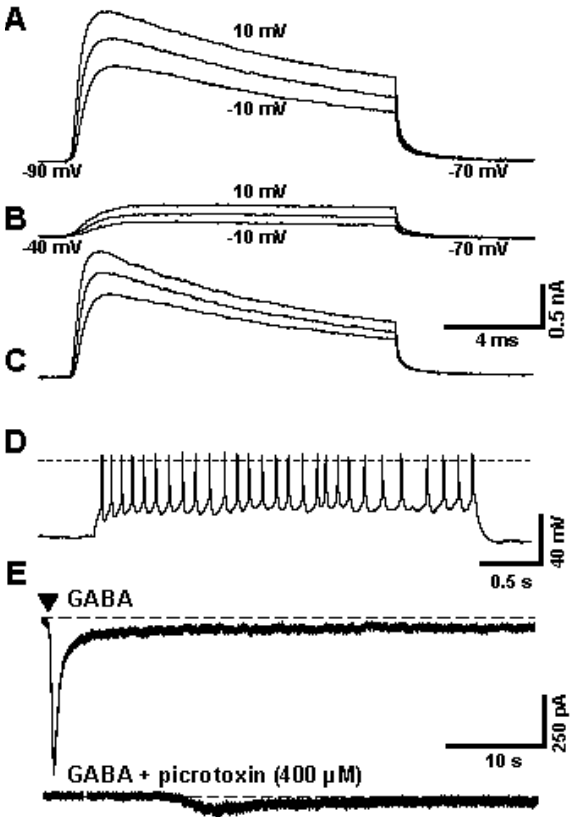
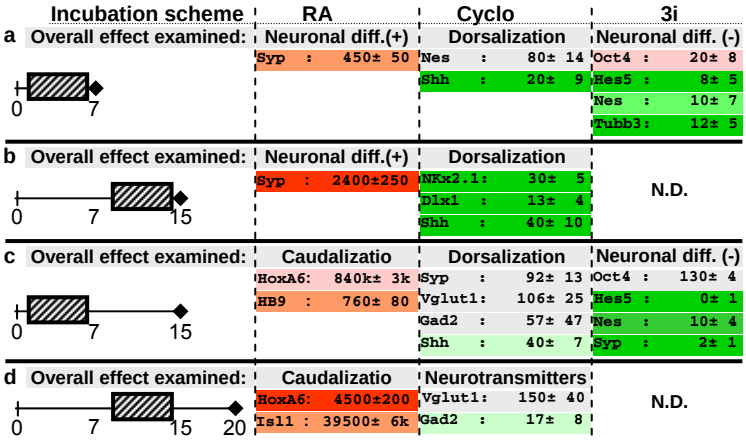
Figure S1: Additional electrophysiological data



Cells were differentiated on glass cover slips towards the neuronal lineage for 20-24 days and then placed into a temperature controlled recording chamber for whole cell patch-clamp studies. A. Whole cell voltage clamp recording: Overall K+ currents were pharmacologically isolated from Na+ currents in the presence of 500 nM tetrodotoxin. The 120 ms cycle involved an initial hyperpolarization phase at -90 mV, a 20 ms ramping step (to -10 mV, 0 mV, 10 mV) and a -70mV repolarization step as indicated. B. A subgroup of K+ channels with slow activation kinetics and no spontaneous inactivation was triggered, when cells were held at -40 mV before the depolarizing voltage step. C. Mathematical subtraction of B from A indicates another group of K channels with fast activation, and spontaneous inactivation characteristics. The scale bars indicate time and current dimensions for figures A-C. D. Action potentials were evoked by triggering with a defined outward current of 20 pA. The trace indicates the voltage signal obtained in current clamp mode. Data are representative for 10 cells from 2 differentiations. Reversal of the current (-10 pA) showed only passive biophysical membrane properties on the voltage trace. Injection of a smaller current (5 pA) triggered action potentials with lower frequency (in 15 out of 19 cells; not shown). E. Current traces were recorded after stimulation of neurons with GABA in the presence or absence of the specific antagonist picrotoxin. The scale bars represent the current and time dimensions of the experiment. Data are representative for $N \geq 10$ neurons (agonists) and $n = 3$ for antagonists.

