

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/13623283>

Loss of adenylyl cyclase I activity disrupts patterning of mouse somatosensory cortex

Article in *Nature Genetics* · August 1998

DOI: 10.1038/980 · Source: PubMed

CITATIONS

149

READS

85

10 authors, including:



Wey L Leong

University Health Network

85 PUBLICATIONS 1,623 CITATIONS

SEE PROFILE



Leonard C Schalkwyk

University of Essex

220 PUBLICATIONS 12,591 CITATIONS

SEE PROFILE



Daniel R Storm

University of Washington Seattle

352 PUBLICATIONS 28,967 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Paternal age alters social development in offspring [View project](#)



Investigations of the budding yeast 2-micron plasmid [View project](#)

Loss of adenylyl cyclase I activity disrupts patterning of mouse somatosensory cortex

Raja M. Abdel-Majid¹, Wey L. Leong^{2,3}, Leonard C. Schalkwyk⁴, Donald S. Smallman³, Scott T. Wong⁵, Daniel R. Storm⁵, Alan Fine⁶, Melanie J. Dobson⁷, Duane L. Guernsey³ & Paul E. Neumann^{1,3}

The somatosensory (SI) cortex of mice displays a patterned, nonuniform distribution of neurons in layer IV called the 'barrelfield' (ref. 1). Thalamocortical afferents (TCAs) that terminate in layer IV are segregated such that each barrel, a readily visible cylindrical array of neurons surrounding a cell-sparse center, represents a distinct receptive field. TCA arbors are confined to the barrel hollow and synapse on barrel-wall neurons whose dendrites are oriented toward the center of the barrel². Mice homozygous for the barrelless (*brl*) mutation, which occurred spontaneously in ICR stock at Université de Lausanne (Switzerland), fail to develop this patterned distribution of neurons, but still display normal topological organization of the SI cortex³. Despite the absence of barrels and the overlapping zones of TCA arborization, the size of individual whisker representations, as judged by 2-deoxyglucose uptake, is similar to that of wild-type mice. We identified adenylyl cyclase type I (*Adcy1*) as the gene disrupted in *brl* mutant mice by fine mapping of proximal chromosome 11, enzyme assay, mutation analysis and examination of mice homozygous for a targeted disruption of *Adcy1*. These results provide the first evidence for involvement of cAMP signalling pathways in pattern formation of the brain.

We constructed a high-resolution genetic map of the region around the *brl* locus on proximal chromosome 11 (ref. 3) using intercross and backcross offspring (Fig. 1). Candidate genes Ca^{2+} /calmodulin protein kinase type II (*Camk2b*) and leukaemia inhibitory factor (*Lif*), from the region of conserved synteny with human chromosome 22 (ref. 4), were excluded on the basis of sequence analysis and map position. To identify other candidates, we made a sequence tagged site (STS)-content map of a yeast artificial chromosome (YAC) contig spanning the *brl* critical region (Fig. 1). Four genes (*Adcy1*, *Gk*, *Igfbp1*, and *Igfbp3*) from the region of conserved synteny with human chromosome 7 (refs 4,5) were placed on this physical map.

The physical map identified *Adcy1* as a candidate gene for *brl*. *Adcy1* (EC 4.6.1.1) is a membrane-bound enzyme that catalyzes the formation of cAMP, an important second messenger. *Adcy1* is neurospecific and is expressed in areas of the brain that are associated with neuroplasticity, such as the hippocampus and cerebral cortex⁶. *Adcy1* activity is directly stimulated by Ca^{2+} and calmodulin *in vivo* with half-maximal stimulation at 150 nM free Ca^{2+} (refs 7,8). Although it is not stimulated by activation of G_s -coupled receptors alone *in vivo*, it is stimulated by receptor activation when paired with Ca^{2+} (ref. 9).

