The Subplate and Early Cortical Circuits

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Key Words

cerebral cortex, development, plasticity, cortical column, neuronal network, connectivity, cell death, neuropeptides, MAP2, neurotransmitters

Abstract

The developing mammalian cerebral cortex contains a distinct class of cells, subplate neurons (SPns), that play an important role during early development. SPns are the first neurons to be generated in the cerebral cortex, they reside in the cortical white matter, and they are the first to mature physiologically. SPns receive thalamic and neuromodulatory inputs and project into the developing cortical plate, mostly to layer 4. Thus SPns form one of the first functional cortical circuits and are required to relay early oscillatory activity into the developing cortical plate. Pathophysiological impairment or removal of SPns profoundly affects functional cortical development. SPn removal in visual cortex prevents the maturation of thalamocortical synapses, the maturation of inhibition in layer 4, the development of orientation selective responses and the formation of ocular dominance columns. SPn removal also alters ocular dominance plasticity during the critical period. Therefore, SPns are a key regulator of cortical development and plasticity. SPns are vulnerable to injury during prenatal stages and might provide a crucial link between brain injury in development and later cognitive malfunction.

Contents

INTRODUCTION

The subplate represents a transient layer in the developing cerebral cortex, which is located directly under the cortical plate and which consists of a heterogeneous neuronal population according to morphology and neurotransmitter identity (Kostovic & Rakic 1980, 1990; Luskin & Shatz 1985). The subplate plays an important role in the pathfinding of corticopetal and corticofugal axonal projections (Ghosh et al. 1990, McConnell et al. 1989), in the development of the cortical columnar architecture (Ghosh & Shatz 1992a, 1994; Kanold 2004; Kanold et al. 2003), in developmental plasticity, and in the maturation of cortical inhibition (Kanold 2009, Kanold & Shatz 2006). SPns (subplate neurons) possess a number of structural and functional properties, which put them in an ideal position to be critically involved in all these developmental processes, i.e., they show relatively mature electrophysiological properties and they are well interconnected in the developing cortical network (Friauf et al. 1990, Hanganu et al. 2002).

In 1994, Allendoerfer and Shatz published a review in *Annual Review of Neuroscience* (Allendoerfer & Shatz 1994) summarizing the role of the subplate in the development of connections between the thalamus and neocortex. Over the past 15 years, we have learned a lot more about the function and diverse roles of SPns in neocortical development. The present review aims to provide a summary of our current knowledge on the development, connectivity, function, and plasticity of SPns in the cerebral cortex.

DEVELOPMENTAL ORIGINS OF SUBPLATE NEURONS

Cortical neurons are generated in the ventricular zone (VZ). The first postmitotic neurons are the preplate cells (Bystron et al. 2008) (**Table 1**). The subsequent rounds of cell divisions give rise to neurons forming cortical layers 2–6. Via radial migration, these neurons split

Table 1 Subplate neurons across species

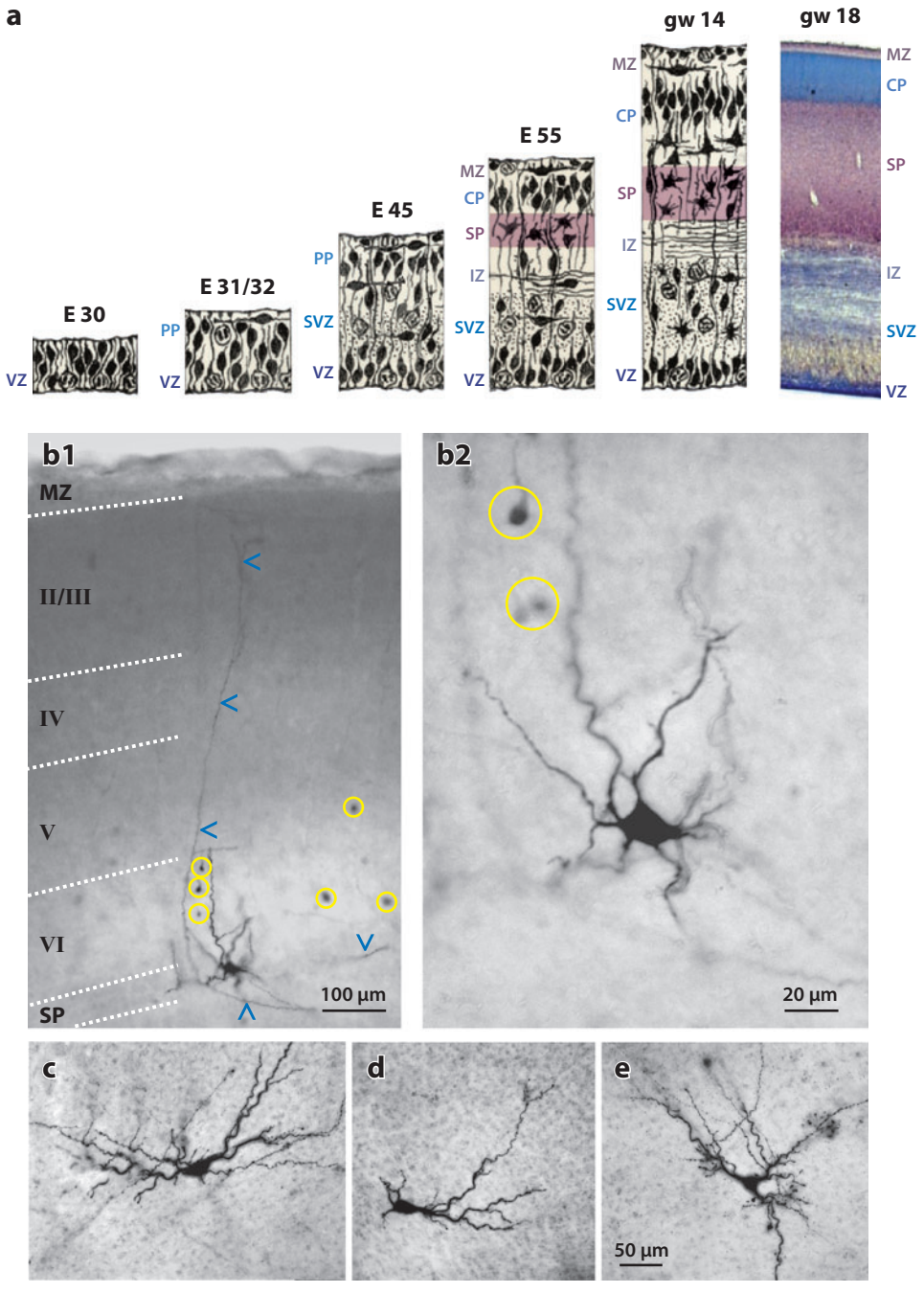
Species	Mouse	Rat	Cat	Primate	Human
Gestation	19.5	21	65	167	40GW
Birth	E11–13 (visual, somato, auditory) (Del Rio et al. 2000, Price et al. 1997, Wood et al. 1992, Zeng et al. 2009)	E12–15 (Al-Ghoul & Miller 1989, Bayer & Altman 1990)	E24–E30 (visual) (Allendoerfer et al. 1990, Luskin & Shatz 1985)	E38–E43 (somato) (Kostovic & Rakic 1980) E43–E45 (Visual) (Kostovic & Rakic 1980)	GW5–6 (Bayer et al. 1993, Kostovic & Rakic 1990)
Waiting	E14–P0 (Del Rio et al. 2000, Deng & Elberger 2003)	E16–17, none (Catalano et al. 1991, Erzurumlu & Jhaveri 1992, Kageyama & Robertson 1993)	E36–E50 (Ghosh & Shatz 1992b)	E78–E124 (Kostovic & Rakic 1984)	GW20–26 (Hevner 2000, Kostovic et al. 2002, Kostovic & Judas 2002, Kostovic & Rakic 1984)
Death (0–80%)	E18–P21 (McQuillen et al. 2002, Price et al. 1997, Torres-Reveron & Friedlander 2007, Wood et al. 1992)	E20–P30 (Al-Ghoul & Miller 1989, Ferrer et al. 1990, Robertson et al. 2000)	P0–P28 (Chun & Shatz 1989a)	E104–P7 (visual) (Kostovic & Rakic 1990) E120–P7 (Somato) (Kostovic & Rakic 1990)	GW34–41 (Kostovic & Rakic 1990, Samuelsen et al. 2003) 2 years (PFC) (Delalle et al. 1997)
Birth.%GP	56%–66%	57%	36%–46%	22%–27%	13%–15%
Waiting%GP	71%–100%	76%–80%	55%–76	45%–85	50%–65%
Death%GP	200%	200%	150%	110%	100%–240%