Figure S2: Additional data (standard deviations for figure 3)



Cultures of mESC were neuronally differentiated for 7, 15 or 20 days as indicated in a-d. They were exposed to retinoic acid (RA), cyclopamine (Cyclo) or 3i for the time periods indicated by the hatched boxes. RNA was isolated at the end of the incubations and used for quantitative RT-PCR analysis of selected differentiation and patterning markers. The data indicate relative expression levels (in %) compared to untreated cultures at the same time point and are means ± SD from two to three cultures for each treatment and exposure schedule. Non-significant expression differences with untreated differentiation cultures are indicated by grey shading; Up- and downregulations are color-coded: $p < 0.05$ (light green,down), , down),$p < 0.01$(green, down), $p < 0.001$ (dark green, down), $p < 0.05$ (light red, up), $p < 0.01$ (red, up), $p < 0.001$ (dark red, up). Headings indicate the overall biological effect, such as accelerated neuronal differentiation (e.g. Neuronal diff. (+)), altered patterning (e.g. Caudalization) or evidence for altered cell composition (e.g. Neurotransmitters or Diff. block). Names are the official gene names, apart from the following: Vglut1 = Slc17a7, Oct4 = Pou5f1, HB9 = Mnx1. N.D.: not determined, as cells did morphologically not differentiate.

Figure S3: Complete listing of the assignment of genes to the different clusters

The genes are named according to their official NCBI PubMed annotation and can be used without further modification for different bio-informatic analyses, such as testing for overrepresented gene ontologies with g:Profiler (http://biit.cs.ut.ee/gprofiler/). Note that the separator (komma, or blank) needs to be adjusted, depending on the software used.

Cluster Ia

0610005K03Rik, 0610039J04Rik, 1110002N22Rik, 1110006G06Rik, 1110030K22Rik, 1110033J19Rik, 1190003J15Rik, 1190005I06Rik, 1300013J15Rik, 1500001L15Rik, 1520401A03Rik, 1600021C16Rik, 1700007J06Rik, 1700012H05Rik, 1700013B16Rik, 1700013H19Rik, 1700019D03Rik, 1700019H03Rik, 1700028N11Rik, 1700029P11Rik, 1700071K01Rik, 1810008K03Rik, 1810015A11Rik, 2010001H14Rik, 2010111I01Rik, 2200001I15Rik, 2310003C23Rik, 2310008H09Rik, 2310042N02Rik, 2310045B01Rik, 2310073E15Rik, 2410004A20Rik, 2410004F06Rik, 2410008K03Rik, 2410012C07Rik, 2410018M08Rik, 2410081M15Rik, 2410116G06Rik, 2410127L17Rik, 2600005C20Rik, 2610103J23Rik, 2610305D13Rik, 2700038I16Rik, 2810410M20Rik, 4432409M07Rik, 4632409L19Rik, 4732467B22, 4922503E23Rik, 4930447C04Rik, 4930504E06Rik, 4930539E08Rik, 4930572J05Rik, 4931417G12Rik, 4933405K07Rik, 4933409I22, 4933437F05Rik, 5430425C04Rik, 5730436H21Rik, 5730593N15Rik, 5830436D01Rik, 5830482F20Rik, 5830482G23Rik, 6030457N17Rik, 6330406L22Rik, 6330579B17Rik, 8430410A17Rik, 9030407H20Rik, 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Cluster Ib

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Cluster IIa

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Cluster IIb

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Cluster IIIa

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Cluster IIIb

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Cluster IV

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Cluster V

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Figure S4: Listing and assignment of the genes graphed in figure 5

All regulated genes were individually examined on the basis of the pertinent literature for identification of those involved in chromatin structure and epigenetic regulation. The genes were grouped into categories as indicated in the first column. Further functional information was added in the last column, where this was unambiguously available. Genes related to cell cycle, DNA replication and DNA repair were extracted from the relevant GO categories.