Basal adenylyl cyclase activity in brain membrane preparations from *brl/brl* mice was one-half that of wild-type mice, but this difference was not statistically significant (Fig. 2). There was a significant difference in enzyme activity in the presence of Ca^{2+} and calmodulin ($t=5.7$, $\text{df}=3.0$, $P=0.01$); adenylyl cyclase activity

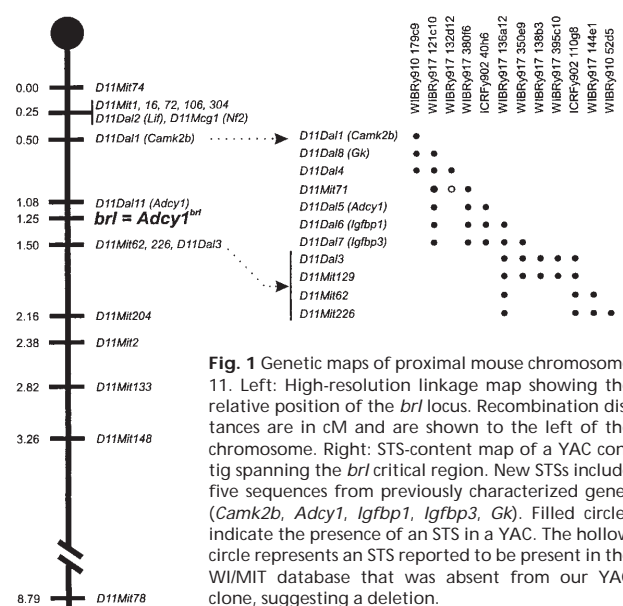


Fig. 1 Genetic maps of proximal mouse chromosome 11. Left: High-resolution linkage map showing the relative position of the *brl* locus. Recombination distances are in cM and are shown to the left of the chromosome. Right: STS-content map of a YAC contig spanning the *brl* critical region. New STSs include five sequences from previously characterized genes (*Camk2b*, *Adcy1*, *Igf1bp1*, *Igf1bp3*, *Gk*). Filled circles indicate the presence of an STS in a YAC. The hollow circle represents an STS reported to be present in the WI/MIT database that was absent from our YAC clone, suggesting a deletion.

increased sixfold in wild-type mice, whereas no increase in enzyme activity was observed in mutants. Comparable results were reported in *Adcy1* knockout mice¹⁰. Sequencing of *Adcy1* cDNA from B6 and *brl* mutant mice revealed two differences: a polymorphism in the 5' end of the coding region (*D11Dal11*) found in other ICR-derived lines that mapped proximal to *brl*, and an early retrotransposon (ETn) insertion that co-segregated with the *brl* phenotype (Fig. 3). Insertion of an ETn has been associated with loss-of-function mutations in other genes due to alter-

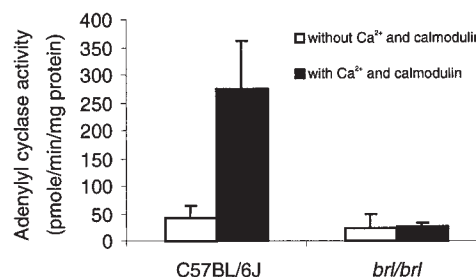


Fig. 2 Adenylyl cyclase activity in two-week wild-type and *brl* mutant mice. Enzyme activity in brain membrane protein fractions was measured in the absence (white bars) or presence (black bars) of free Ca^{2+} (estimated to be 300–500 nM) and calmodulin.

Departments of ¹Anatomy & Neurobiology, ²Surgery, ³Pathology, ⁶Physiology & Biophysics, ⁷Biochemistry, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7. ⁴Max-Planck-Institut für Molekulare Genetik, Abteilung Lehrach, Berlin-Dahlem, Germany. ⁵Department of Pharmacology, University of Washington, Seattle, Washington, USA. R.M.A. and W.L.L. contributed equally to this work. Correspondence should be addressed to P.E.N. e-mail: neumannp@tupdean1.med.dal.ca.