the preplate into two regions, the marginal zone (containing Cajal-Retzius cells) and the subplate zone (**Figure 1*a***). The relative size of the subplate in comparison to the overlying cortical plate varies among species (Aboitiz & Montiel 2007). The subplate is largest in monkeys and humans (Molnár et al. 2006), suggesting that SPns are not a vestige of earlier neuronal structures but rather a key structure enabling radial organization and higher intercortical connectivity (Aboitiz 1999, Aboitiz et al. 2005). SPns have been identified in placental mammals such as rodents, cats, ferrets, primates, and humans (Molnár et al. 2006). The existence of a subplate in marsupials is controversial (Harman et al. 1995, Marotte & Sheng 2000, Reep 2000, Reynolds et al. 1985). Since all experiments discussed in this review have been performed on placental animals, we focus on placental mammals.

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF SUBPLATE NEURONS

In all mammalian species studied so far, SPns are characterized by their relatively mature structural and functional properties. In newborn rodents and fetal cats, subplate neurons show an extensive axonal and dendritic arborization pattern (**Figure 1*b***) (Friauf et al. 1990; Hanganu et al. 2001, 2002). SPns are capable of integrating synaptic inputs over their large dendritic tree, which in rodents can span in horizontal and vertical directions over a few hundred micrometers (**Figure 1*c–e***). In human prenatal cortex, the dendrites of SPns may extend up to 1 mm and significantly exceed the size of the basal dendrites of pyramidal neurons (Mrzljak et al. 1988, 1992). Short dendritic spines can be observed on the

dendrites of a subpopulation of SPns (Mrzljak et al. 1988), indicating an excitatory function. SPns are characterized not only by their large diversity in the expression pattern of molecular markers (**Table 2**) (Hoerder-Suabedissen et al. 2009, Osheroff & Hatten 2009), but also by their variability in morphological appearance (**Figure 1*c–e***). On the basis of their



somato-dendritic morphology (i.e., the form of the soma and the orientation of the dendritic tree), at least five to six different neuronal types of SPns can be distinguished in rodent (Hanganu et al. 2002) and human cerebral cortex (Mrzljak et al. 1988), i.e., bitufted and monotufted horizontal, multipolar, inverted pyramidal, polymorphous, and fusiform SPns.

SPns show not only a dense axonal arborization within the subplate and axonal projections to the cortical plate and marginal zone/layer I (**Figure 1*b1***) (Clancy & Cauller 1999, Finney et al. 1998, Friauf et al. 1990) but also long-range axons to the thalamus (De Carlos & O'Leary 1992; Kim et al. 1991; McConnell et al. 1989, 1994) and to more distant neocortical regions (Higo et al. 2007, Tomioka et al. 2005). Some of these long-distance projections arise from GABAergic subplate cells (Luhmann et al. 2009). A subset of SPns persisting in adult rats, called subgriseal neurons by Clancy & Cauller (1999), have cortico-cortical projections of more than 4 mm. SPns are connected not only extensively via chemical synapses, but also locally via electrical synapses. The spatial extent of this gap junction–coupled syncytium can be visualized after intracellular filling of a single subplate neuron with a dye that passes through gap junctions (**Figure 1*b***). In newborn rats, one SPn is on average electrically coupled to about 9 other neurons in the subplate or cortical plate (Dupont et al. 2006). The average distance of the coupled neurons is ~100 μm in the medio-lateral direction and ~125 μm in the dorso-ventral direction, thereby forming a columnar network of about 100 μm in diameter. The average coupling conductance between two neighboring subplate neurons is in the range of 1.2 nS (Dupont et al. 2006).

In addition to this high level of morphological differentiation, SPns also have rather mature functional properties, as judged by their ability to fire repetitive overshooting action potentials (**Figure 2*a***) and by the presence of chemical synaptic inputs with fast kinetics. Intracellular or whole-cell patch-clamp recordings in neocortical slices from mice (Hirsch & Luhmann 2008), rats (Hanganu et al. 2001, 2002; Luhmann et al. 2000), cats (Friauf et al. 1990), and humans (Moore et al. 2009) demonstrated relatively mature passive and active membrane properties. When compared to other neurons at the same developmental stage, SPns reveal the largest amplitudes and fastest kinetics in voltage-dependent sodium and calcium currents (Luhmann et al. 2000).

THALAMIC INNERVATION, CORTICAL MICROCIRCUITRY, AND NEUROMODULATION OF SUBPLATE NEURONS

SPns receive prominent synaptic inputs from various presynaptic sources (**Figure 2*b***). Electron microscopical studies in different species have documented the presence of symmetrical and asymmetrical synapses on subplate cells (Chun & Shatz 1988b; Herrmann et al. 1994;

Figure 1

Development of the subplate and morphological properties of SPn. (*a*) Prenatal development of the human cerebral cortex from embryonic day (E) 30 to gestational week (gw) 18. Photograph to the right shows coronal section of gw 18 human cortex stained with cresyl violet. CP, cortical plate; IZ, intermediate zone; MZ, marginal zone; PP, preplate; SP, subplate; SVZ, subventricular zone; VZ, ventricular zone. Drawing from Pasko Rakic, reproduced and modified with permission from Bystron et al. (2008). Photograph of gw 18 human cortex reproduced with permission from Kostovic et al. (2002). (*b–e*) Morphology of biocytin-stained subplate neurons in newborn rat cerebral cortex. (*b1*, *b2*) Subplate neuron in a coronal section of a P3 rat. Note axonal collaterals ascending into upper layers and projecting horizontally within subplate (marked by blue <). Several cells are dye coupled and are marked by yellow circles. (*c*) Postnatal day (P) 3 horizontal bitufted subplate neuron with horizontal dendrites. (*d*) P2 horizontal monotufted subplate cell. (*e*) P2 inverted pyramidal neuron with triangular soma and dendrite oriented towards white matter. Scale bar in *e* corresponds to *c* to *e* and pial surface is located at the top. Panel *b* is reproduced and modified with permission from Luhmann et al. (2003); panels *c–e* are reproduced with permission from Hanganu et al. (2002).