	cluster	accession	symbol	comment	Ref.
chromosome	Ib	NM_001077712.1	Stag2 (SA2)	cohesin subunit	1
		NM_021886.1	Cenph	kinetochor organization	2
		NM_009282.2	Stag1 (SA1)	cohesin subunit	1
		NM_025795.2	Ncaph2 (H2)	condensin subunit	1
		NM_012039.1	Zw10	kinetochor organization	4
		NM_016692.1	Incenp	centromere protein	2
		NM_027263.1	Apitd1 (Cenps)	Kinetochor organization	6
		NM_019710.1	Smc1a	cohesin subunit	1
		NM_027263.1	Apitd1	centromere protein	
		NM_145924.2	Cenpi	centromere protein	3
		NM_025495.1	Cenpp	centromere protein	6
		XM_127861.2	Cenpj	centromere protein	
	IIb	NM_080470.1	Smc1b	cohesin subunit (meiosis)	1
		NM_016964.1	Stag3 (SA3)	cohesin subunit (meiosis)	8
heterochromatin	Ib	NM_001076789.1	Cbx5 (HP1 alpha)	heterochromatin structure	4
		NM_172663.2	Epc2	enhancer of polycomb	10
		NM_025900.1	Dek		11
	IIa	NM_007622.2	Cbx1 (HP1 beta)	heterochromatin structure	4
		NM_009122	Satb1	x-inactivation regulates ESC differentiation and nanog expression	12
		NM_010470.1	Hp1bp3	heterochromatin protein 1, binding protein 3	
euchromatin	Ia	NM_178017.1	Hmgb2l1 (Hmgxb4)	HMG box domain containing 4	
	Ib	NM_016660.1	Hmga1	Highly expressed during embryonic development	5
		NM_008252.2	Hmgb2		6
		NM_016710.1	Nsbp1		7
	IIa	NM_016957.3	Hmgn2		8
	IIIa	NM_175074.1	Hmgn3		8
chromatin remodeling	Ia	NM_009211.1	Smarcc1 (BAF155)	Component of esBAF	17
		XM_132597.3	Smarcad1 (Etl1)		9
		NM_009530.1	Atrx	SNF2-related helicase, Interneuronal survival	19, 20
		NM_009031.2	Rbbp7 (Rbap46)	Histone chaperone	21
		NM_001081267.1	Rsf1	Spacing factor	21, 22
		NM_024184.1	Asf1b	nucleosome assembly	21
		NM_015781.2	Nap1l1	nucleosome assembly	21, 23
		NM_013733.2	Chaf1a	nucleosome assembly	21
		NM_025541.2	Asf1a	nucleosome assembly	21