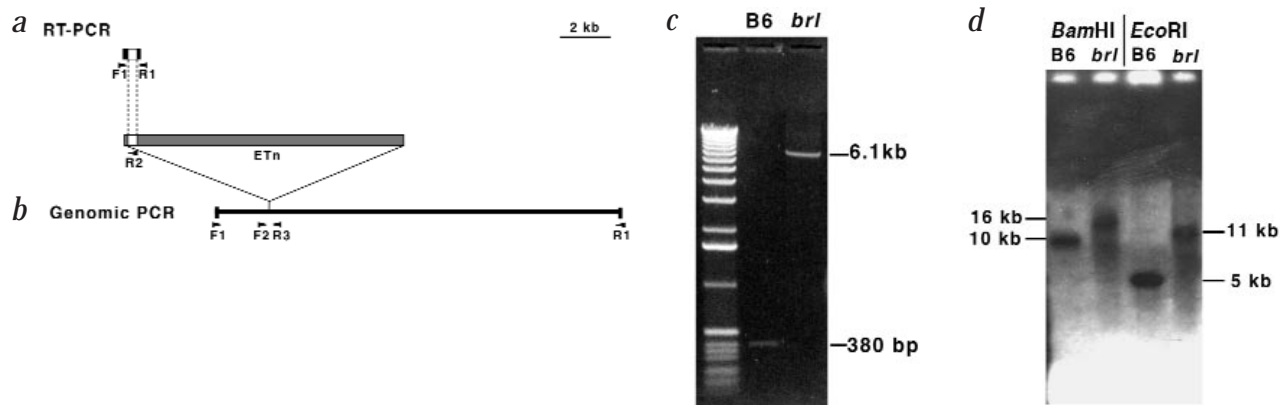


Fig. 3 Mutational analysis of *Adcy1* in *brl* mutant mice. **a**, RT-PCR of *brl* brain RNA using primers F1 and R1 yielded an aberrant amplicon with a 183-bp insert (white box) after nucleotide 2158 of our partial cDNA sequence. The sequence of the 183-bp insert was identical to part of the long terminal repeat (LTR) of ETn. **b**, Extended genomic PCR and DNA sequencing with primers F1 and R2 of *brl* genomic DNA was used to amplify the 5' end of the intron and to develop primer F2, which was used with primer R1 to amplify the 3' end of the intron from B6 mice. **c**, Primers F2 and R3 were used to amplify the inserted sequence in *brl* mice. The amplicon from *brl* genomic DNA is approximately 5.7 kb larger than the amplicon from B6, consistent with the presence of a complete ETn. **d**, Southern-blot analysis of genomic DNA confirmed the presence of a 5.7-kb insert in *brl* mutant mice. The probe was generated from PCR of B6 genomic DNA with primers F2 and R3 (see Methods). There were no *Bam*HI or *Eco*RI restriction sites in ETn.

native splicing and premature termination of transcripts^{11,12}. The causal relationship between *Adcy1* and the *brl* phenotype was confirmed by the absence of barrels in mice homozygous for a targeted disruption of *Adcy1* (Fig. 4). In contrast, mice homozygous for a targeted disruption of *Adcy8*, another neurospecific Ca^{2+} /calmodulin-responsive adenylyl cyclase gene, have barrels indistinguishable from wild-type mice (Fig. 4).

The cAMP signaling system plays an important role in neuroplasticity in both invertebrates and vertebrates. In *Drosophila melanogaster*, disruption of genes involved in cAMP pathways, including adenylyl cyclase type I (*rutabaga*), impairs learning and memory¹³. In *Aplysia*, calmodulin-sensitive adenylyl cyclase may play a role in the association of conditioned and unconditioned stimuli in short-term and long-term sensitization, perhaps through synergistic activation by serotonin and Ca^{2+} (refs 14,15). Similarly, disruption of *Adcy1* in mice alters performance in the Morris water task and depresses long-term potentiation⁸.