Table 2 Expression of markers on subplate neurons

Marker	Subtype	Species, cortical area, age	Reference for mRNA expression	Reference for immunocytochemistry
Ca^{2+} binding proteins	Calbindin	Mouse, >E13		Del Río et al. 2000
	Calbindin	Rat, >E18		Liu & Graybiel 1992
	Calbindin	Ferret, visual cortex		Antonini & Shatz 1990
	Calbindin	Human, >g.w. 20		Ulfig 2002
	Calretinin	Mouse, >E13		Del Río et al. 2000, Hevner et al. 2003
	Calretinin	Human, >g.w. 20		Ulfig 2002
	Parvalbumin	Rat, E16-P10		Csillik et al. 2002
	Parvalbumin	Ferret, visual cortex, >P28		Finney et al. 1998
	Hippocalcin	Mouse, >E14.5	Osheroff & Hatten 2009	Osheroff & Hatten 2009
Extracellular matrix-associated proteins	Connective tissue growth factor	Mouse, visual and somatosensory cortex, >E18 (in situ), >P8 (immuno)	Hoerder-Suabedissen et al. 2009	Hoerder-Suabedissen et al. 2009, Molyneaux et al. 2007
	Connective tissue growth factor	Rat, >E16	Heuer et al. 2003	
	Chondroitin sulfate proteoglycan	Mouse, >E16		Bicknese et al. 1994
	Chondroitin sulfate proteoglycan (neurocan)	Rat, >E16		Fukuda et al. 1997, Miller et al. 1995
	Fibronectin	Cat, >E50	Chun & Shatz 1988a	Chun & Shatz 1988a
Growth factors	p75NTR	Monkey, visual cortex, >E56		Meinecke & Rakic 1993
	p75NTR	Mouse, >E14	McQuillen et al. 2002	
	p75NTR	Rat, >E17	DeFreitas et al. 2001, Koh & Higgins 1991	DeFreitas et al. 2001, Koh & Higgins 1991
	NGF receptor	Human, >g.w. 16		Kordower & Mufson 1992
	NGF receptor	Cat, >E43		Allendoerfer et al. 1990
	NGF receptor	Ferret, >P2		Allendoerfer et al. 1990
Transcription factors, guidance molecules	Nuclear receptor-related 1/Nr4a2	Mouse, visual and somatosensory cortex, >E18 (in situ), >E20 (immuno)	Hoerder-Suabedissen et al. 2009	Arimatsu et al. 2003, Hoerder-Suabedissen et al. 2009, Molyneaux et al. 2007
	SOX5	Mouse, >E14	Kwan et al. 2008	Kwan et al. 2008
	Dlx	Mouse, >E13		Hevner et al. 2003
	Ephrin-A5	Rat, >E17	Mackarehtschian et al. 1999	
	Ephrin-A4, -A7	Mouse, >E15	Yun et al. 2003	

(*Continued*)

Table 2 (*Continued*)

Marker	Subtype	Species, cortical area, age	Reference for mRNA expression	Reference for immunocytochemistry
Nitric oxide synthase		Ferret, visual cortex, >P28		Finney et al. 1998
		Rat E16-P10		Csillik et al. 2002
		Rat, visual cortex, >P4		Clancy et al. 2001
		Human, >g.w. 15		Judas et al. 1999
	nNOS	Cat		Higo et al. 2007
Chemokine, cytokine	Cxcr4	Mouse, >E14	Tissir et al. 2004	
	TNF-alpha and IL-1beta	Sheep, >E40		Dziegielewska et al. 2000
Steroid hormones	Beta-estradiol (estrogen)	Mouse, >E15	Osheroff & Hatten 2009	
	Progesterone receptor	Rat, >E18	López & Wagner 2009	López & Wagner 2009, Wagner 2008
Others	Monooxygenase Dbh-like 1	Mouse, visual and somatosensory cortex, >E18 (in situ), >P8 (immuno)	Hoerder-Suabedissen et al. 2009	Hoerder-Suabedissen et al. 2009
	Subplate −1	Cat, visual cortex		Dunn et al. 1995, Wahle et al. 1994
	Subplate −1	Rat, mouse, >E18	Fairen et al. 1992	Fairen et al. 1992
	Paired-immunoglobulin–like receptor B (PirB)	Mouse	Syken et al. 2006	
	Complexin 3	Mouse, visual and somatosensory cortex, >E18 (in situ), >P8 (immuno)	Hoerder-Suabedissen et al. 2009	Hoerder-Suabedissen et al. 2009
	Cadherin-related neuronal receptor (CNR)/protocadherin (Pcdh)	Mouse	Morishita et al. 2004	Morishita et al. 2004
	G protein-gated inwardly rectifying K-channels (GIRK)	Mouse	Wickman et al. 2000	
	Phosphodiesterase 1C	Mouse, >E13.5	Osheroff & Hatten 2009	Osheroff & Hatten 2009

Kostovic & Rakic 1980, 1990), indicating that SPns receive GABAergic as well as glutamatergic synaptic inputs. As initially suggested by Kostovic & Rakic, glutamatergic inputs onto SPns arise from the thalamus and other neocortical areas, whereas GABAergic synaptic inputs originate from GABAergic interneurons located in the subplate (Kostovic & Rakic 1980). GABAergic and glutamatergic receptors and markers can be demonstrated in various species at the earliest developmental stages (**Table 3**). Functionally, spontaneous synaptic inputs with fast kinetics mediated by AMPA, NMDA, and $GABA_A$ receptors have been recorded in SPns

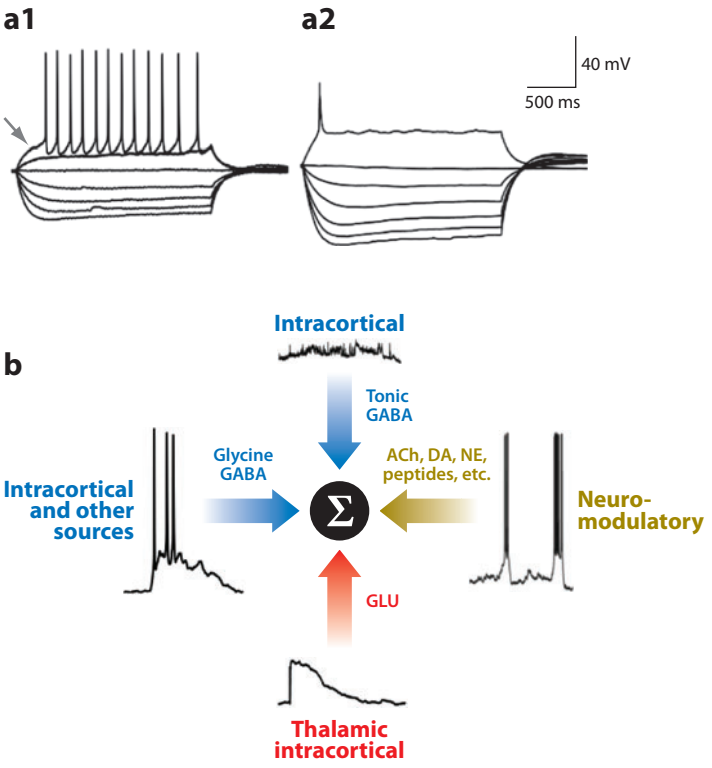


Figure 2

Firing pattern and synaptic inputs of SP. (*a*) Whole-cell patch-clamp recordings from a subplate neuron (*a*1) and in comparison from an immature cortical plate pyramidal cell (*a*2) in newborn rat neocortical slice. Current–voltage relationship and firing pattern illustrates the relative mature electrophysiological properties of the subplate cell with large and repetitive action potentials. Note presence of an A-current in the SPn (*arrow*). Reproduced and modified with permission from Luhmann et al. (2003). (*b*) Subplate neurons integrate afferent inputs from various presynaptic sources. A glutamatergic input innervates the subplate from the thalamus and cerebral cortex. A cortical phasic and tonic GABAergic input depolarizes SPn. The origin of the glycinergic input is unknown, but glycinergic receptors may be tonically activated by taurine. Various neuromodulatory inputs (ACh, DA, 5-HT, NE, peptides, etc.) transiently innervate the subplate and have a profound influence on cortical network activity.

in newborn rats (Hanganu et al. 2001). SPns receive a glutamatergic input from the thalamus mediated via ionotropic glutamate receptors (Hanganu et al. 2002, Herrmann et al. 1994, Higashi et al. 2002, Hirsch & Luhmann 2008). Thalamic axons arrive in the subplate around the time that layer 4 cells are born and wait in the subplate before growing into layer 4 (**Figure 3*a***). The duration of the waiting period varies considerably between species and is longer in species with longer gestation times (**Table 1**). In marsupials, however, thalamic axons seem to directly innervate layer 4 neurons without waiting in the subplate (Molnár et al. 1998, Pearce & Marotte 2003).