	IIa	NM_020618.3	Smarce1 (BAF57)	SWI/SNF-related	10
		NM_053123.3	Smarca1 (Snf2L)	SWI/SNF-related, specific for postnatal neurons and in adult brain	25
	IIIa	NM_011416.2	Smarca2 (brahma, Snf2a)	SWI/SNF-related	10
	IIIb	NM_133741.1	Snrk	SNF-1 related kinase	26
histone modification	Ia	NM_011791.2	Ash2l	H3K4Me	11
		NM_001035123.1	Setd6	Set-domain containing protein	
		NM_021876.1	Eed	H3K27Me	11
		NM_017479	Myst4 (querkopf)	histone acetyl transferase, cerebral cortex development	28
		NM_173001.1	Jmjd1a	H3K9Me1 and 2 demethylation	11
		NM_144787	Jmjd2c	H3K36Me1, 2 and 3 demethylation	11
		NM_029441.1	Cdyl2	chromodomain potein	29
		NM_009881	Cdyl	chromodomain potein	29
	Ib	NM_199196.1	Suz12	PRC1 associated protein	4
		NM_007971.1	Ezh2	K27Me3	11
		NM_008739	Nsd1	H3K36Me, H4K20Me	11
		NM_145414.1	Nsun5	Putative methyltransferase	12
		NM_172567.1	Mettl2	Putative methyltransferase	31
		NM_144918.1	Smyd5	Putative methyltransferase, Set-domain containing	
		NM_172545.1	Ehmt1	H3K9Me2	11
		NM_133740.1	Prmt3	arginine methylation	32
		NM_145404.1	BC006705 (PRMT7)	arginine methylation	13
		NM_178891.4	Prmt6	H3R2Me2	14
		NM_007415.2	Parp1	poly-(ADP-ribosyl)ation	15
		NM_009483.1	Utx	lysine demethylation, H3K27Me	16
		NM_008228.1	Hdac1	histone deacetylation	17
		NM_019812.1	Sirt1	histone deacetylation	17
		NM_021788.1	Sap30	histone deacetylation	38
		NM_181586.2	Sirt6	histone deacetylation	17
		NM_177239.2	Mysm1	histone de-ubiquitination	39
		NM_027432.3	Wdr77 (MEP-50)	interaction with H2A	18
		NM_026539.1	Chd1l	chromodomain protein	19
		NM_007690.1	Chd1	chromodomain protein	19
		NM_145125.1	Brwd1	bromodomain protein	20
	IIa	NM_007552.3	Bmi1	PRC complex	4
		NM_080560.2	Ube2n (Ubc13)	histone ubiquitination	21
		NM_021554.2	Mettl9	Methyl transferase like protein	
		NM_007623.2	Cbx2 (Pc)	chromodomain protein, H3K27Me3 binding	9, 44
		NM_172605.2	Tdrd3	tudor domain protein, binds RMe2	45
		XM_131021.5	Tdrkh	tudor domain protein	46
	IIIa	NM_146142.1	Tdrd7	tudor domain protein	47
	IIIb	NM_011514.1	Suv39h1	H3K9Me3	11
		NM_133182	Hrmt1l1	Prmt2 variant 1	48

		NM_001077638.1	Prmt2	Prmt2 variant 2	48
		NM_001017426.1	Jmjd3	H3K27Me demethylase, Required for neural commitment	22
		NM_019572.2	Hdac7a	histone deacetylation	17
		NM_013926.1	Cbx8	chromo domain protein; PRC1 complex	9, 44
		NM_001045523.1	Bahd1	bromo domain protein	50
	IV	NM_144919.1	Hdac11	histone deacetylation, expressed during murine brain development	51
	V	NM_054054	Brdt	bromo domain protein	52
histones	Ia	NM_178187.2	Hist1h2ae	H2A consensus	23
		NM_175665.1	Hist1h2bk	histone H2B, S124A	23
		NM_178197.1	Hist1h2bh	H2B consensus	23
		NM_178204	Hist1h3d	H3.2	23
		NM_178205.1	Hist1h3e	H3.2	23
		NM_013550.3	Hist1h3a	H3.1	23
		NM_178210.1	Hist1h4j	H4	23
		NM_175656	Hist1h4i	H4	23
		NM_175657.1	Hist1h4m	H4	23
		NM_178211.1	Hist1h4k	H4	23
	Ib	NM_016750.1	H2afz	H2A variant H2AZ	23
		NM_175660.1	Hist1h2ab	H2A consensus	23
		NM_178186.2	Hist1h2ag	H2A consensus	23
		NM_178188.3	Hist1h2ad	H2A consensus	23
		NM_175653.1	Hist1h3c	H3.1	23
		NM_175655.1	Hist1h4f	H4	23
	IIa	NM_198622.1	H1fx	H1.X	24
	IIb	NM_178198.1	Hist1h2bj	H2B consensus	23
	V	NM_015786.1	Hist1h1c	H1.2	24
		NM_023422.1	Hist1h2bc	H2B; S75G	23
DNA methylation	Ia	NM_019448.2	Dnmt3l	accessory Dnmt	4
	Ib	NM_010068.1	Dnmt3b	de novo Dnmt	4
		NM_010066.2	Dnmt1	maintenance Dnmt	4
		NM_013595.1	Mbd3	binding to DNAMe	4
	IIa	NM_010788.1	Mecp2	binding to DNAMe	4

component of the Polycomb repressive complex (PRC), neuro-specific

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Figure S5: Listing, assignment, and literature references of the genes graphed in figure 6D.

