 TAKVIVVFSSGPDLEPLIKEIVRRNITGRXXXXXXXXXXXXXXXXMPEVFHVVGDTIGFGLKAGQIPGFREFLQKVHPRKSVHNGFAKEFWEETFNCHLQEGAKGPLPVDTFVRSHEEGGN 382
           A+ I++F++  D+  +++   R N TG                +     V G +    K  + GF +          + N +  EFWE+ F+C L    A   L  + ++
MGluR: 277 NARGIIFANEDDIRRVLEAARRANQTGHFFWMGSDGWGSKSAPVLRLEEVAEGAVTILPKRMSVRGFDRYFSSRTLDNNRRNIWFAEFWEDNFHCKLSRHA---LKKGSHIKK------ 387

CaR:   383 RLLNSSTAFRPLCTGDENISVETPYMDYEHLRISYNVYLAVYSIAHALQDIYTCL-PGRGLFTNGSCADIKKVEAWQVLKHLRHLNFTNNMGEQVTFDECGDLVGNYSIINWHLSPEDG 501
           +L  S+    +  +  E I              YE     +  V  AVY++ HAL  ++  L PGR    G C  +  V+  Q+LK++R+NF+   G VTF+E GD  G Y I  + L  +G
MGluR: 388 ------------CTNRERIGQDSA----YEQEGKVQFVIDAVYAMGHALHAMHRDLCPGR----VGLCPRMDPVDGTQLLKYIRNVNFSGIAGNPVTFNENGDAPGRYDIYQYQL--RNG 485

CaR:   502 SIVFKEVGYYNVYAKKGERLFINEEKILWSGFSREVPFSNCSRDCQAGTRKGIIEGEPTCCFECVECPDGEYSGETDASACDKCPDDFWSNENHTSCIAKEIEFLAWTEPFGIALTLFAV 621
           S  +K +G +          + L + E++ W G  ++++P S CS  CQ G RK  ++G   CC+ C  C  +Y  + D  C CP D    EN TSC     I  L W  P+ +     AV
MGluR: 486 SAEYKVIGSWT------DHLHLRIEEMQWPGSGQQDPRSICSLPCQPGERKKTVKG-MAC CWHCEPCTGYQYY--QVDRYTCKTCPYDMRPTENRTSCQPIPIVKLEWDSPWAVLPLFLAV 596

CaR:   622 LGIFLTAFVLGVFIKFRNTPIVKATNREXXXXXXXXXXXXXXXXXIGEPQDWTCRLRQPAFGISFVLICSICILVKTNRVLLVFEAKIPTSFHRKWWGLNLQFLLVFLCTFMQILIGI 741
           +GI  T FV+  F+++ +TPIVKA+ RE                 I EP  TC LR+  G+  + +L KTNR+ +FE  +  ++      Q  + F+  +QHL  +
MGluR: 597 VGIAATLFVVTFVPVNDTPIVKASGRELSYVLLAGIFLCYATTFLMIAEPDLGTCSLRRIFLGLGMSISYAALLTKTNRIYRIFEQGKRSVSAPRFISPASQLAITFILISLQLGIDVC 716

CaR:   742 WLYTAPPSSYRNWHELEDEII------FITCHEGSLMALGSLIGYTXXXXXXXXXXXXSRKLPENFNEAKFITFSMLIFFIVFISFIPAYASTYGKF------VSAVEVAILAASFGLL 849
           W    P  S  + +  +  +      + C + S ++L  L+GY+                K+R +PE FNEAK I F+ML   +V  S  P  +  +G+      V+ V  L+AS  L
MGluR: 717 WFVVDPSHSVVDPQQRTLDPRFARGVLKC-DISDLSLICLLGYSMLLMVTCTVVAIKTRGVPETFNEAKPIGFTMYTTCIVWLAFIPIFFGTSQSADKLYIQTTTLTVSVSLSLSASVS-L 834