Due to the changing nature of subplate circuits, the pattern of thalamocortical activation of cortex varies over development (**Figure 3*a***), as shown by studies in brain slices of cat visual (Friauf & Shatz 1991) and rodent somatosensory cortex (Higashi et al. 2002, Molnár et al. 2003). Electrical white matter stimulation in cat visual cortex at birth results in short latency responses in the subplate and long latency responses in layer 4. This latency difference likely indicates disynaptic responses, suggesting that SPns strongly excite layer 4 neurons (Friauf & Shatz 1991). At later ages, short latency responses to white matter stimulation start to emerge in layer 4. This indicates that now thalamic activity directly activates layer 4 neurons, consistent with mature thalamocortical circuits. Similar results were obtained from imaging experiments and current source density analyses in slices from rodent somatosensory cortex (Higashi et al. 2002, Molnár et al. 2003). In rodents thalamic stimulation activates SPNs by embryonic day 16 (E16) while cortical plate activation is seen at E21. However, in these studies, disynaptic cortical activation was absent. The delay in the emergence of cortical responses in both species reflects the "waiting period" and time needed for synapses to mature. The difference in timing (prenatal versus postnatal) between these studies might reflect an early maturation of the somatosensory relative to the visual system or species differences. The absence of disynaptic responses in the imaging studies could be due to different stimulation sites (thalamus versus white matter) recruiting fewer thalamocortical fibers. However, these data together show that thalamocortical transmission undergoes a functional reorganization from activating subplate neurons to activating layer 4 neurons.

Another glutamatergic input onto SPn arises from the cortical plate and from glutamatergic

Table 3 Expression of transmitter receptors and subtypes on subplate neurons

Transmitter	Receptor subtype	Species, cortical area, age	Reference for mRNA expression	Reference for immunocytochemistry	Reference for electrophysiology
GABA		Mouse, >E13		Del Río et al. 2000	
		Rat, >E16		Lauder et al. 1986, Robertson et al. 2000	
		Cat, >E50		Chun & Shatz 1989b	
		Human, gestation week >7		Zecevic & Milosevic 1997	
	Glutamic acid decarboxylase (GAD)	Rat, >E18		Arias et al. 2002	
	GAD-67	Ferret, visual cortex, >P28		Finney et al. 1998	
	GABA-A	Rat, somatosensory cortex, >P0			Hanganu et al. 2001, 2002
Glycine		Rat, somatosensory cortex, >P0			Kilb et al. 2008
Glutamate	VGLUT1, VGLUT2	Mouse, >E13	Ina et al. 2007		
	AMPA (GluR 2/3)	Rat, >E18		Arias et al. 2002	
	AMPA (GluR2/3)	Sheep, >E60		Furuta & Martin 1999	
	Glutamate	Ferret, visual cortex, >P28		Finney et al. 1998	
	AMPA, kainate	Mouse, somatosensory cortex >P0			Hirsch & Luhmann 2008
	AMPA, kainate	Rat, somatosensory cortex >P0			Hanganu et al. 2001, 2002
	Kainate (GluR6/7)	Sheep, >E60		Furuta & Martin 1999	
	NMDA, kynurenine aminotransferase (KAT)-I	Rat E16-P7		Csillik et al. 2002	
	NMDA	Rat, visual and somatosensory cortex, >P0			Hirsch & Luhmann 2008; Hanganu et al. 2001, 2002; Torres-Reveron & Friedlander 2007
	NR2A	Rat P1-P7		Csillik et al. 2002	
	NR2A, NR2B, NR2D	Mouse, somatosensory cortex >P0	Hirsch & Luhmann 2008		Hirsch & Luhmann 2008

(*Continued*)

Table 3 (*Continued*)

Transmitter	Receptor subtype	Species, cortical area, age	Reference for mRNA expression	Reference for immunocytochemistry	Reference for electrophysiology
Acetylcholine, nicotinic	Alpha4	Human, frontal cortex, >17 weeks of gestation	Schröder et al. 2001	Schröder et al. 2001	
	Alpha4, beta2	Rat, somatosensory cortex >P0			Hanganu & Luhmann 2004
	Alpha5	Rat >E18	Winzer-Serhan & Leslie 2005		
	Alpha7	RAT, >P1		Csillik et al. 2002	
Acetylcholine, muscarinic	M1–m5	Rat, somatosensory cortex >P0	Hanganu et al. 2009		Dupont et al. 2006, Hanganu et al. 2009
Dopamine	DOPA decarboxylase	Mouse, visual and somatosensory cortex, >E18 (in situ), >P8 (immuno)	Hoerder-Suabedissen et al. 2009	Hoerder-Suabedissen et al. 2009	
Neuro-peptides	NPY	Mouse, >E16		Del Río et al. 2000	
	NPY	Rat, >E18, P7-P10		Arias et al. 2002, Csillik et al. 2002, Robertson et al. 2000	
	NPY	Ferret, visual cortex, >P28		Antonini & Shatz 1990, Finney et al. 1998	
	NPY	Cat, >E50		Chun & Shatz 1989b	
	NPY	Monkey, visual cortex, >E75		Mehra & Hendrickson 1993	
	NPY	Human, >14 weeks of gestation		Delalle et al. 1997	
	CCK	Mouse, >E16		Del Río et al. 2000	
	CCK	Cat, >E60		Chun & Shatz 1989b	
	Somatostatin	Rat		Robertson et al. 2000	
	Somatostatin	Ferret, visual cortex, >P28		Antonini & Shatz 1990, Finney et al. 1998	
	Somatostatin	Cat, >E50		Chun & Shatz 1989b	
	Somatostatin	Human, frontal cortex, >22 weeks of gestation		Kostovic et al. 1991	
	Substance P	Mouse, >P0		Del Rio et al. 1991	
	Substance P	Monkey, visual cortex, >E90		Mehra & Hendrickson 1993	
	Hypocretin-orexin (Hcrtr2-OX2)	Rat, different cortical areas, >P15			Bayer et al. 2004

