CHAPTER

28

The Basal Forebrain Cholinergic Projection System in Mice

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OUTLINE

INTRODUCTION

The basal forebrain is composed of an affiliation of heterogeneous structures and includes the medial septum, ventral pallidum (VP), diagonal band nuclei, substantia innominata/extended amygdala, and peripallidal regions. The basal forebrain is located close to the medial and ventral surfaces of the cerebral hemispheres that develop from the subpallium. This highly complex brain region has been implicated in cortical activation, attention, motivation, memory, and neuropsychiatric disorders such as Alzheimer's disease

(AD), Parkinson's disease, schizophrenia, and drug abuse (Blanco-Centurion et al., 2007; Detari, 2000; Conner et al., 2003; Goard and Dan, 2009; Jones, 2008; Kauer et al., 2008; Lin and Nicolelis, 2008; Parikh and Sarter, 2008; Weinberger, 2007). Part of the difficulty in understanding the role of the basal forebrain in these functions, as well as the processing characteristics of these disease states, lies in the anatomical complexity of this region. The basal forebrain contains a heterogeneous mixture of cell types that differ in transmitter content, morphology, and projection pattern. One of the most prominent features of the mammalian basal forebrain is the presence of a collection of aggregated and non-aggregated, large, hyperchromic neurons, many of them containing choline acetyl transferase (ChAT), the critical enzyme in the synthesis of acetylcholine (ACh); these neurons project to the cerebral cortex. However, cholinergic corticopetal neurons in rodents represent only about 20% of the total cell population in the basal forebrain. Other basal forebrain neurons utilize a number of different neuroactive substances, including GABA, glutamate and neuropeptides (Duque et al., 2000; Gritti et al., 2006; Hur and Zaborszky, 2005; Jones, 2008; Zaborszky and Duque, 2000, 2003; Zaborszky et al., 1999).

The large, corticopetal neurons are often referred to as the 'magnocellular basal forebrain system' (Hedreen et al., 1984) or the basal nucleus of Meynert (NBM) in primates (Koelliker, 1896). The clusters of large neurons in the basal forebrain, first illustrated by Theodor Meynert in 1872 (Meynert, 1872), have long been a focus of attention, as these neurons degenerate in AD (Brockhaus, 1942; Kodama, 1927; Pilleri, 1966; Perry et al., 1984; Price et al., 1986). Cholinergic neurons extend rostrally and medially from the septum and caudally to the amygdala, largely in an area that was named the substantia innominata more than two centuries ago (Reil, 1809). This latter term, however, lost its significance in light of tracer and histochemical studies in the early 1980s that indicated that the main portion of the basal forebrain, previously called the substantia innominata (SI), belongs to nearby and better defined anatomical systems. The rostral, subcommissural part of the SI is primarily occupied by the ventral extensions of the globus pallidus and striatum, i.e. the VP and the core/shell subdivisions of the nucleus accumbens (ventral striatum). More caudally, the sublenticular part of the SI is occupied by the 'extended amygdala' (EA), which refers to the subpallidal cell bridges extending from the centromedial amygdala to the bed nucleus of the stria terminalis (Heimer, 2000; Heimer and van Hoesen, 2006; Heimer et al., 1985; 1999; de Olmos et al., 2004; de Olmos and Heimer, 1999; Riedel et al., 2002; Sakamoto et al., 1999; Zaborszky et al., 1985).

Cholinergic neurons are located in other parts of the rat brain beyond the basal forebrain. They are found in the striatum, the medial habenular nucleus, mesopontine tegmentum, cranial nerve motor nuclei and the ventral horn of the spinal cord (for ref. see Semba, 2004). Cholinergic intrinsic neurons are absent in the cortex of the BALB/c ByJ mouse (Kitt et al., 1994) but present in the rat cortex. Various cholinergic cell groups in the brain can be identified with numbers and letters Ch based upon their projection target. In the basal forebrain of mammals cholinergic neurons are located in the medial septum (Ch1), the vertical (Ch2) and horizontal (Ch3) limbs of the diagonal band and in the substantia innominata/nucleus basalis (Ch4) (Mesulam et al., 1983a). Since the projection target of the cholinergic neurons is poorly determined based upon their topography in the basal forebrain, the Ch nomenclature has met with considerable criticism (see Butcher and Semba, 1989). Corticopetal basal forebrain neurons often form dense clusters that are interrupted by regions of low cellular density but there are no easily defined borders that would justify use of the term nucleus. When describing the various compartments of the cholinergic neurons we will use topographical terms and well-known fiducial markers, unless there is not enough information provided in the original paper, in which case we refer to the authors' usage of the term (nucleus basalis, basal nucleus of Meynert) in the publication. Usually neurons projecting to the hippocampus that are located rostrally in the basal forebrain are not included in the term nucleus basalis, although amygdalopetal neurons that are intermingled with corticopetal cells may be included.

Whereas ACh serves as a direct neurotransmitter at the neuromuscular junction where it opens sodium channels and initiates muscle contraction, in the brain ACh acts primarily as a neuromodulator. ACh can activate nicotinic or G-protein coupled muscarinc receptors. A number of different nicotinic and muscarinic receptors have been cloned. The synthesis and release of ACh requires the expression of three genes, encoding ChAT, the vesicular acetylcholine transporter (VAChT) and the choline transporter 1 (Brandon et al., 2004; Ferguson et al., 2003). ACh is hydrolyzed by cholinesterases (AChE) that is expressed both in cholinergic and cholinoceptive neurons, thus AChE is not a definitive marker for cholinergic neurons. Late stages of cholinergic differentiation are regulated by the neurotrophin nerve growth factor (NGF) through binding to its high and low affinity receptors (TrkA and $p75^{NTR}$, respectively), both of which are expressed in basal forebrain cholinergic neurons (Fagan et al., 1997; Yuen et al., 1996). Finally, basal forebrain cholinergic neurons in rodents express several neurotransmitter receptors, including

adrenergic, glutamatergic, GABAergic (De Souza Silva et al., 2006; Kiss et al., 1993; Zaborszky et al., 2004), receptors for estrogen (Miettinen et al., 2002) and endocannabinoids (Harkany et al., 2003).

In this chapter, we first present a series of figures depicting the distribution of basal forebrain cholinergic neurons, overlaid on Nissl images of the same sections with standard anatomical delineations corresponding to the Franklin-Paxinos mouse atlas. Other sections in this chapter review the molecular specification and maintenance of cholinergic neurons in mice. The input-output relations of cholinergic and other local neurons will be discussed mainly based upon data in rats, supplemented with mouse data when available. Finally, we attempt to give an overview of mouse models of AD relating to loss or degeneration of cholinergic neurons.

NEURON TYPES IN THE BASAL FOREBRAIN

In the basal forebrain, cholinergic neurons are co-distributed with several other cell populations, including GABAergic and various CBP (calcium binding protein) containing neurons such as calbindin, calretinin or parvalbumin (Gritti et al., 2003; Henderson et al., 2010; Zaborszky et al., 1999; Zaborszky and Duque, 2003). More recently, glutamate and neuropeptides including neuropeptide Y (NPY) and somatostatin have been described in projection neurons and interneurons (Hur and Zaborszky, 2005; Zaborszky and Duque, 2000, 2003).