A barrelless phenotype similar to that observed in *Adcy1*^{brl} mutants has also been found in mice homozygous for a targeted disruption of monoamine oxidase A (*Maoa*; refs 16,17). In these knockout mice, a ninefold increase in serotonin (5-HT) concentrations in the brain has been implicated in the pathogenesis of the barrelless phenotype. Early depletion of 5-HT delays the development of barrels, reduces their size and decreases the growth of TCAs (ref. 18). The shared phenotypic features of

Maoa and *Adcy1* mutants suggest the involvement of serotonergic and cAMP signaling pathways in the formation of barrels. Thalamic neurons transiently express the serotonin transporter¹⁹ and the 5-HT_{1B} receptor²⁰ during the perinatal period, coincident with barrel formation. As the 5-HT_{1B} receptor decreases adenylyl cyclase activity²¹, both *Maoa* and *Adcy1* loss-of-function mutations should reduce cAMP levels and cAMP-dependent protein kinase (PKA) activity in TCAs. Decreased *Adcy1* activity may lead to absence of barrels through reduced glutamate release, as 5-HT_{1B} mediates serotonin's presynaptic inhibition of thalamocortical transmission²² and NMDA receptor antagonists produce a barrelless phenocopy²³. Alternatively, the barrelless phenotype may result from loss of *Adcy1* in cortical neurons; because cAMP directly stimulates neurite outgrowth *in vitro*²⁴, any contribution of cortical neurite outgrowth to barrel formation may be impaired. The cAMP second messenger system may influence barrel formation and other forms of neuroplasticity in SI cortex through phosphorylation of protein targets and/or changes in gene expression.

The demonstration that the barrelless phenotype results from disruption of adenylyl cyclase type I provides the first evidence that cyclic nucleotide signal transduction systems are important for barrel formation. As many mechanisms of cortical pattern formation are not specific to the barrel cortex, cAMP may play a more general role in cortical specification.

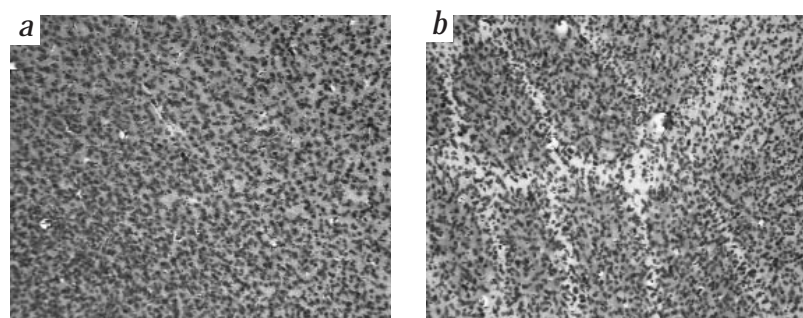


Fig. 4 Digitized images of layer IV of SI cortex from seven-month *Adcy1* (**a**) and *Adcy8* (**b**) knockout mice. *Adcy1* knockouts display a barrelless phenotype, whereas *Adcy8* knockouts have the normal pattern of barrels separated by cell-sparse septa. Sections (50 μm) were cut parallel to the pial surface overlying SI and stained with cresyl violet (Nissl).

Methods

Linkage map. Independent linkage maps for the intercross ($n=469$) and backcross ($n=607$) populations were constructed and combined by calculating the weighted averages. In addition to the marker *D11Mcgl* (ref. 25), thirteen of the microsatellites from proximal chromosome 11 in the WI/MIT database (<http://www-genome.wi.mit.edu>; refs 4,26) were found to be polymorphic between *brl/brl* and C57BL/6J inbred mice. PCR products were separated in 15% polyacrylamide gels and visualized by a reducing silver stain method. *Camk2b* was mapped using an amplicon length polymorphism in the 3' untranslated region (*D11Dal1*), which corresponds to nucleotides 3203–3499 in the cDNA sequence. The C57BL/6J-derived allele was 14 bases longer than the allele in *brl* mutant mice, which

was identical to the published sequence. *Lif* was mapped using a restriction fragment length polymorphism (RFLP, *D11Dal2*); an additional *AluI* restriction site was found in the second intron of the allele in *brl* mutant mice. Primers that correspond to bases 2493 and 3723 in the genomic sequence were used to generate amplicons for restriction digestion. After *Adcy1* was physically mapped to this genomic region, it was placed on the genetic map using RFLP (*D11Dal1*); a G→T transition at nucleotide 143 in the cDNA sequence of ICR-derived lines, including barrelless, is associated with loss of a *BanI* restriction site. *D11Dal1* primers correspond to nucleotides 99 and 435 in the cDNA sequence.