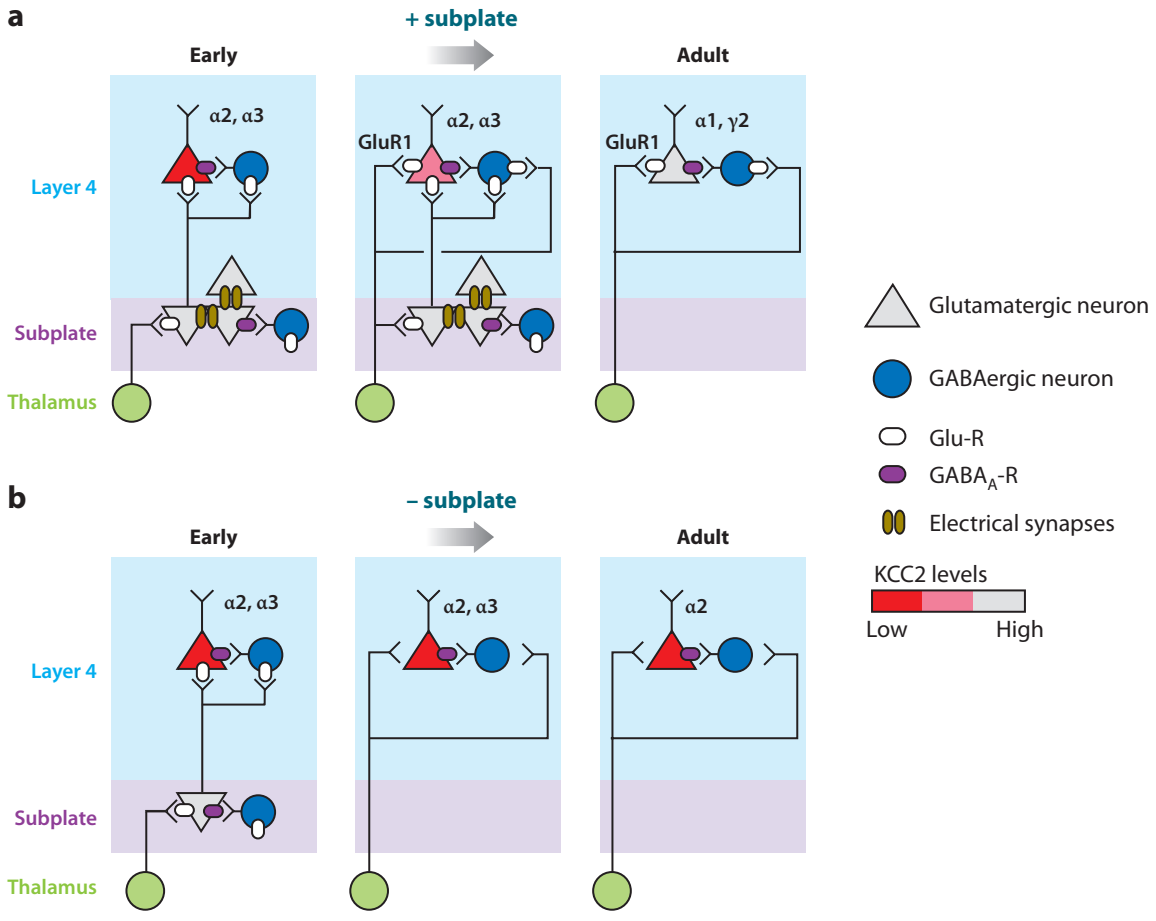


Figure 3

Subplate neurons affect thalamocortical circuit development and inhibitory maturation (*a*) Developmental changes in thalamocortical projections and intracortical inhibition over development. Early (*left*): Thalamus projects to subplate, which in turn projects to layer 4. Potential targets for subplate neurons are both GABAergic and excitatory layer 4 neurons. Subplate neurons and cortical neurons are coupled via electrical synapses. At these ages GABA is depolarizing in layer 4 due to low KCC2 levels (see cell shading). Subplate neurons have higher KCC2 levels due to their advanced maturity. Over development (*middle*) thalamic axons grow into layer 4 and contact layer 4 cells. KCC2 levels in layer 4 increase. Adult (*right*): The thalamocortical synapse has matured. KCC2 levels are high, thus GABA is hyperpolarizing. At early ages GABAergic receptors are composed of α2 and α3 containing subunits, whereas mature receptors contain the α1 and γ2 subunit. (*b*) Summary of circuit changes after early subplate ablation. The α2 and α3 receptor mRNA are expressed at high levels in layer 4, whereas KCC2, α1, γ2, and Glur1 mRNA levels remain low. Since Glur1 mRNA levels are low, the cortex is decoupled from its inputs.

SPns (Hanganu et al. 2002, Hirsch & Luhmann 2008). Both inputs are mediated via ionotropic glutamate receptors, but a significant proportion of the intrasubplate input is mediated via NMDA receptors, which can be activated at more hyperpolarized membrane potentials (Hanganu et al. 2002). These intrasubplate synaptic connections show a pronounced paired-pulse facilitation and temporal summation and at postnatal day 0 (birth) (P0) contain a large amount of the NR2D subunit (Hirsch & Luhmann 2008).

A GABAergic input may originate from local as well as from remote GABAergic subplate neurons, which in the mouse cerebral cortex can project over distances of up to 2 mm

(Higo et al. 2007, Tomioka et al. 2005). Activation of $GABA_A$ receptors as well as glycine or taurine receptors elicits a depolarizing response in newborn rat cerebral cortex (Hanganu et al. 2002, Kilb et al. 2008) due to high intracellular chloride concentrations (Yamada et al. 2004). Besides this phasic GABAergic input, SPns also receive a tonic activation via ambient nonsynaptically released GABA, which facilitates the generation of up states in the neonatal cortex (Hanganu et al. 2009). GABAergic SPns may contribute to this tonic GABA release, thereby modulating proliferation and migration of neuronal progenitors (Maric et al. 2001). Nonsynaptically released taurine may have a similar role by tonic activation of glycine receptors (Flint et al. 1998).

SPns receive a diversity of neuromodulatory inputs from various presynaptic sources. Anatomical and immunocytochemical studies have demonstrated a selective innervation of the subplate by cholinergic fibers arising from the basal forebrain in the newborn rat (Calarco & Robertson 1995, Mechawar & Descarries 2001) and in the 18–22 gestational week in human cortex (Kostovic 1986). The expression of nicotinic acetylcholine transcripts and receptors on SPn have been documented in the rat (Csillik et al. 2002, Winzer-Serhan & Leslie 2005) and human (Schröder et al. 2001) cortex. Patch-clamp recordings from SPns in neonatal rats showed a strong nicotinic excitation mediated by alpha4beta2 receptors (Hanganu & Luhmann 2004). Nicotine, at concentrations similar to the amount that reaches the developing human brain through maternal smoking, induced in SPns a prominent desensitization of nicotinic acetylcholine receptors (Hanganu & Luhmann 2004), suggesting that exposure to nicotine during prenatal stages may disturb developmental processes that are influenced by acetylcholine. SPns in neonatal rat cortex also have M1 to M5 muscarinic receptors, as shown by single-cell PCR studies (Hanganu et al. 2009). Activation of muscarinic M1 receptors causes a membrane depolarization and repetitive ~20 Hz burst discharges in SPns (**Figure 2*b***) (Hanganu et al. 2009). Due to their intense coupling via chemical and electrical synapses to other SPns and to cortical plate neurons, these burst discharges are efficiently transmitted to a local neuronal network (see next section). The first fibers approaching the subplate before the arrival of the cholinergic and thalamic afferents seem to be monoaminergic (Mrzljak et al. 1988). Monoaminergic fibers reach the subplate in human cortex at 12 weeks of gestation and immunohistochemical studies in rodents have demonstrated that these monoaminergic fibers are serotonergic, noradrenergic, and dopaminergic (Kalsbeek et al. 1988, Molliver 1982). The function of these monoaminergic inputs onto SPns is currently unknown, but activation of metabotropic receptors may cause subplate-driven network oscillations similar to those shown for muscarinic receptors (Dupont et al. 2006, Hanganu et al. 2009).

SPns also express various peptide receptors (**Table 3**) and are the source of the earliest peptidergic activity in the cortex. Somatostatin-immunoreactive SPns can be identified in the human cortex at 22 weeks of gestation (Kostovic et al. 1991). The exact functional role of the different peptides on SPns is poorly understood. In juvenile rat cortex, application of cholecystokinin (CCK) to layer 6b neurons (subplate) causes a strong excitation via CCK(B) receptors (Chung et al. 2009). It has been further demonstrated that hypocretin-orexin neurons in the lateral hypothalamus innervate layer 6b and that activation of Hcrtr2-OX2 receptors causes a closure of a potassium conductance, thereby promoting widespread activation of layer 6b/SPns (Bayer et al. 2004).

Subplate Projections

SPns have diverse axononal output patterns. Subplate axons project into the developing cortical plate (Friauf et al. 1990; Friauf & Shatz 1991; Hanganu et al. 2001, 2002; Hanganu & Luhmann 2004; Luhmann et al. 2000; Piñon et al. 2009) and also pioneer the corticogeniculate projection (De Carlos & O'Leary 1992, McConnell et al. 1989, Molnár & Cordery

1999). In higher mammals, but not in rodents, SPns also project through the corpus collosum (Antonini & Shatz 1990, deAzevedo et al. 1997, Del Rio et al. 2000). However, it is unknown if these three different projection patterns are subserved by different classes of SPns. Of the three projection targets, the feed-forward cortical projection is the best-studied projection. Subplate projections to the cortical plate are radially oriented, show some collateral axon branches, and predominantly target layer 4 (Dupont et al. 2006, Friauf et al. 1990, Friauf & Shatz 1991, Piñon et al. 2009). Most SPns projecting to the cortical plate are glutamatergic (Finney et al. 1998), and recent physiological experiments show that selective subplate stimulation evokes excitatory synaptic currents in layer 4 (Zhao et al. 2009).