Cholinergic Neurons

In the mouse, different mRNA species are transcribed by a combination of three distinct promoters together with alternative splicing of noncoding exons from the ChAT gene (Misawa et al., 1994). These different forms of ChAT mRNAs, all containing the same coding regions, differ only in their 5′ noncoding end and encode the same ChAT protein. There are pronounced differences in the relative expression of splice variants in various brain regions. Of the seven splice variants, basal forebrain cholinergic neurons express mostly the R1 and R2 types, while cranial motor nuclei express high levels of five variants (R1,R2,R3,R4, N1) (Trifonov et al., 2009). The number of cholinergic neurons in the nucleus basalis was estimated to be around 6,632 ± 1,105 in C57BL/6J non-transgenic mice (Perez et al., 2007). By comparison, in rats, the number of cholinergic neurons in the medial septum/vertical diagonal band nucleus (MS/VDB) was reported to be 9,647 ± 504, with 26,390 ± 1097 cholinergic neurons found in the entire basal forebrain (Miettinen et al., 2002). Cholinergic neurons in the caudal part of the basal forebrain, similar to cholinergic cells in the septum are slow-firing neurons (Duque et al., 2000; Simon et al., 2006). In head-fixed rats, cholinergic neurons of the basal forebrain show the highest firing rate during the wake state as compared to slow-wave sleep or REM sleep (Hassani et al., 2009).

GABAergic Neurons

GABAergic neurons are a diverse cell population in the basal forebrain and are divided into several subtypes based on their morphology, spontaneous or evoked firing pattern, and neuromodulatory function. Various CBPs are often co-expressed in a high percentage of GABAergic neurons in the cortex and hippocampus and serve to distinguish subpopulations of GABAergic interneurons. Tamamaki et al. (2003) created a transgenic mouse line (GAD67-GFP knock-in) that expresses GFP specifically in GABAergic neurons by using gene-targeting methods that have proven to be an important tool for both developmental as well as for electrophysiological and anatomical studies (Tamamaki et al., 2003). GABAergic cells in the medial septum-diagonal band complex have recently been described using this transgenic line (Henderson et al., 2010). This study confirmed the presence of a heterogeneous population of septo-hippocampal GABAergic neurons (Castaneda et al., 2005), many of them expressing parvalbumin and the KV3.1 potassium channel that contribute to the fast-spiking properties of these neurons. GABAergic terminals frequently surround GABAergic and glutamatergic neurons in rat and mice (Hajszan et al., 2004; Henderson et al., 2010). Reciprocally interconnected parvalbumin-containing GABAergic and glutamatergic neurons in the septum are important in hippocampal theta generation (Freund, 2003). In the rat MS/VDB a small percentage of cholinergic neurons has been suggested to coexpress GAD (Brashear et al., 1986; Sotty et al., 2003), however, such colocalization was not found in GAD67-GFP transgenic mice (Henderson et al., 2010). GAD+ neurons comprise multiple sleep-wake subgroups in rat basal forebrain (Hassani et al., 2009).

Calcium Binding, Protein-Containing Neurons

Ca^{2+} binding proteins such as parvalbumin, calretinin, calbindin and the newly described secretagogin are valuable phenotypic markers for differentiating various cell types in the brain. Ca^{2+} signaling in neurons

is extremely important, and defines the ability of these cells to release neurotransmitter and regulate intracellular signaling pathways. During development, CBPs show different temporal and spatial patterns.

Parvalbumin containing neurons are abundant in the cortex, the hippocampus and the thalamus of mice as revealed by both immunohistochemistry and in situ hybridization using parvalbumin-transgenic mice (Tanahira et al., 2009). In the rat basal forebrain, a substantial proportion of parvalbumin cells contain GABA and project to the cerebral cortex (Celio, 1990; Gritti et al., 1993; Zaborszky et al., 1999).

Calbindin and Calretinin. Calbindin has proven to be a useful marker of tangential migratory cells during cortical development in mice (Jimenez et al., 2002). Most cholinergic neurons in the monkey and human basal forebrain are immunoreactive for calbindin-D-28, a vitamin D-dependent calbindin; on the other hand, none of the rat basal forebrain cholinergic neurons express calbindin immunoreactivity (Celio and Norman, 1985; Chang and Kuo, 1991; Smith et al., 1994). In AD there is a significant loss of cholinergic neurons in the basal forebrain and the remaining cholinergic neurons display a substantial loss of calbindin immunoreactivity when compared with aged normal controls (Geula et al., 2003; Wu et al., 2005). Since cholinergic neurons which display a loss of calbindin in AD show immunoreactivity for the apoptotic signal Fas-associated death domain and for abnormally phosphorylated tau protein, the loss of calbindin and concomitant increase of intracellular Ca^{2+} may be an important process in the pathologic cascade leading to degeneration of basal forebrain cholinergic neurons in this disease (Wu et al., 2005). A small percentage of calbindin and calretinin cells in the rat project to the cortex (Zaborszky et al., 1999), although their transmitter content remains to be determined.

Secretagogin is a recently discovered CBP and is widely distributed in the developing and adult mouse brain (Mulder et al., 2009). Immunoreactivity for *secretagogin* was found in the amygdaloid complex as well as in the basal forebrain. In the basal forebrain, scattered *secretagogin* neurons were found in the interstitial nucleus of the posterior limb of the anterior commissure, in the VP, horizontal limb of the diagonal band nucleus and the dorsal part of the SI/EA. In the primate (Mulder et al., 2009) and in the mouse basal forebrain, cholinergic neurons coexist with secretagogin (Gyengesi et al., 2010).

Glutamatergic Neurons

Cells that use glutamate as a fast excitatory neurotransmitter contain one of three vesicular transporters, vGluT1, -2, or -3, and can be identified by expression of one of these glutamate vesicular transporters. Rat basal forebrain areas rich in cholinergic neurons contain vGluT2 cells, a small proportion of which project to the prefrontal and somatosensory cortices (Hur and Zaborszky, 2005). Similarly, in the medial septum of mice a small proportion of vGluT2 cells project to the hippocampus (Henderson et al., 2010). Interestingly, a small proportion of vGluT2 cells are colocalized with GAD67 in rat and in transgenic vGluT2-GFP mice (Sotty et al., 2003; Henderson et al., 2010). Some of the vGluT2 cells in transgenic mice also express the Kv3.1 potassium channel (Henderson et al., 2010). A portion of the vGluT2 cells in the basal forebrain may act as local interneurons, as has been suggested in the septum (Hajszan et al., 2004). In C57Bl/6N mice and rats, Harkany et al. (2003) reported that a significant proportion of cholinergic neurons expressed Vglut3 immunoreactivity in the medial septum, diagonal band and nucleus basalis, and were in close apposition to vGluT3-immunoreactive terminals. In the rat, many cholinergic neurons projecting to the basolateral nucleus of the amygdala express Vglut3 and are located in the VP (Nickerson-Poulin et al., 2006). Interestingly, these amygdalopetal cholinergic neurons do not contain $p75^{NTR}$, the low affinity NGF receptor (Heckers et al., 1994).

In the MS/VDB region of vGluT2-GFP transgenic mice, GnRH (Gonadotropin-Releasing Hormone) was colocalized in a subpopulation of vGluT2 neurons; some of these double-labeled neurons were exquisitely sensitive to kisspeptin, a puberty-initiating peptide. A different population of GnRH/vGluT2 neurons responded to group 1 metabotropic glutamate receptor agonists (Dumalska et al., 2008). These GnRH/vGluT2 neurons also receive GnIH (avian gonadotropin inhibitory peptide) innervation, and are inhibited by this neuropeptide (Wu et al., 2009a). The same cells receive an innervation from lateral hypothalamic neurons that synthesize melanin concentrating hormone (MCH), and show a substantial inhibitory response to MCH (Wu et al, 2009b).