Physical map. The YAC contig was initiated by screening two YAC libraries, ICRFy902 (ref. 27) and WIBRy910 (ref. 28) with *D11Dal1* and *D11Mit226*, extended by screening these libraries and the WIBRy917 library²⁹ with STSs from YAC end-clones (*D11Dal3* and *D11Dal4*) and completed after the addition of Whitehead Institute contig WC11.0. All YAC clones were obtained from the RZPD, Ressourcenzentrum/Primaerdatenbank des deutschen Humangenomprojektes. PCR products were visualized using 1.5–2.5% agarose gels stained with ethidium bromide.

Generation of knockout mice. *Adcy1* knockouts⁸ used in this study were taken from a congenic inbred strain that was produced by backcrossing to 129/JR2448 inbred mice for 12 generations. Targeted disruption of *Adcy8* was achieved using the method described for the *Adcy1* knockout⁸. Briefly, an isogenic *Adcy8* clone was isolated from a 129/Sv murine genomic library (Stratagene). A 6.2-kb fragment of *Adcy8*, which included DNA sequences 4.7-kb upstream and 1.8-kb downstream of the translational start codon, were replaced by a *neo^r* cassette. To enrich for homologous recombinants, a herpes simplex viral thymidine kinase (TK) gene was ligated to the 3' end of the construct. ES cells containing the disrupted *Adcy8* gene were injected into blastocysts. Chimeric mice were obtained and bred for germline propagation. Disruption of *Adcy8* was confirmed by PCR analysis. Ca^{2+} -stimulated adenylyl cyclase activity in the neocortex and hypothalamus were reduced 30% and 100%, respectively.

Adenylyl cyclase assay. Membrane protein fractions were prepared from whole brains of two-week mice as described³⁰. Protein concentration was determined using the BCA Protein Assay Reagent (Pierce). The enzyme assay was performed, with and without free Ca^{2+} (estimated to be 300–500 nM) and calmodulin (2.4 μ M), in a 250 μ l volume containing membrane preparation (30 μ g), Tris-HCl (20 mM, pH 7.4), ATP (1 mM), $MgCl_2$ (5 mM), 3-isobutyl-1-methyl-xanthine (1 mM), EDTA (1 mM), BSA (0.1%), creatine phosphate (20 mM), creatine phosphokinase (60 U/ml),

myokinase (20 U/ml) and adenosine deaminase (8 U/ml). Free Ca^{2+} concentration was estimated using the Bound and Determined program. The assay was incubated at 30 °C for 20 min, and the reaction was stopped by the addition of 2% SDS (250 μ l) followed by boiling for 2 min. A cAMP enzyme-immunoassay system (Amersham) was used to determine cAMP levels.

RT-PCR. RNA was prepared from brains of six-week-old mice, reverse transcribed into cDNA and PCR amplified by standard techniques. The primers were F1, 5'-GCTATCCTGCTGTTCTCATGC-3' and R1, 5'-GGCAGGAGATTGAAGAGGATC-3'. Cycling parameters were 94 °C (3 min), [94 °C (45 s)–57 °C (45 s), 72 °C (90 s)] \times 35, 72 °C (5 min), 15 °C. Amplicons were analysed by 1.5% agarose gel electrophoresis and DNA sequencing using a Cyclist *Taq* DNA sequencing kit (Stratagene).