THE SUBPLATE: AN ACTIVE HUB STATION

Numerous studies have demonstrated with tracing methods that the subplate receives a transient input from the specific thalamic nuclei and that the subplate serves as a waiting station for the ingrowing thalamocortical axons (see above). Friauf et al. were the first to demonstrate a functional synaptic input from the thalamus onto SPns (Friauf et al. 1990). In subsequent studies, different groups confirmed these results for rats and mice (Hanganu et al. 2002, Higashi et al. 2002, Molnár et al. 2003, Zhao et al. 2009). Furthermore, electrophysiological studies demonstrated functional intracortical GABAergic inputs (Hanganu et al. 2001, 2002, 2009), an intracortical and thalamocortical glutamatergic input (Hanganu et al. 2002, Hirsch & Luhmann 2008), and a cholinergic input mediated via muscarinic receptors (Dupont et al. 2006, Hanganu et al. 2009). In addition, functional nicotinic alpha4beta2 receptors (Hanganu & Luhmann 2004) and glycinergic (Kilb et al. 2008) receptors have been demonstrated on SPns in newborn rodent cortex. All these functional data demonstrate that the subplate may have a more important function than just serving as a rather passive waiting station of the ingrowing thalamocortical afferents. Voigt and colleagues (Voigt et al. 2001) have demonstrated in dissociated neuronal cell cultures from embryonic rat cerebral cortex that a distinct population of large GABAergic neurons is a key element in the generation of synchronous oscillatory network activity. The authors have suggested that SPns function as an integrating element that synchronizes neuronal activity by collecting incoming extrinsic and intrinsic signals and distributing them effectively throughout the developing cortical plate. A minimal number of two large GABAergic SPns per square millimeter were required for the occurrence of synchronous activity. The pivotal role of SPns in generating synchronous oscillatory network activity has been confirmed by multichannel recordings from acute neocortical slices of newborn rodents. Electrical stimulation of the subplate in 800–1000 μm thick slices with a sufficiently preserved neuronal network elicits synchronized oscillatory activity (Sun & Luhmann 2007). Carbachol-induced synchronized network oscillations with similar properties can be elicited in intact cortices of the newborn rat only when the subplate is intact (Dupont et al. 2006). These in vitro observations have been recently confirmed by in vivo experiments demonstrating a clear participation of the subplate in generating locally synchronized oscillatory network activity (Yang et al. 2009).

Together these data show that SPns play a very active role in cortical processing. The subplate functions not only as a passive relay or waiting zone, but rather as an active hub station of the developing cortical network! This is due to their unique anatomical properties (extensive dendritic arborization, widespread axonal projections), their relative mature functional state (firing pattern, etc.), their gap junction mediated electrical coupling to other SPns and cortical plate neurons, their substantial glutamatergic or GABAergic synaptic inputs from thalamic, intrasubplate and cortical plate sources, and their strong synaptic inputs from neuromodulatory systems (e.g., the selective innervation of the SP by the cholinergic basal

forebrain). Thus SPns possess key attributes and are in a key position to affect cortical development.

ROLE OF THE SUBPLATE IN REGULATING MATURATION OF CORTICAL INHIBITION (AND EXCITATION)

The maturation of cortical circuits involves the functional maturation of neurons, the maturation of their capability to release neurotransmitters, and the increased expression of excitatory and inhibitory neurotransmitter receptors.

Role of Subplate Neurons in Maturation of Thalamocortical Synapses

Selective lesioning of SPns can be achieved by excitotoxic injections (Ghosh et al. 1990, Ghosh & Shatz 1992a, Kanold et al. 2003, Kanold & Shatz 2006, Lein et al. 1999) or by exploiting the selective expression of p75 in subplate neurons (**Table 2**) and using injections of p75-immunotoxin (Kanold et al. 2003, Kanold & Shatz 2006). Such selective subplate lesions have been used to elucidate the role of SPns in cortical circuit maturation. Ablations in cat during the first postnatal week when thalamocortical excitation is immature revealed a profound effect of SPns on thalamocortical maturation when animals were examined ~3–4 weeks later (Kanold et al. 2003).

Ablation of SPns in visual cortex prevents the developmental increase in expression of glutamate receptor subunits (GluR1) mRNA specifically in layer 4 (Kanold et al. 2003) (**Figure 3*b***). The low mRNA levels are paralleled functionally by weak thalamocortical synapses (Kanold et al. 2003). However, despite the low thalamocortical synaptic strength, an increase in spontaneous synaptic events and spiking activity is seen (Kanold et al. 2003). Thus, visually driven thalamic activity is unable to strongly drive cortical neurons, and therefore the visual cortex becomes functionally decoupled from the visual thalamus (LGN). SPns might strengthen thalamocortical synapses by interacting with synaptic plasticity rules. Simulations using a computational model have shown that strong subplate input to layer 4 can entrain correlations between thalamic activity and layer 4 activity that lead to strengthening of thalamocortical synapses via Hebbian plasticity rules (Kanold & Shatz 2006).

Role of Subplate Neurons in Maturation of Cortical Inhibition

Maturation of inhibition involves the maturation of inhibitory neurons to express GABA synthesizing enzymes (glutamate-decarboxylase, GAD) and postsynaptic expression of a mature complement of GABA receptors. In addition, fast GABAergic inhibition via $GABA_A$ receptors involves the influx of chloride (Cl^-) ions. Thus the intracellular Cl^- concentration and thereby E_{Cl} determine the functional effect of GABAergic inhibition. KCC2 removes Cl^- from the cytosol and thus can control Cl^- levels (and E_{Cl}) (Blaesse et al. 2009). Low E_{Cl} renders GABA hyperpolarizing, whereas high E_{Cl} renders GABA depolarizing, which can act excitatory or inhibitory (shunting), depending on the size of the depolarization (Achilles et al. 2007, Blaesse et al. 2009). KCC2 levels increase over development and render $GABA_A$ receptors hyperpolarizing (Blaesse et al. 2009) (**Figure 3*a***). KCC2 expression can be regulated by neuronal activity (Fiumelli et al. 2005, Ganguly et al. 2001, Kriegstein & Owens 2001, Ludwig et al. 2003), BDNF (Aguado et al. 2003; Rivera et al. 2002, 2004), or injury (Cramer et al. 2008, Rivera et al. 2004, Shimizu-Okabe et al. 2007).

Subplate ablations in cat during the first postnatal week when intracortical inhibition is immature revealed a profound effect of SPns on inhibitory maturation when animals were examined ~3–4 weeks later (Kanold & Shatz 2006). Ablation of SPns prevents the developmental increase in expression of KCC2 and "mature" $GABA_A$ receptor subunits such as the alpha1 and gamma2 subunit (Kanold & Shatz 2006) (**Figure 3*b***). This immature expression pattern is paralleled functionally by a

sustained presence of depolarizing responses to GABAergic stimulation (Kanold & Shatz 2006). SPns might regulate the expression levels of KCC2 and GABA receptors by providing depolarization to layer 4 that is able to increase the expression of these genes. This view is supported by in vivo experiments in which glutamatergic signaling was blocked during development. In these experiments, KCC2 mRNA levels also failed to increase, suggesting that a glutamatergic input is required to induce KCC2 and $GABA_A$ alpha1 mRNA expression (Kanold & Shatz 2006). During development, there are three sources of glutamatergic excitation to cortical neurons (see above): thalamic inputs, intracortical inputs, and subplate inputs. Since both thalamic and intracortical inputs are present after subplate ablation, but fail to cause increased expression of KCC2 and $GABA_A$ alpha1 (Kanold & Shatz 2006), these data suggest that specific glutamatergic input from the subplate is needed for their increased expression.