Neuropeptide-Containing Neurons

Neuropeptide-Y

There is a substantial amount of data that has been collected that focuses on the anatomy and function of NPY neurons in the mouse hypothalamus and their role in food intake regulation, maintenance of energy homeostasis and obesity (Pinto et al., 2004). NPY acts as a potent and direct inhibitory peptide in the thalamus and hypothalamus (Acuna-Goycolea et al., 2005; Fu et al., 2004; Sun et al., 2003). NPY neurons are colocalized with GABA in the forebrain (Aoki and Pickel 1989). A reversed phase relationship exists between

basal forebrain NPY and cholinergic cell firing as studied with cortical electroencephalogram (EEG) *in vivo* (Duque et al., 2000). Furthermore, NPY injection into the basal forebrain induces changes in cortical EEG in both anesthetized and freely moving rats (Toth, et al., 2005; 2007); together these data suggest a possibility of regulation of cholinergic output by local NPY neurons. With the creation of an NPY-GFP mouse line (van den Pol et al., 2009) it is possible to visualize most of the known NPY-containing neurons in the brain, without using the toxic and destructive colchicine treatment that blocks axonal transport, and enhances detection of peptidergic neurons using immunocytochemistry. NPY neurons are particularly rich in cortical areas and the striatum, and modest-to-medium density NPY-containing cells are intermingled with cholinergic neurons in the SI/EA and HDB. Rich pockets of NPY neurons can be found in various locations of the lateral hypothalamus (Figs. 28.1–28.3). NPY acts via specific receptors, including Y1, Y2, Y4, and Y5 and possibly others. Recently, a number of transgenic mice have been generated to investigate the expression pattern and function of these receptors (Edelsbrunner et al., 2009a, b; Oberto et al., 2007; Painsipp et al., 2008; Tasan et al., 2009). In the mouse basal forebrain, Stanic et al. (2006) reported strongly-to-moderately labeled Y2R positive neurons in the bed nucleus of stria terminalis, VP and the nucleus accumbens. Y2R positive fibers were described in almost every area of the forebrain; however, they are clearly missing from the globus pallidus, the horizontal and vertical diagonal band nuclei, medial septum and the islands of Calleja. NPY neurons in the rat arborize heavily in basal forebrain areas and synapse on both cholinergic and non-cholinergic neurons (Mosca et al., 2005; Zaborszky et al., 2009). Based on preliminary *in vitro* electrophysiological studies in rat slices, NPY inhibits the majority of cholinergic neurons, with this effect being mediated via Y1 receptors (Zaborszky et al., 2009).

Somatostatin and Galanin

Somatostatin, a 14- or 28-amino acid-containing neuropeptide, has been identified in synapses on cholinergic projection neurons (Zaborszky, 1989b). A portion of these somatostatin-containing terminals may originate from local neurons distributed mainly in the VP, SI and around the HDB (Zaborszky and Duque, 2000). Using *in vitro* patch clamp techniques, our studies suggest that somatostatin presynaptically inhibits both GABA and glutamate release onto basal forebrain cholinergic neurons (Momiyama and Zaborszky, 2006).

The neuropeptide galanin (GAL) is widely distributed in the mammalian central nervous system (Perez et al., 2001). GAL-positive fibers were found innervating cholinergic neurons in the basal forebrain

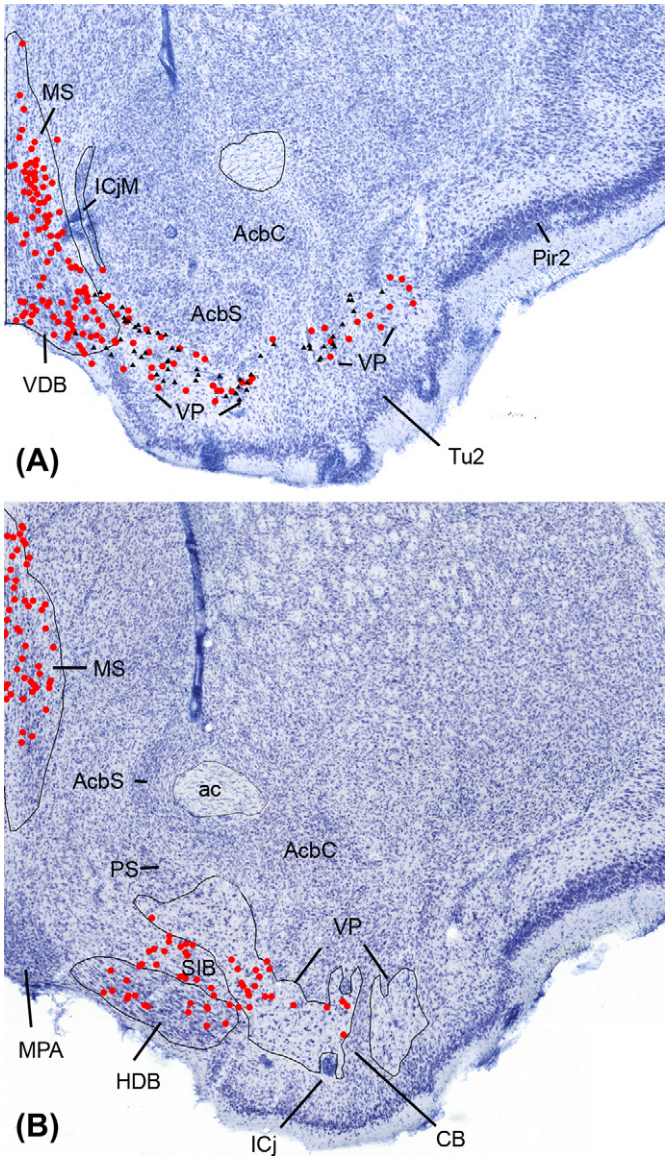

FIGURE 28.1 **Distribution of cholinergic projection and associated NPY neurons in the basal forebrain.** Brain sections from NPY-GFP mice that were also processed for ChAT immunostaining using fluorescent-tagged antibodies were counterstained with Nissl after mapping cholinergic (red circles) and NPY cells (black triangles) in extrastriatal areas. Cholinergic neurons were labeled with a sheep anti-ChAT and Cy3-antisheep IgG. (A) approximately 1.3 mm, (B) 0.7 mm rostral to the bregma according to the Franklin and Paxinos (2008) atlas. For better orientation the vertical (VDB), the horizontal diagonal band (HDB) and the ventral pallidum (VP) are outlined using dark filed illumination for locating heavily myelinated fiber tracts. Additional abbreviations: ac, anterior commissure; AcbC, accumbens nucleus, core; AcbS, accumbens nucleus, shell; cell bridge connecting the nucleus accumbens with the layer of the olfactory tubercle; ICj, island of Calleja; ICjM, island of Calleja, major island; MPA, medial preoptic area; MS, medial septal nucleus; Pir2, piriform cortex, layer 2; PS, parastrial nucleus; SIB, substantia innominata, basal part; Tu2, olfactory tubercle, dense-cell layer.