Extended genomic PCR. GeneAmp XL PCR kit (Perkin-Elmer) was used for extended PCR on genomic DNA templates as detailed by the manufacturer. Cycling parameters were 94 °C (3 min), [94 °C (45 s), 62 °C (45 s), 72 °C (8 min)] \times 15, [94 °C (45 s), 62 °C (45 s), 72 °C (8 min+10 s increment/cycle)] \times 20, 72 °C (10 min), 15 °C. The following primers (Fig. 3b) were used to generate extended PCR products: F1, R1 (see above); F2, 5'-TCCCAA-CCCAAGTTGCCCCAGA-3'; R2, 5'-GCTCCGATAGTCCGATACGA-3'; and R3, 5'-TACAGTGGACGGACAGTCCA-3'. Amplicons were analysed by 0.8% agarose gel electrophoresis and DNA sequencing as above.

Southern-blot analysis. Genomic DNA (10 μ g) digested with *Bam*HI and *Eco*RI was fractionated by electrophoresis in a 0.6% agarose gel and transferred to a charged nylon membrane by Southern blotting. The probe was generated by PCR using primers F2 and R3 on B6 genomic DNA. [³²P]-labelled by random priming (1.5 \times 10⁶ cpm/100 ng) and used in a standard hybridization reaction. The blot was visualized using autoradiography.

GenBank accession numbers. *Adcy1* coding sequence (partial) from C57BL/6J inbred mice, AF053980; sequence of 183 bp insert in *Adcy1* cDNA from *brl* mutant mice, AF053979; sequence of an ETn, U06639; mouse *Camk2b* cDNA, X63615; genomic sequence of mouse *Lif*, M63419.

Acknowledgements

We thank A. Blackadar, B. Cusack and K. Furue for technical assistance, and H. Lehrach and F.B. Palmer for support. This work has been supported by the Medical Research Council of Canada (grant MT-12941).

Received 23 March; accepted 27 May, 1998.