The lower expression levels of GluR1, KCC2, GABA receptors, and increased spontaneous activity levels are paralleled by increased expression of BDNF mRNA (Lein et al. 1999). Since BDNF mRNA levels can be regulated by neural activity (Castren et al. 1998, Lein et al. 2000), overall activity levels in layer 4 after ablation might be higher than normal, despite reduced thalamic inputs. Alternatively, the regulation of BNDF might be altered after subplate ablation. Subplate ablation also results in increased levels of GAD (Lein et al. 1999) suggesting that interneurons are present and active. The increased cortical GAD levels after ablation might indicate that GABAergic neurons are hyperactive. In unmanipulated cortex, increased activity can lead to increased inhibitory tone and decreased excitatory tone possibly via BDNF signaling (Turrigiano 2007). However, BDNF levels can also lead to a reduction in KCC2 levels (Molinaro et al. 2009; Rivera et al. 2002, 2004). Thus, increased BDNF levels after subplate ablation might prevent inhibitory maturation. The observed increased inhibitory activity together with high BDNF levels after ablation might be indicative of dysfunctional homeostatic regulation of cortical activity (Turrigiano 2007) due to immature inhibitory maturation. Because levels of KCC2 remain low after subplate ablation, increased GABAergic activity due to possibly hyperactive GABAergic neurons (Lein et al. 1999) can further increase cortical activity levels, possibly contributing to seizure activity following SPn ablation (Lein et al. 1999).

ROLE OF THE SUBPLATE IN SCULPTURING NEOCORTICAL ARCHITECTURE (COLUMNS)

One hallmark of neocortical organization especially primary sensory cortices are functional columns that group neurons with similar stimulus selectivity (Mountcastle 1997). On a small scale, columns are formed by grouping several microcolumns (radial units) and on a larger scale, columns are organized into cortical maps. SPns are involved in setting up this architecture at multiple levels and at multiple developmental time points.

Role of the Subplate in Area Identity and Radial Unit Formation

SPns aid in the guidance of thalamic axons into layer 4. Removal of SPns before thalamic axons enter layer 4 redirects these axons to an area where SPns are present (Ghosh et al. 1990). In addition to providing guidance cues, SPns might contain direct cues directing the positioning of cortical maps. FGF8 gradients are involved in positioning sensory maps in the rostro-caudal axis (Fukuchi-Shimogori & Grove 2001). Misexpressing FGF8 in the cortical plate causes thalamocortical axons to enter the cortical plate and then turn posterior to innervate layer 4 (Shimogori & Grove 2005). However, if FGF8 was also misexpressed in subplate then thalamocortical axons travel further posterior within the subplate and innervate the cortical plate radially, suggesting that SPns contained positional information (Shimogori & Grove 2005). Such position information can be conveyed by graded

expression of guidance molecules in the subplate, such as p75 (McQuillen et al. 2002) and ephrinA5 (Mackarehtschian et al. 1999, Yun et al. 2003) (**Table 2**).

In addition to radial glia cells (Rakic 1988), SPns may represent an additional cell type contributing to the radial organization of the cerebral cortex (Mountcastle 1997). SPns are coupled via electrical synapses to cells in the cortical plate (Dupont et al. 2006) and could aid in establishing cortical microcolumns by defining coupled radial units (Mountcastle 1997). This is consistent with evolutionary hypotheses about the role of SPns in allowing the radial organization of the mammalian cerebral cortex (Aboitiz 1999, Aboitiz et al. 2005).

Role of Subplate Neurons in Establishing the Functional Cortical Architecture

Neurons in the visual cortex respond selectively to lines of a particular orientation and in higher mammals these neurons are grouped in orientation columns and orientation maps (Hubel & Wiesel 1977). In binocular animals, thalamic afferents segregate into ocular dominance columns (ODCs) in layer 4 (Hubel & Wiesel 1977) (**Figure 4*a***). The development of ODCs and orientation tuning has been a model system to investigate mechanisms of development. Subplate ablation after thalamocortical axons have innervated layer 4, but before ODCs and orientation maps have formed, prevents the formation of ODCs and functional orientation maps, even though both thalamic fibers and layer 4 neurons are present (Ghosh & Shatz 1992a, Kanold et al. 2003) (**Figure 4*b***). Single unit recordings show that subplate ablation prevents the acquisition of normal visual responses (Kanold et al. 2003). While a large fraction of neurons is unresponsive to visual stimuli after ablation, consistent with weak thalamocortical synapses, the remaining neurons show weak orientation tuning (Kanold et al. 2003). Since orientation-tuned responses and ODCs require the refinement of thalamocortical projections, these results suggest that the refinement of LGN projections to layer 4 did not occur.

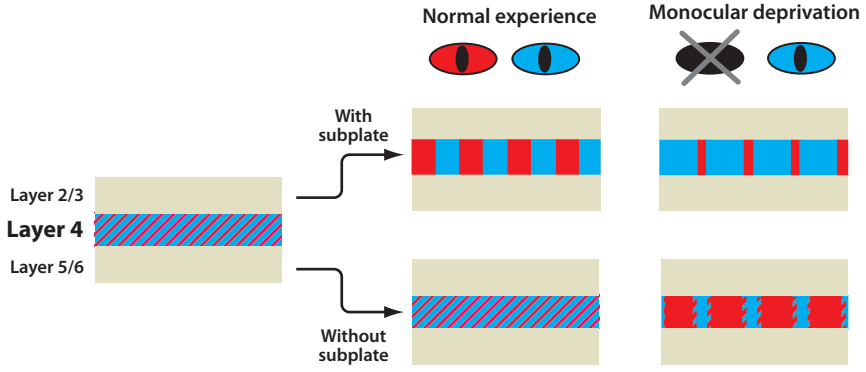


Figure 4

Subplate neurons are required for formation and normal plasticity of ocular dominance columns. A schematic of the development of ocular dominance columns (ODCs) under two conditions with and without subplate neurons: normal visual experience and monocular deprivation. Initially in development, thalamocortical projections representing the two eyes (*red* and *blue*) overlap. With normal experience, equally spaced ODCs emerge in V1 (*top left*). If one eye is closed during the critical period with subplate neurons present, then open eye projections expand and closed eye projections contract (*top right*). Without subplate neurons thalamocortical projections do not segregate, and no ODCs are observed (*bottom left*). If one eye is closed in the absence of subplate neurons, then deprived eye projections are retained and open eye projections are removed (*bottom right*).

Neuronal activity and normal sensory experience are required for ODCs and orientation maps to emerge (Crair et al. 1998, Hensch 2004, Reiter et al. 1986, Stryker & Harris 1986). Since ablation of subplate prevents the maturation of thalamocortical synapses, the visual cortex is decoupled from its inputs and thus deprived of visual inputs (Kanold et al. 2003). This deprivation prevents the emergence of the functional architecture of the visual cortex. Alternatively, computational modeling studies have suggested that the functional architecture of the cortex might develop in the subplate and be transferred into the developing cortical plate (Grossberg & Seitz 2003). If such a scenario were true, then subplate ablation would remove the organizational template in the subplate.

Thus, SPns contain molecular cues that direct thalamic axons to the right cortical area and also enable cortical neurons to respond to early spontaneous and later sensory evoked neuronal activity in order to develop the functional architecture of the cortex.