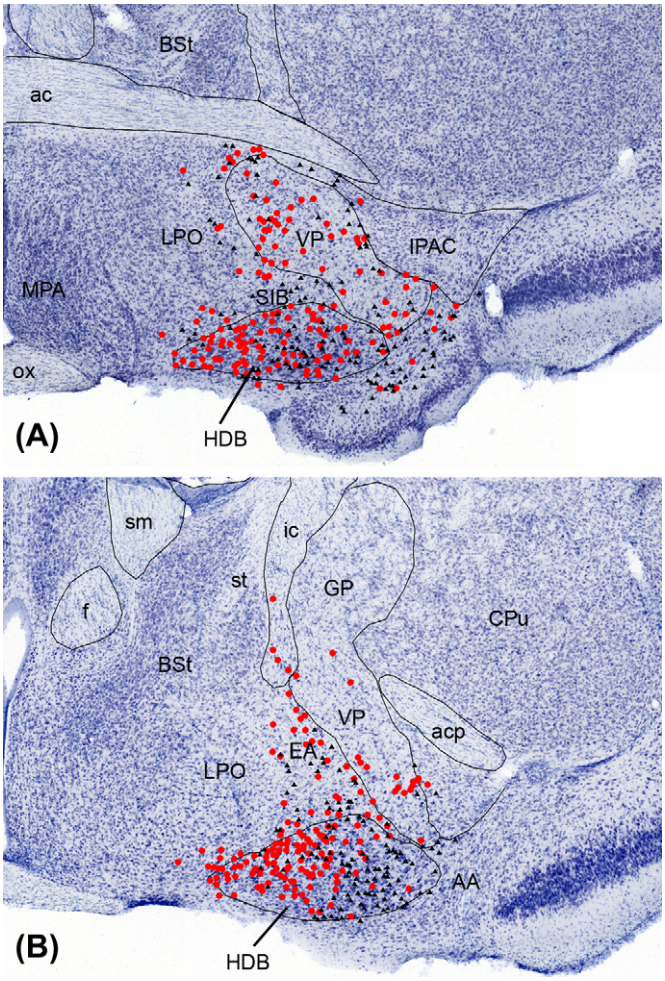

FIGURE 28.2 **Distribution of cholinergic and associated NPY neurons.** (A) approximately 0.15 mm rostral, (B) 0.25 mm caudal to the bregma. Abbreviations: AA, anterior amygdaloid area; ac, anterior commissure, anterior part; acp, anterior commissure, posterior part; BSt, bed nucleus of the stria terminalis; CPu; caudate putamen; f, fornix; EA, extended amygdala; GP, globus pallidus; HDB, nucleus of the horizontal limb of the diagonal band; ic, internal capsule; IPAC, interstitial nucleus of the posterior limb of the anterior commissure; LPO, lateral preoptic area; MPA, medial preoptic area; ox, optic chiasm; SIB; substantia innominata, basal part; sm, stria medullaris; st, stria medullaris; VP, ventral pallidum. Note the lateral part of the HDB is labeled as magnocellular preoptic nucleus in the Franklin-Paxinos atlas.

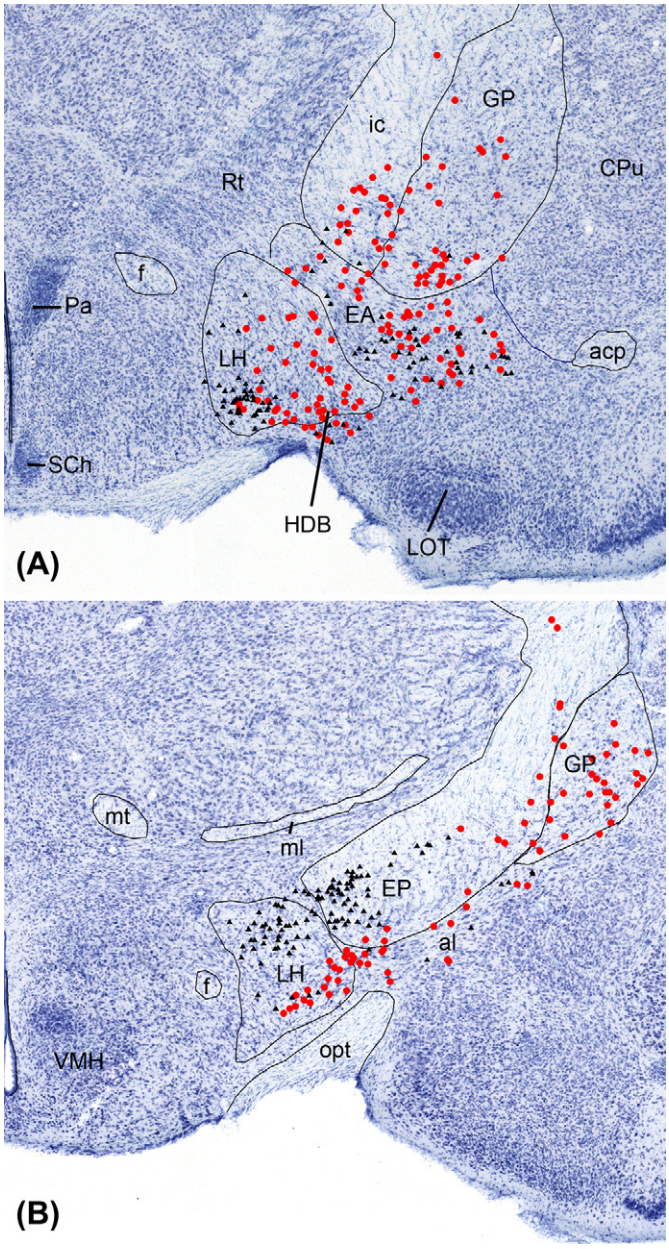

FIGURE 28.3 **Distribution of cholinergic and associated NPY neurons.** (A) approximately 0.7mm, (B) 1.3 mm behind the bregma. The lateral hypothalamic area/medial forebrain bundle is delineated using dark field illumination to discern heavily myelinated fiber bundles. Abbreviations: acp, anterior commissure, posterior part; al, ansa lenticularis; CPu, caudate putamen; EA, extended amygdala; EP, entopeduncular nucleus; GP, globus pallidus; ic, internal capsule; LOT, nucleus of the lateral olfactory tract; ml, medial lemniscus; mt, mammillothalamic tract; opt, optic tract; Pa, paraventricular hypothalamic nuleus; Rt, reticular thalamic nucleus; SCh, suprachiasmatic nucleus; VMH, ventromedial hypothalamic nucleus.

(Henderson and Morris, 1997; Mufson et al., 2003). In addition, high and low affinity GAL receptors were also found in the basal forebrain. Transgenic mice overexpressing GAL display hyperinnervation of cholinergic basal forebrain neurons and are associated with a reduction in the number of cholinergic neurons in the HDB (Steiner et al., 2001). GAL was also shown to inhibit cholinergic transmission in the hippocampus and impair spatial memory in rodent models (Elvander et al., 2004). The functional consequence of GAL hyperinnervation around basal forebrain cholinergic neurons is controversial (Mufson et al., 2005). Using single cell expression analysis in AD, a recent study suggested that GAL might exert a neuroprotective effect upon basal forebrain cholinergic neurons (Counts et al., 2009).

Distribution of Cholinergic and Associated NPY Neurons in the 'Cytoarchitectonic Space' of the Basal Forebrain