- Woolsey, T.A. & Van der Loos, H. The structural organization of layer IV in the somatosensory region of mouse cerebral cortex: The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* **17**, 205–242 (1970).
- Woolsey, T.A., Dierker, M.L. & Wann, F. Mouse Sml Cortex: Qualitative and quantitative classification of Golgi-impregnated Barrel neurons. *Proc. Natl Acad. Sci. USA* **72**, 2165–2169 (1975).
- Welker, E. et al. Modified tactile processing in somatosensory cortex of a new mutant mouse, barrelless. *Science* **271**, 1864–1867 (1996).
- Copeland, N.G. et al. Genome Maps IV. *Science* **262**, 67–82 (1993).
- Edelhoff, S., Villacres, E.C., Storm, D.R. & Distche, C.M. Mapping of adenylyl cyclase genes type I, II, III, IV, V, VI in mouse. *Mamm. Genome* **6**, 111–113 (1995).
- Xia, Z., Choi, E.J., Wang, F., Blazynski, C. & Storm, D.R. Type I Calmodulin-Sensitive Adenylyl cyclase is neural specific. *J. Neurochem.* **60**, 305–311 (1993).
- Wu, Z., Wong, S.T. & Storm, D.R. Modification of the Ca^{2+} /Calmodulin sensitivity of the type I adenylyl cyclase by mutagenesis of its calmodulin binding domain. *J. Biol. Chem.* **268**, 23766–23768 (1993).
- Wu, Z.L. et al. Altered behavior and long-term potentiation in type I adenylyl cyclase mutant mice. *Proc. Natl Acad. Sci. USA* **92**, 220–224 (1995).
- Wayman, G.A., et al. Synergistic activation of the type I adenylyl cyclase by Ca^{2+} and G_{α} -coupled receptors in vivo. *J. Biol. Chem.* **269**, 25400–25405 (1994).
- Villacres, E.C. et al. Developmentally expressed Ca^{2+} -sensitive adenylyl cyclase activity is disrupted in the brains of type I adenylyl cyclase mutant mice. *J. Biol. Chem.* **270**, 14352–14357 (1995).
- Steinmeyer, K. et al. Inactivation of muscle chloride channel by transposon insertion in myotonic mice. *Nature* **354**, 304–308 (1991).
- Mitrea, K. et al. Disruption of the murine p53 gene by insertion of an endogenous retrovirus-like element (ETn) in a cell line from radiation-induced osteosarcoma. *Virology* **200**, 837–841 (1994).
- Davis, R.L. Physiology and biochemistry of *Drosophila* learning mutants. *Physiol. Rev.* **76**, 299–317 (1996).
- Byrne, J.H. et al. Neural and molecular basis of nonassociative and associative learning in Aplysia. *Ann. N. Y. Acad. Sci.* **627**, 124–149 (1991).
- Yovell, Y. & Abrams, W. Temporal asymmetry inactivation of Aplysia adenylyl cyclase by calcium and transmitter may explain temporal requirements of conditioning. *Proc. Natl Acad. Sci. USA* **89**, 6526–6530 (1992).
- Cases, O. et al. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science* **268**, 1763–1766 (1995).
- Cases, O. et al. Lack of barrels in somatosensory cortex of Monoamine Oxidase A-deficient mice: role of a serotonin excess during the critical period. *Neuron* **16**, 297–307 (1996).
- Bennett-Clarke, C.A., Leslie, M.J., Lane, R.D. & Rhoades, R.W. Effect of serotonin depletion on vibrissa-related patterns of thalamic afferents in the rat's somatosensory cortex. *J. Neurosci.* **14**, 7594–7607 (1994).
- Lebrand, C. et al. Transient uptake and storage of serotonin in developing thalamic neurons. *Neuron* **17**, 823–835 (1996).
- Bennett-Clarke, C.A., Leslie, M.J., Chiala, N.L. & Rhoades, R.W. Serotonin 1B receptors in the developing somatosensory and visual cortices are located on thalamocortical axons. *Proc. Natl Acad. Sci. USA* **90**, 153–157 (1993).
- Bouhelal, R., Smounya, L. & Bockaert, J. 5-HT_{1B} receptors are negatively coupled with adenylyl cyclase in rat substantia nigra. *Eur. J. Pharmacol.* **151**, 189–196 (1988).
- Rhoades, R.W., Bennett-Clark, C.A., Shi, M.-Y. & Mooney, R.D. Effects of 5-HT on thalamocortical synaptic transmission in the developing rat. *J. Neurophysiol.* **72**, 2438–2450 (1994).
- Mitrovic, N., Mohajeri, H. & Schachner, M. Effects of NMDA receptor blockade in the developing rat somatosensory cortex on the expression of the glia-derived extracellular matrix glycoprotein tenascin-C. *Eur. J. Neurosci.* **8**, 1793–1802 (1996).
- Song, H.-J., Ming, G.-I. & Poo, M.-m. cAMP-induced switching in turning direction of nerve growth cones. *Nature* **388**, 275–279 (1997).
- Claudio, J.O., Malo, D. & Rouleau, G.A. The mouse neurofibromatosis type 2 gene is highly conserved. *Genomics* **21**, 437–439 (1994).
- Dietrich, W.F. et al. A comprehensive genetic map of mouse genome. *Nature* **380**, 149–152 (1996).
- Larin, Z., Monaco, A.P., Meier-Ewert, S. & Lehrach, H. Construction and characterization of yeast artificial chromosome libraries from the mouse genome. *Methods Enzymol.* **225**, 623–637 (1993).
- Kusumi, K., Smith, J.S., Segre, J.A., Koos, D.S. & Lander, E.S. Construction of a large-insert yeast artificial chromosome library of the mouse genome. *Mamm. Genome* **4**, 391–392 (1993).
- Haldi, M.L. et al. A comprehensive large-insert yeast artificial chromosome library for physical mapping of the mouse genome. *Mamm. Genome* **7**, 767–769 (1996).
- Johnson, R.A. & Sutherland, E.W. Detergent-dispersed adenylyl cyclase from rat brain. *J. Biol. Chem.* **248**, 5114–51121 (1973).