CaR:   850 ACIFFNKVYIILFKPSRNTIEEVRSSTA 877
           ++  KVYIILF P +N  +  RS  A
MGluR: 835 GMLYMPKVYIILFHPEQNVPKRKRSLKA 862
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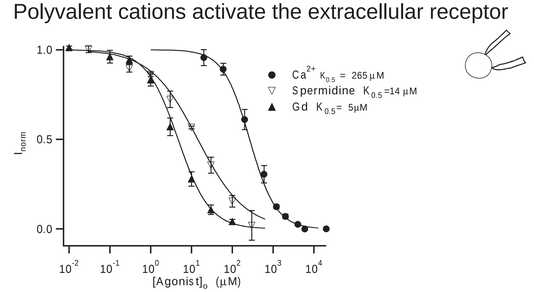
Polyvalent cations activate the extracellular receptor



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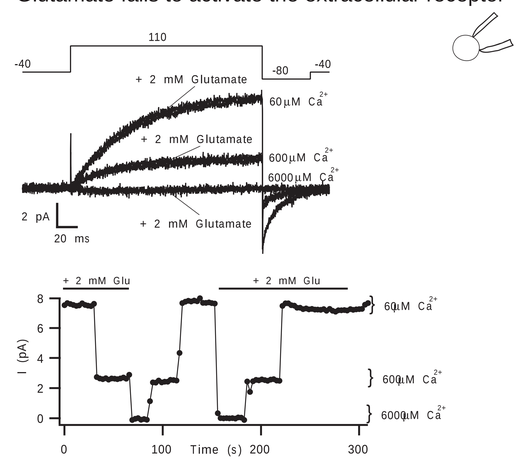
Glutamate fails to activate the extracellular receptor



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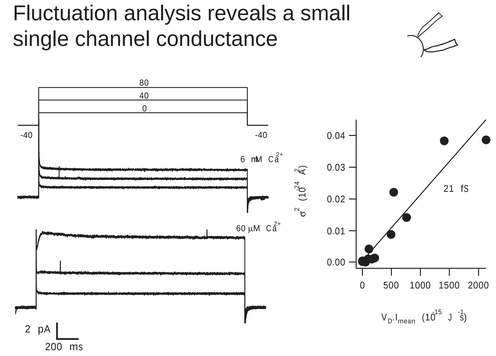
Fluctuation analysis reveals a small single channel conductance



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Conclusions

Since it is well recognized that the release probability of the nerve terminal is proportional to $[Ca^{2+}]_o^4$, physiological reductions of cleft $[Ca^{2+}]$ may significantly reduce synaptic efficacy. Mechanisms that have been postulated to compensate for drops in cleft $[Ca^{2+}]_o$ include release of Ca^{2+} from synaptic vesicles and dissociation of Ca^{2+} from plasma membrane. We have identified with direct electrophysiological recordings a new pathway that can be used by presynaptic terminals to sense and respond to changes in the level of $[Ca^{2+}]_o$. The system revealed by our experiments may be dormant until periods of high activity, when presynaptic depolarizations and associated decreases in cleft $[Ca^{2+}]$ might activate the NSCC.

- Physiological decreases in $[Ca^{2+}]_o$ and action-potential-like stimulation can activate significant amounts of this current.
- The rank order of potency of four polyvalent cations in inhibiting the NSCC at the nerve terminal was Mg^{2+}<Ca^{2+}<spermidine<Gd^{3+}, the same as previously shown for the CaR. The EC_{50} of glutamate for mGluRs is ~10 μM. Glutamate up to 2 mM had no effect on NSCC current activated in low $[Ca^{2+}]_o$ in nerve terminals. These results suggest that the $[Ca^{2+}]_o$ sensor at the nerve terminal is not an mGluR and is likely to be the same as or similar to a CaR.
- Using fluctuation analysis we have estimated the single channel conductance of the NSCC in nerve terminals to be ~21 fS, at least 3 orders of magnitude smaller than identified in other experiments. Nevertheless there are sufficient numbers of these channels at the nerve terminal that this remains the dominant current in our recordings

The NSCC currents may exert an influence on electrical activity or intracellular ion levels. Possible effects include broadening of the presynaptic action potential, or decreasing Ca^{2+} efflux as a result of decreased Na^+/Ca^{2+} exchange and increased intraterminal $[Na^+]$. Thus, the function of the current may be compensatory, allowing active synapses to continue signaling despite lowered cleft $[Ca^{2+}]$.