The literature was searched for patterning-related genes (by function and/or expression). Those found to be regulated here were listed and assigned a role in forebrain, midbrain or hindbrain development (black boxes). The relevant literature sources are indicated. N.B.: sometimes functional importance (e.g. knockout phenotype) does not match the criteria for region markers and *vice versa*. Multiple regional assignments were allowed, where this was supported by the literature.

Gene name	Accession number	Cluster	full name	for-brain	mid-brain	hind-brain	literature
App	NM_007471.1	IIIa	Amyloid beta (A4) precursor protein	■			52
Ascl1	NM_008553.2	IIIb	Achaete-scute complex homolog 1	■		■	9, 12, 58, 59
Bcan	NM_007529.1	V	Brevian	■			76
Bcl11b	NM_021399	V	B-cell leukemia/lymphoma 11B	■			4, 5 ,6
Calb2	NM_007586.1	V	Calbindin 2	■			4, 38
Chrd	NM_009893.1	IIIb	Chordin	■	■	■	65
Cxcl12	NM_001012477.1	V	Chemokine (C-X-C motif) ligand 12	■			72, 73, 74
Dlx1	NM_010053.1	IV	Distal-less homeobox 1	■			1, 2 ,3
Egr2	NM_010118.1	Iia	Early growth response 2			■	31, 32
En1	NM_010133.1	IV	Engrailed 1		■	■	25, 27, 28
En2	NM_010134.1	IV	Engrailed 2		■	■	28, 29, 30
Fabp7	NM_021272.2	IIIb	Fatty acid binding protein 7, brain	■		■	53, 54
Fez1	NM_007586.1	IIIa	Fasciculation and elongation protein zeta 1	■			39
Fgf8	NM_010205.1	IIIb	Fibroblast growth factor 8	■	■	■	27, 29, 90, 91
Fgfr2	NM_201601.1	IIIb	Fibroblast growth factor receptor 2	■	■	■	49, 88
Fgfr3	NM_008010.2	Iia	Fibroblast growth factor receptor 3	■	■	■	48, 49
Foxg1	NM_008241.1	Iia	Forkhead box G1	■			9, 10
Gata2	NM_008090.3	V	GATA binding protein 2		■	■	92, 93, 94
Gli3	NM_008130	Iia	GLI-Kruppel family member GLI3	■	■	■	44, 45, 46, 47
Hes3	NM_008237.1	Iia	Hairy and enhancer of split 3	■	■	■	18, 41, 43
Hoxa1	NM_010449.1	IV	Homeo box A1			■	36
Hoxa2	NM_010451.1	Iia	Homeo box A2			■	33
Hoxb2	NM_134032.1	Iia	Homeobox B2			■	20
Irx2	NM_010574.2	IIIa	Iroquois related homeobox 2			■	60, 62
Irx3	NM_008393	IIIa	Iroquois related homeobox 3		■	■	60, 61
Irx5	NM_018826.2	IIIa	Iroquois related homeobox 5		■	■	60, 61
Isl1	NM_021459.2	Iia	ISL1 transcription factor, LIM/homeodomain	■			9, 11, 12
Lhx1	NM_008498	IIIa	LIM homeobox protein 1			■	12, 80
Lhx5	NM_008499.2	IV	LIM homeobox protein 5	■	■	■	80
Lmx1a	NM_033652.2	IIIb	LIM homeobox transcription factor 1 alpha		■	■	22, 23, 81, 82, 83
Mecp2	NM_010788.1	Iia	Methyl CpG binding protein 2	■	■	■	68
Msx1	NM_010835.1	IV	Homeobox, msh-like 1	■	■		18, 22, 23, 24
Ndst1	NM_008306.2	IIIb	N-deacetylase/N-sulfotransferase 1	■			75
Neurog2	NM_009718.2	IV	Neurogenin 2	■	■		12, 17, 18, 19
Nfib	NM_008687.2	V	Nuclear factor I/B	■		■	55, 56, 57
Nkx6-1	NM_144955.1	IIIa	NK6 homeobox 1		■		22, 40, 41
Nog	NM_008711.1	IIIb	Noggin	■	■	■	65
Notch3	NM_008716.1	IIIb	Notch gene homolog 3	■			50, 51
Nr2f1	NM_010151.1	IIIa	Nuclear receptor subfamily 2, group F, member 1	■			13, 14, 15
Nr2f2	NM_183261.3	IIIb	Nuclear receptor subfamily 2, group F, member 2	■		■	14, 63, 64
Nrg	NM_178591.2	Iia	Neuregulin1	■			6, 79
Otx1	NM_011023.2	IIIa	Orthodenticle homolog 1	■	■		20, 21
Pitx2	NM_011098.2	IIIb	paired-like homeodomain transcription factor 2	■	■	■	25, 89
Ptx3	NM_008987.2	IIIb	Pentraxin related gene		■		25, 26
Reln	NM_011261	IIIb	Reelin	■			4, 16
Rfx4	NM_001024918.1	IIIa	Regulatory factor X, 4	■	■	■	69, 70, 71
Rora	NM_013646.1	V	RAR-related orphan receptor alpha			■	34, 35
Shh	NM_009170	IIIa	Sonic hedgehog	■	■	■	4, 27
Smo	NM_176996.3	Iia	Smoothened homolog	■			37
Sox5	NM_011444.1	IIIb	SRY-box containing gene 5	■			4, 7, 8
Tal2	NM_009317.2	IV	T-cell acute lymphocytic leukemia 2	■	■	■	12, 66, 67
Tgfb2	NM_009367	IIIb	Tgf-beta2	■	■	■	85, 86, 87
Wnt1	NM_021279.1	IIIb	Wingless-related MMTV integration site 1	■	■	■	22, 27
Wnt3a	NM_009522.1	IIIb	3a/wingless-related MMTV integration site 3A		■		77, 78
Wnt7a	NM_009527.2	IIIb	Wingless-related MMTV integration site 7A	■			84