Figs. 28.1–28.3 are a series of Nissl-stained sections with mapped cholinergic and NPY neurons from NPY-GFP mice that were also processed for ChAT staining. After mapping cholinergic neurons and the surrounding NPY neurons in extra-striatal areas, sections were stained for Nissl substance. At the level of Fig. 28.1A (approx. 1.3 mm anterior to the bregma according to the Franklin-Paxinos mouse atlas), the majority of cholinergic cells are located in the nucleus of the vertical limb of the diagonal band (VDB) with a few scattered cells in the VP. At this level the VP consists of a few small compartments between the dense cell layer of the olfactory tubercle and the ventral part of the shell of the nucleus accumbens. At the next level (Fig. 28.1B; approx. 0.7 mm anterior to the bregma) the medial preoptic area separates the cholinergic cells into a dorsal component that occupies the medial septal nucleus and a ventral component located in the nucleus of the horizontal limb of the diagonal band (HDB). Scattered cholinergic cells can be seen in the VP and in the space between the VP and the HDB that corresponds to the basal part of the substantia innominata (SIB). Fig. 28.2A is at the level of the crossing of the anterior commissure, corresponding to 0.15 mm rostral to the bregma. The majority of cholinergic cells occupy the HDB, with scattered cells appearing in the VP and SIB. Occasionally, cholinergic cells are found in the lateral preoptic area and in the ventrolateral part of the bed nucleus of the stria terminalis. Fig. 28.2B is approximately 0.25 mm posterior to the bregma; cholinergic cells occupy the dorsal part of the HDB, the territory of the sublenticular substantia innominata-extended amygdala (EA/SI) and the VP. Few cholinergic cells are in the lateral hypothalamus. At the next level (Fig. 28.3A), that is approximately 0.7 mm behind the bregma and is characterized by the prominent suprachiasmiatic and paraventricular nuclei in the hypothalamus and the nucleus of the lateral olfactory tract in the amygdala, cholinergic cells occupy the caudo-lateral part of the HDB, the dorsal aspect of lateral hypothalamus and most of EA/SI. Cholinergic cells are prominent in the ventro-lateral part of the internal capsule and are scattered in the globus pallidus. The last section from this series (Fig. 28.3B) is approximately 1.3 mm behind the bregma. Prominent at this level are the ventromedial hypothalamic nucleus and various amygdaloid nuclei. Cholinergic neurons are located ventrolaterally in the lateral hypothalamus from where scattered cells extend into the area of the ansa lenticularis. A few cholinergic cells can be seen in the lateral portion of the internal capsule and the globus pallidus. Fig. 28.4 shows the co-distribution of NPY and cholinergic neurons from a section at a level slightly rostral to Fig. 28.3A.

EFFERENT, AFFERENT, INTRINSIC CONNECTIONS AND ORGANIZATION

Efferent Projections

Many of the output relations of basal forebrain cholinergic neurons are known from studies in rats (Carlsen et al., 1985; Gritti et al., 1997; Hur and Zaborszky, 2005; Mesulam et al., 1983a; Semba et al., 1988; Zaborszky et al., 1999; Zaborszky and Duque, 2003; Zaborszky et al., 1986a; 1991) and primates (Mesulam et al., 1983b; Pearson et al., 1983). Additional observations are based on cases of AD that had relatively selective cell loss in various regions of the basal forebrain (Arendt et al., 1985; Mesulam and Geula, 1988). Neurons within the medial septum and nucleus of the vertical limb of the diagonal band (MS/VDB; also termed Ch1/Ch2 according to the classification of Mesulam et al., 1983a) provide the major cholinergic innervation of the hippocampus. Cholinergic neurons within the horizontal limb of the diagonal band and magnocellular preoptic nucleus (HDB/MCPO; Ch3) project to the olfactory bulb, piriform, and entorhinal cortices. Cholinergic neurons located in the VP, sublenticular substantia innominata-extended amygdala (SI/EA), globus pallidus, internal capsule, and nucleus ansa lenticularis, collectively termed the Ch4 group of Mesulam, project to the basolateral amygdala, and innervate the entire neocortex according to a rough medio-lateral and antero-posterior topography. Cholinergic neurons, mainly in the MS/VDB, HDB and MCPO also project to orexin/hypocretin neurons in the lateral hypothalamus (Sakurai et al., 2005). In addition to cholinergic neurons, the basalocortical projection system consists of various amounts of GABAergic, glutamatergic and peptidergic projections (Gritti et al., 1997; Hur and Zaborszky, 2005; Zaborszky et al., 1999). The ratio of cholinergic to non-cholinergic projection neurons varies systematically according to the cortical target area. This value is lower in frontal (0.3 on average) than in the posterior cortical areas (0.6) (Zaborszky, unpublished). According to a recent study in the rat, axons in the prefrontal cortex originating from the basal forebrain give rise to 19% cholinergic, 52% GABAergic and 15% glutamatergic terminals (Henny and Jones, 2008).

Cortical Cholinergic Innervation Pattern and Receptors

Cholinergic varicosities are present in all cortical layers in rats and mice with regional and laminar

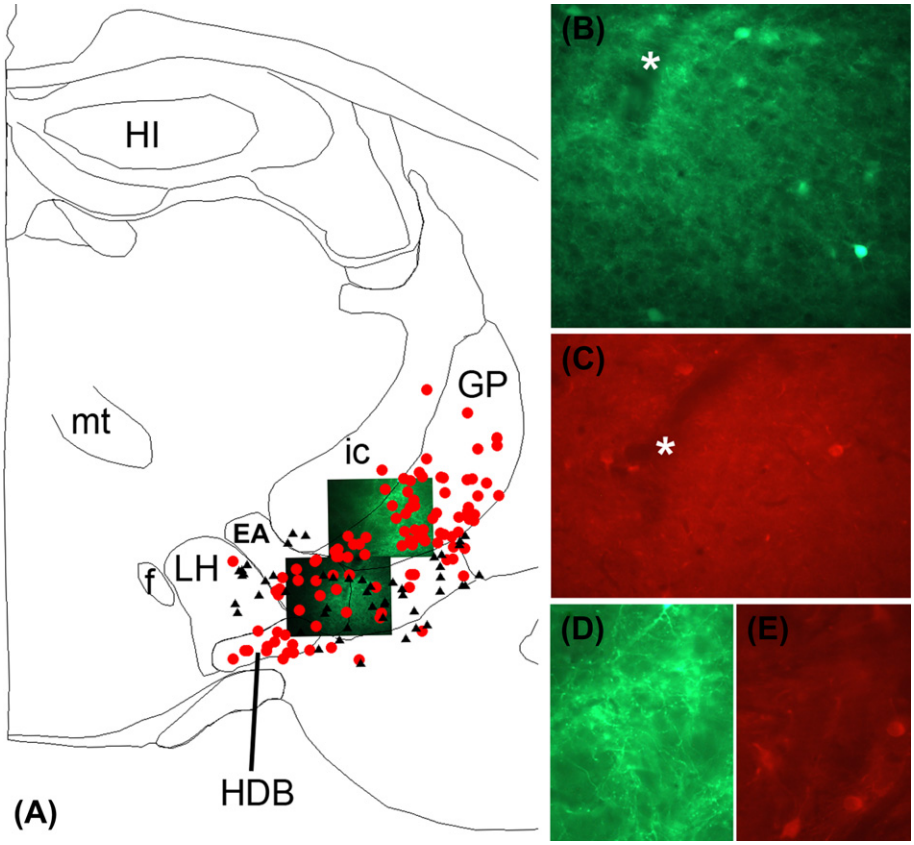

FIGURE 28.4 **Codistribution of cholinergic and NPY neurons.** (A) section mapped with the Neurolucida[R] system, located between sections depicted in Figs. 28.3A and 28.3B. Cholinergic cells are represented by solid red, NPY neurons by black triangles. Panels (B) and (C) are from the lower green boxed area in (A). Panels (D–E) are from the upper green box in (A) which is populated by cholinergic cells only, but contains rich NPY axonal network. On panels (B) and (C) star labels the same vessel. Abbreviations: f, fornix; EA, extended amygdala; GP, globus pallidus; HDB; nucleus of the horizontal limb of the diagonal band; HI, hippocampus; ic, internal capsule; LH, lateral hypothalamus; mt, mammillothalamic tract; LH, lateral hypothalamus.

differences in fiber densities (Mechawar et al., 2002; Avendano et al., 1996; Kitt et al., 1994; Lysakowski et al., 1988; Umbriaco et al., 1994). Considering the density of cholinergic innervation in the rat, the frontal cortex has the densest ACh innervation, followed by the occipital and parietal cortex (5.4; 4.6 and 3.8 x 10^6 varicosities per mm^3). The average density of cholinergic varicosities is about four times higher than that of noradrenaline (Descarries et al., 2004). Cholinergic varicosities in the cortex are endowed with clearly identifiable synapses, although the percentage of synapse per varicosity is reported to vary between 15% in rat parietal cortex (Umbriaco et al., 1994) and 44% in monkey prefrontal cortex (Mrzljak et al., 1995) and 67% in human temporal cortex (Smiley et al., 1997). Both pyramidal and non-pyramidal cells receive cholinergic synapses (Houser et al., 1985). The low proportion of synaptic attachments in the rodent cortex prompted Descarries to propose that ACh acts in the cortex primarily by volume transmission through diffusion in the extracellular space. This hypothesis is supported to some extent by reports showing that both muscarinic and nicotinic receptor subtypes are often localized peri- and extra-synaptically in the cortex and hippocampus (Lubin et al., 1999; Mrzljak et al., 1998; Rouse et al., 2000). Another complication is that a single ultrathin section may not reveal an existing synapse that is out of the plane of section; serial section synaptic reconstruction is the ideal way of determining synapse per bouton probability, and that has not always been done in these studies.