ROLE OF THE SUBPLATE IN DEVELOPMENTAL PLASTICITY

The lack of functional cortical organization following subplate ablation does not imply that sensory experience has no influence on cortical organization. Sensory imbalances such as monocular deprivation (MD) during early life, in particular during the critical period, are able to alter the functional organization of the cortex (Hensch 2004). Following MD, there is an expansion of the cortical territory innervated by thalamic projections representing the open eye, whereas there is a loss of projections representing the closed eye. This results in a OD shift toward the open eye (**Figure 4*a***). The maturation of inhibition has been shown to be a crucial regulator in allowing OD shift to occur in the critical period (Hensch 2004). If inhibitory circuits are weakened, then no OD shift is observed (Hensch 2004). However, even though inhibitory circuits remain immature after subplate removal, sensory imbalances can change ODCs in a "paradoxical" manner (Kanold et al. 2003). In contrast to normal OD plasticity, after ablation concurrent with MD, thalamocortical projections representing the deprived eye are retained, whereas thalamocortical projections representing the open eye are removed (Kanold et al. 2003) (**Figure 4*b***). Thus, mechanisms underlying OD plasticity still operate in the absence of SPns. A paradoxical shift of OD toward the less active eye is also observed in experiments where the cortex has been pharmacologically silenced (Hata et al. 1999, Hata & Stryker 1994).

Simulations using a computational model of ODC development (Kanold & Shatz 2006) based on circuits shown in **Figure 3*a*** suggest that decorrelation of thalamic and cortical activity after subplate removal can lead to such paradoxical shifts. One assumption of the model is that a spike-time–dependent learning rule (STDP) exists in layer 4 (Kanold & Shatz 2006). Cortical STDPs show a longer time window for synaptic depression (LTD) than for synaptic strengthening (LTP) (Abbott & Nelson 2000). Thus, if synaptic inputs are uncorrelated with cellular firing, a net weakening occurs and more active synapses become weakened than less active synapses. Thus open eye projections would be weakened more than closed eye projections and paradoxical plasticity results (Kanold & Shatz 2006). These simulations point to a key role of SPns in promoting the correlation of cortical activity with thalamic activity that enables the strengthening and refinement of thalamocortical connections by Hebbian learning rules such as STDP (Kanold & Shatz 2006). SPns can promote such correlations by providing excitatory inputs to layer 4 (Finney et al. 1998, Zhao et al. 2009) and also by controlling the balance of excitation and inhibition within layer 4 (Kanold & Shatz 2006).

Therefore, by controlling the maturation of excitatory and inhibitory circuits, SPns enable cortical circuits to reorganize correctly following sensory manipulations. The disappearance of SPns over development might restrict this ability and might restrict the observed circuit plasticity to a limited critical period.

CONSEQUENCES OF EARLY HYPOXIA, ISCHEMIA, ETC., ON SUBPLATE (DYS-)FUNCTION

Animal studies and clinical evidence indicate that the subplate is critically involved in various brain developmental disorders including cerebral palsy, periventricular leukomalacia (PVL), autism, schizophrenia, and epilepsy. An enhanced vulnerability of subplate neurons to early hypoxia-ischemia resulting in PVL has been documented in neonatal rats (Csillik et al. 2002, McQuillen et al. 2003). In vitro electrophysiological recordings in neocortical slices from newborn rats have demonstrated a pronounced functional impairment of SPns following a combined oxygen and glucose deprivation (Albrecht et al. 2005). In humans, the peak of subplate development coincides with the gestational age of highest vulnerability to perinatal brain injury in the premature infant (McQuillen & Ferriero 2005). It has been postulated that the second trimester represents the "window of vulnerability" for selective subplate injury and that defects in prefrontal cortical regions are related to schizophrenia (Bunney et al. 1997). An immunohistochemical analysis on neonatal telencephalon samples obtained postmortem from infants with white matter lesions and born at 25–32 weeks of gestation has shown a significant loss of GABAergic SPns, indicating that this subpopulation of SPns may be more vulnerable to perinatal systemic insults (Robinson et al. 2006). The mechanisms of SPn selective susceptibility are unknown, but their unique molecular, structural and functional properties may well explain this vulnerability. SPns express glutamate receptors at the earliest stages (**Table 3**), receive functional glutamatergic synaptic inputs (**Figure 2*b***), and possess NMDA receptors that can be activated at resting membrane potentials (Hirsch & Luhmann 2008).

Disturbances in the programmed cell death of SPns may also cause long-term neurological deficits. It has been proposed that cortical dysplasia associated with pharmaco-resistant epilepsy could be the consequence of postnatal retention of some SPns (Cepeda et al. 2007). An increased density of interstitial cells in the white matter have been found in the frontal and temporal cortex of schizophrenic patients (Kirkpatrick et al. 1999) and this has been attributed to alterations in the pattern of programmed cell death (Akbarian et al. 1996). If these surviving SPns maintain their extensive local and long-range synaptic connections, they may disturb cortical processing (Bunney & Bunney 2000) or may function as pacemaker regions for the generation of epileptic activity (Luhmann et al. 2003) via their feed forward excitatory projections (**Figure 3*a***) (Zhao et al. 2009).

SUMMARY AND PERSPECTIVES

Accumulating evidence points to a key role of SPns in neocortical development. The number of SPns increases with increasing brain complexity. Not only is the subplate larger in primates than in rodents, it also persists for a much longer developmental period, the longest being in humans. To date, investigations of SPns have mostly focused on their role in thalamocortical processing. However, SPns also project back to the thalamus and to the opposite hemisphere, but the function of the corticothalamic and callosal projections has not been investigated to date.

Unfortunately, despite their demonstrated importance, SPns are woefully understudied. For example, the role of the various neuromodulatory systems innervating the subplate at earliest stages is mostly unknown. The lack of information about these neurons might derive from the fact that they are only present in very young animals and that they are located deep in the brain and hence not easily accessible. In rodents the subplate is only very thin, making an analysis or manipulation of the subplate difficult. In addition, manipulations of subplate function have to be precisely targeted to avoid affecting other cortical neurons that migrate through the subplate. Thus, better selective markers to specifically target subplate neurons are needed. Genetic profiling of SPns is the

first step to unequivocally identify these neurons and to categorize subpopulations of SPns. The search for SPn-specific genetic markers has recently begun (Hoerder-Suabedissen et al. 2009, Osheroff & Hatten 2009) and most likely will open the door for new experimental strategies to better understand the role of the subplate in the developing and mature cerebral cortex. It is even now possible to immunopurify subplate neurons for in vitro cellular, molecular, and physiological studies of synaptogenesis and to study mechanisms of subplate neuron death (McKeller & Shatz 2009, DeFreitas et al. 2001). Such studies might also contribute to the urgently needed development of genetic techniques to silence or activate subplate neurons to investigate their role in cortical development.

One key question that needs exploration is why SPns exist in the first place and why and how do they die. Ablation data show that SPns are required for the functional maturation and plasticity of thalamocortical connections. However, in many other areas of the brain (such as the thalamus) connections mature and refine without the aid of a transient cell population. Thus the function of the subplate might be related to unique properties of the neocortex, such as its radial organization and increased lateral connectivity. It might be that subplate neurons are needed to generate and control activity patterns to set up areas that show a large-scale systematic organization (such as orientation maps).

Another unsolved question is why a larger fraction of SPns seem to survive in rodents versus carnivores and primates. Whereas in the mature rodent neocortex layer 6b neurons play an important function in cortical processing, surviving SPns in the adult human cortex seem to be involved in pathophysiological disturbances such as epilepsy and schizophrenia. It may be most relevant clinically to identify the genetic disorders and the environmental risk factors that cause SPn dysfunction during early developmental stages.

In summary, subplate neurons are closely intertwined with the developing cortical circuit and play key roles at multiple stages of development to ensure normal emergence of the complex circuitry of the cerebral cortex.

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Contents

Indexes

Errata

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