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Figure S6: Neuroteratogenic effects of Retinoic acid, Cyclopamine and Lead

compound	species	Neuro-teratogenic effect	Ref.
all-trans Retinoic acid (RA)	Xenopus laevis	microcephalic, anterior brain decreased, *hindbrain and spinal cord increased, suppression of anterior CNS*	25,26
	Mesocicetus auratus	Spina bifida, microcephaly, exencephaly, microcephaly	27
		crebellum malformations	28
	Rattus norvegicus	exencephaly, anencephaly, spina bifida	29-31
	Mus musculus	Spina bifida, exophtalmos, exencephaly, *truncation of the anterior brain*	32,33
	Macaca mulatta	microcephaly, hydrocephalus, vermus abnormality	27
	Homo sapiens*	Hydrocephaly, *decline in size of forebrain regions*	28
Cyclopamine	Gallus gallus	cyclopia, fused telencephalon, defects in midline patterning of the neural plate, *holoprosencephaly*	34
	Sheep	cyclopia	35
	Rabbit	cyclopia, cebocephalia	35
	Rattus norvegicus	cebocephalia	35,36
	Mus musculus	Exencephaly, *holoprosencephaly*	35
	Mesocicetus auratus	cebocephalia, exencephaly, encephalocele	
Lead	Rattus norvegicus	Size increase in mossy fibres and the granule cell layer, inhibition of postnatal structuring, reduced VAChT and ChAT mRNA levels, increase in TH activity	8,37-41
	Mus musculus	Changes in the cholinergic system, hyperactive behaviour	37
	Cavia porcellus	Change in glutamate synthesis, reduced glutamine synthetase activity	37,42,43
	Monkeys	Basal forebrain and primary visual cortex damage, memory and learning defects, impaired spatial tasks	37,44
	Homo sapiens	Increased distractability, attention deficit disorder, decreased auditory sensitivity, decreased visumotor performance	37,45,46
Lead	disease correlation	Alzheimers disease[47], Parkinsons disease[48], Schizophrenia[49]	

*: data for 13-*cis*-retinoic acid (Accutane®)

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