Cholinergic receptors. ACh acts through muscarinic (mAChRs) and nicotinic receptors (nAChRs) that are localized in the cortex both pre- and postsynaptically in different layers (Hill et al., 1993; Levey et al., 1991; Palomero-Gallagher and Zilles, 2004; Waada et al., 1989), thus ACh can affect different neuronal classes and thereby could change the direction of information flow within cortical circuits (Xiang et al., 1998). Five subtypes of mAChRs have been cloned. M1 localizes to postsynaptic dendrites and spines, M2 is localized both to cholinergic axons as autoreceptors as well as

postsynaptically to pyramidal cells (Mrzljak et al., 1996; 1998). M1 colocalizes with the NMDA receptor in CA1 pyramidal cell bodies and dendrites can potentiate excitatory transmission and thus play a role in synaptic plasticity (Volpicelli and Levey, 2004). In monkey V1 (visual) cortex single cell recording studies suggest that mAChRs mediate attentional modulation (Herrero et al., 2008).

nAChRs are ligand-gated cationic ion channels; molecular biological studies have identified at least nine subunits (α2–α7; β2–4) that are expressed in the brain to form functional pentameric receptors (Alkondon and Albuquerque, 2004; Chamtiaux and Changeux, 2004). In the prefrontal cortex of rat and mouse cholinergic axons are often colocalized with α7nAChR and frequently apposed to α7nAChR-containing spines (Duffy et al., 2009). Prefrontal cortex nAChRs have been shown to play a role in facilitating transient glutamatergic (likely of thalamic origin)-basal forebrain cholinergic interactions that are necessary for cue detection in attentional processes (Parikh et al., 2008; 2010; Howe et al., 2010). In monkey V1, β2-nAChR subunit is localized in thalamocortical axons synapsing with layer 4c spines. β2-nAChR is also expressed by GABAergic interneurons in V1. Nicotine increases responsiveness and lowers contrast threshold in layer 4c neurons (Disney et al., 2007). Several human neuroimaging studies have used pharmacological agents related to muscarinic or nicotinic cholinergic function to influence memory, learning and attention (for ref see Frackowiak et al., 2004).

In contrast to cholinergic axons, GABAergic basalocortical and septohippocampal neurons appear to exclusively innervate inhibitory neurons in their terminal region (Freund and Gulyas, 1991). GABAergic basal forebrain neurons also innervate GABAergic cells in the reticular thalamic nucleus (Asanuma et al., 1990). By acting via a disinhibitory mechanism, the GABAergic projection from the basal forebrain may participate in the timing and synchrony of the principal cells in the cortex and hippocampus (Dykes, 1997; Lin et al., 2006). Nucleus basalis cholinergic and GABAergic projection to the thalamic reticular nucleus suppress low-frequency (<15Hz) oscillations in thalamocortical networks (Steriade, 2004). The function and the postsynaptic target of the recently described basalocortical glutamatergic projection remains to be elucidated (Hur and Zaborszky, 2005).

Afferent Input

General

The study of inputs to local connections of basal forebrain cholinergic neurons proved to be difficult due to the many ascending and descending fibers that pass through the areas populated by basal forebrain cholinergic neurons. Cholinergic cells that project to a specific cortical area are dispersed throughout an extensive territory of the basal forebrain, including several cytoarchitectonic areas (Rye et al., 1984; Zaborszky et al., 1986a). Thus, the location of a cholinergic neuron within a particular subdivision of the basal forebrain does not necessarily determine its target region. Although the specific topographic arrangement of ascending brainstem and hypothalamic fibers (Geeraedts et al., 1990; Nieuwenhuys et al., 1982) may well give valuable clues regarding the origin of these fibers (see Fig. 28.5 and further discussion below), the verification of actual synaptic contact between the afferent fiber system and the cholinergic projection neurons requires appropriate combinations of double immunocytochemical methods at the ultrastructural level, in which the afferent fiber system and the cholinergic nature of the postsynaptic target can be unequivocally determined (Zaborszky and Heimer, 1989; Zaborszky and Leranth, 1985). The study of these inputs is further complicated by the fact that the dendrites of cholinergic neurons extend for several hundred microns (Duque et al., 2007). The rigorous application of electron microscopy in combination with tracer techniques (for ref see Zaborszky and Duque, 2003) and the reconstruction of single, chemically and electrophysiologically characterized basal forebrain neurons in rats (Duque et al., 2000, 2007; Duque and Zaborszky, 2006; Zaborszky et al., 2009) has begun to unravel the basic circuitry of this region.

On the basis of data obtained using a double strategy of identifying terminals on single cells using electron microscopy, together with mapping the 3D light microscopic distribution of putative contact sites of a given afferent system in relation to cholinergic profiles in their entirety (Cullinan and Zaborszky, 1991; Gaykema and Zaborszky 1996; Hajszan and Zaborszky, 2002; Zaborszky et al., 1993, 1997; Zaborszky and Cullinan, 1996), a number of organizational principles have emerged that are likely to be of general relevance (Zaborszky et al., 1991). These principles can be summarized by the following.

1) The distribution patterns of various terminals on cholinergic neurons correspond in most cases to the general topographical arrangement of the specific fiber systems in the forebrain. For example, various hypothalamic cell groups give rise to ascending terminal varicosities contacting cholinergic neurons (see Fig. 24 in Cullinan and Zaborszky, 1991; Fig. 2 in Zaborszky, 1992) whose location in the basal forebrain can be predicted on the basis of the general topography of fibers in the medial forebrain

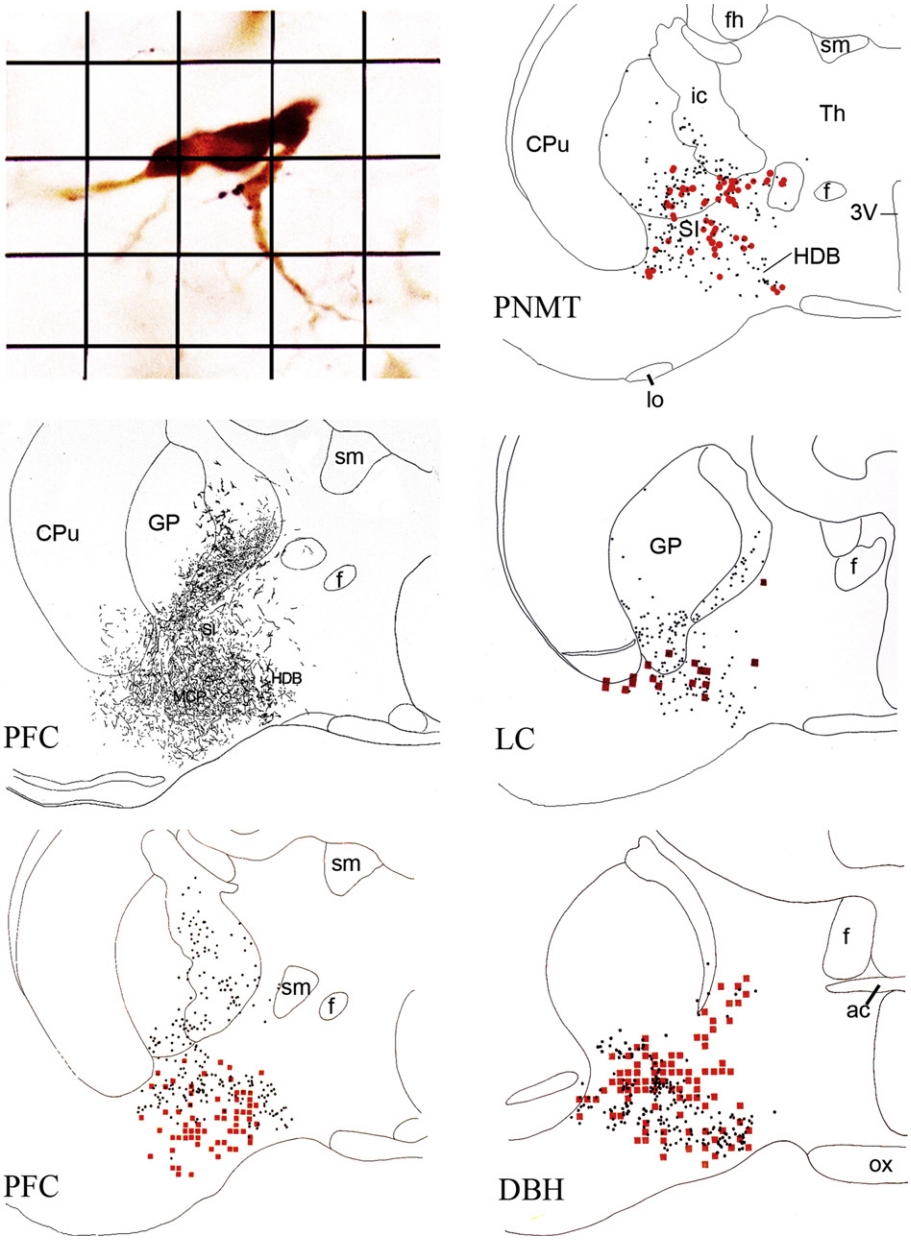

FIGURE 28.5 **Representative cases showing topographical distribution of terminal varicosities in close proximity to cholinergic profiles.** Sections were screened under 63x or 100x for the presence of putative contacts between cholinergic elements and PHA-L-labeled axonterminals from the prefrontal cortex (PFC); or the locus coeruleus (LC), or between cholinergic profiles and catecholaminergic axonal varicosities stained for PNMT (phenylethanolamine-N-methyltransferase), or DBH (dopamine-β-hydroxylase). Zones of putative contacts are labeled with squares or circle. The size of one pixel (or circle) corresponds to about 80 x 80 μm areas in the section. Upper left panel depicts PHA-L labeled varicosities adjacent to proximal portion of a cholinergic dendrite. The grid simulates the proportion of the ocular reticle used to screen sections. One division of the grid is 16 μm. Abbreviations: 3V, 3rd ventricle; CPu, caudate putamen; fh, fimbria hippocampi; HDB, nucleus of the horizontal limb of the diagonal band; f, fornix; GP, globus pallidus; ic, internal capsule; lo, lateral olfactory tract; MCP, magnocellular preoptic nucleus; sm, stria medullaris; stria terminalis; SI, substantia innominata; ox, optic chiasm; Th, thalamus.

bundle, as described by Nieuwenhuys and his colleagues (Geeraedts et al., 1990; Nieuwenhuys et al., 1982). The overwhelming dorso-ventral position of adrenergic and noradrenergic varicosities in close proximity to cholinergic dendrites/cell bodies (Fig. 28.5) tend to correspond to localization of various catecholaminergic ascending axons as described earlier (Bjorklund and Lindvall, 1984; Byrum and Guyenet, 1987; Chang and Kuo, 1989; Jones and Moore, 1977; Jones and Yang, 1985; McKellar and Loewy, 1982; Swanson and Hartman, 1975; Zagon et al., 1994).

2) Inputs to cholinergic neurons are shared with those to adjacent non-cholinergic neurons. In most cases

examined, labeled terminal varicosities detected in the basal forebrain were related to both cholinergic and non-cholinergic neurons; for instance ascending noradrenergic and dopaminergic axons synapse with both cholinergic and non-cholinergic neurons, including parvalbumin-containing cells (Gaykema and Zaborszky, 1996; 1997; Zaborszky et al., 1993).

3) Afferents to the basal forebrain cholinergic system may be restricted or relatively diffuse. Several inputs examined showed a preferential distribution towards a subset of basal forebrain cholinergic neurons. For example, inputs from the nucleus accumbens tend to synapse on ventral pallidal cholinergic neurons (Zaborszky and Cullinan, 1992). Furthermore, the distribution of several peptides in the basal forebrain suggests that peptidergic axons might contact subpopulations of basal forebrain cholinergic neurons (Zaborszky, 1989a, b). On the other hand, if we consider the total noradrenergic and adrenergic projection, using DBH antibody labeling (Fig. 28.5), these afferents apparently contact extended portions of the basal forebrain cholinergic system. However, comparing the location of PNMT, locus coeruleus and the DBH terminals in close proximity to cholinergic profiles suggest that various catecholaminergic cell groups can affect only a subpopulation of cholinergic neurons in spite of the possibility that perhaps most cholinergic neurons receive such input from various sources.
4) Specific vs. quasi-random afferents to basal forebrain cholinergic neurons. Interestingly, a detailed EM study could not identify cortical synapses on cholinergic neurons, and the restricted prefrontal input exclusively contacted non-cholinergic neurons, including parvalbumin-containing neurons in the basal forebrain (Zaborszky et al., 1997). Furthermore, Leranth and Vertes (1999) could not identify serotonin (5HT) containing synapses on cholinergic septal neurons, similar to our studies indicating that 5HT axons seem to avoid cholinergic cells in the basal forebrain, but instead synapse on calretinin-containing neurons (Hajszan and Zaborszky, 2000), indicating some degree of specificity in basal forebrain circuits.

The above data support the notion that cholinergic neurons do not maintain afferent connections distinct from neighboring non-cholinergic cells, but rather participate to some extent in the circuitry of the forebrain regions in which they are located, as suggested by Grove (1988). Thus, the emerging view is that different subsets of cholinergic neurons receive different combinations of afferents according to their location in the basal forebrain. Since there exist only a few studies that have used triple-labeling at the EM level (input-, output- elements and ChAT for the post-synaptic neuron), we have relatively limited knowledge about the specific input-output relations of cholinergic neurons. Only one study established directly that cholinergic neurons that project to the amygdala receive GABAergic input (Zaborszky et al., 1986b). Additionally, a study using high magnification light microscopic screening for putative contact sites suggests that various portions of the prefrontal cortex are in reciprocal connection with basal forebrain projecting neurons to the prefrontal cortex, although EM studies need to confirm this notion (Spiga and Zaborszky, 2006).

Identified Synapses on Basal Forebrain C Neurons

The afferent input to basal forebrain cholinergic neurons has been reviewed in several earlier papers (e.g. Zaborszky, 1992; Zaborszky et al., 1991, 1999). More recently, the inputs were discussed in terms of their significance in sleep-wake regulation exerted by the cholinergic neurons (Zaborszky and Duque, 2003). Based on electron microscopic studies, basal forebrain cholinergic neurons receive ascending brainstem input from adrenaline containing neurons of the medulla (Hajszan and Zaborszky, 2002), from the locus coeruleus (Zaborszky et al., 1993), and from the dopaminergic substantia nigra and ventral tegmental area (Gaykema and Zaborszky, 1996; Zaborszky and Cullinan, 1996). Various hypothalamic nuclei, including orexin/hypocretin neurons, synapse with cholinergic and non-cholinergic neurons in the basal forebrain (Cullinan and Zaborszky, 1991; Wu et al., 2004; Zaborszky and Cullinan, 1989). Histaminergic axons from the tuberomamilary nucleus surround basal forebrain cholinergic neurons, although electron microscopic evidence to confirm synapses is lacking (Turi et al., 2004; see Fig. 28.6E). Histaminergic and orexin /hypocretin-containing neurons represent key nodes in the circuit regulating arousal (Blanco-Centurion et al., 2007; Murillo-Rodriguez et al., 2008). Cholinergic neurons in the nucleus basalis and both cholinergic and GABAergic neurons in the septum are excited by histamine and orexin/hypocretin (Eggermann et al., 2001; Khateb et al., 1995; Wu et al., 2004).

Forebrain afferents originate in the nucleus accumbens (Zaborszky and Cullinan, 1992) and the amygdala (Jolkonnen et al., 2002; Paré and Smith, 1994; Zaborszky et al., 1984). Cortical inputs to the basal forebrain originate in the rat only in restricted portions of the cortex, including medial, lateral and the orbitofrontal part of the prefrontal cortex, with a small contribution from the insular-piriform cortices (Zaborszky et al., 1997). Interestingly, prefrontal fibers synapse only with non-cholinergic neurons, including parvalbumin-containing cells in the VP. In spite of extensive work, it is unclear whether some of the local interneurons

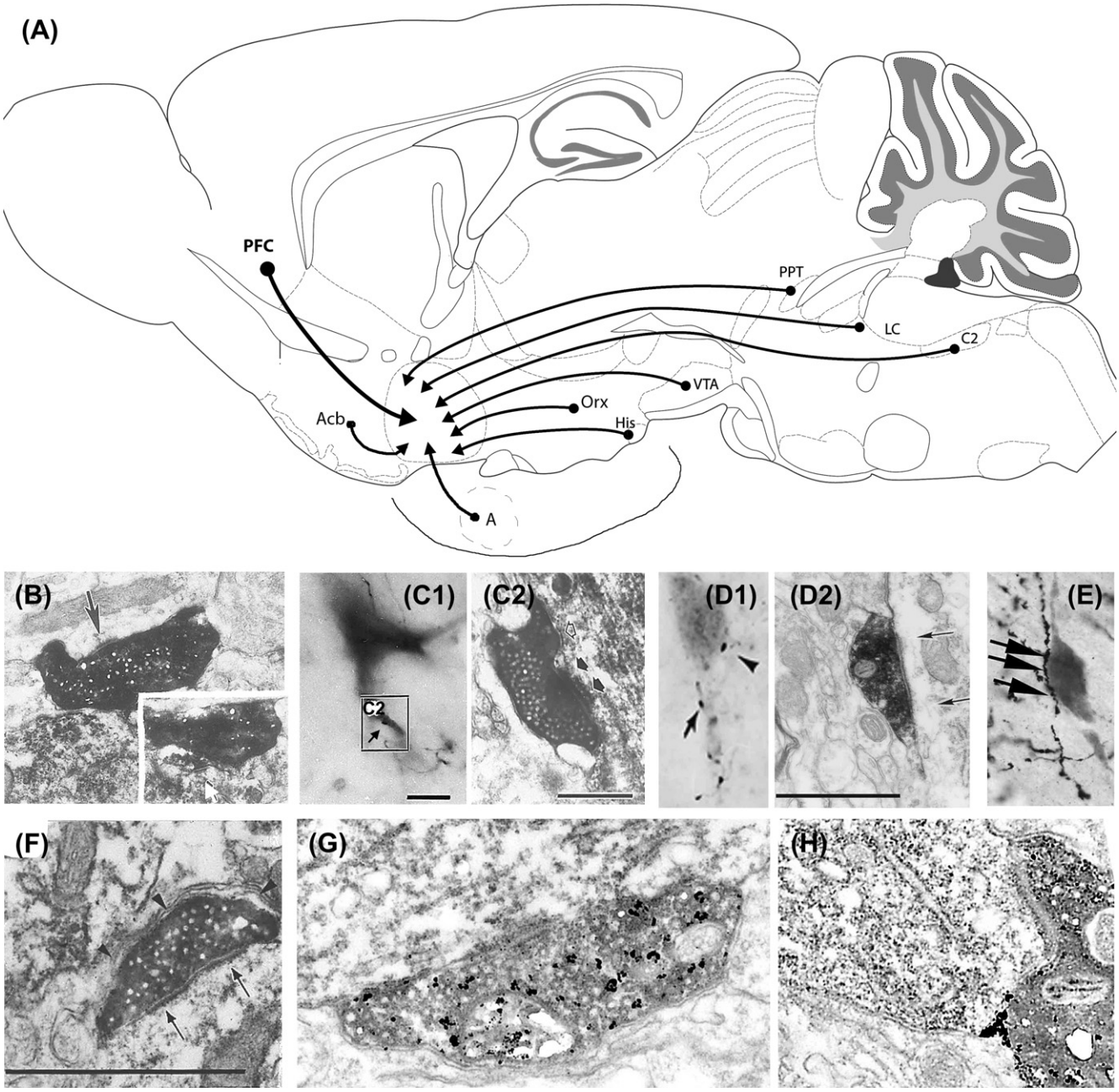

FIGURE 28.6 (A) Schematic illustration showing the major inputs to the basal forebrain cholinergic area projected on a sagittal section (Fig. 110 from the Franklin-Paxinos atlas). (B) PHA-L labeled axon terminal from the locus coeruleus (LC) enter into synaptic contact with an unlabeled (black arrow) and a cholinergic profile (white arrow). From Zaborszky and Heimer, 1989. (C1–C2) Asymmetric synaptic contact between a PHA-L labeled axon terminal, originating in the ventral tegmental area (VTA), and a cholinergic dendrite. Boxed area in C1 is shown under the electron microscope in C2 (from Gaykema and Zaborszky, 1996). (D1–D2) PHA-L labeled axon varicosities, arising from the mesopontine tegmentum (PPT), climb a parvalbumin-containing neuron (Zaborszky et al., 1999). Left arrow in D1 is shown under the electron microscope in panel (D2). (E) Histamin-containing (His) axon varicosities around a cholinergic cell body (Turi and Zaborszky, unpublished observation). (F) PHA-L labeled synaptic bouton from the prefrontal cortex (PFC) in asymmetric synapse with a parvalbumin-containing dendrite. Thin arrows point to the postsynaptic thickening. From the material from Zaborszky et al., (1997). (G) PNMT-positive axon terminal, originating in the medullary C2 cell group, in synapse with a cholinergic dendrite (from the material form Hajszan and Zaborszky, 2002). (H) orexin-labeled (Orx) bouton in synaptic contact with a cholinergic dendrite in the septum (from the material from Wu et al., 2004). A, amygdala; Acb, accumbens nucleus. Scale in C1= 10 μm; C2= 0.5 μm; D2= 1 μm; C= 1 μm.

(NPY, somatostatin, etc) receive prefrontal input. Fig. 28.6 summarizes some of the long-distance inputs that were identified by electron microscopy in rats.

Glutamatergic synapses, using both vGluT1 and vGluT2 transporters to cholinergic and non-cholinergic basal forebrain neurons, have been recently described (Hur et al., 2009). According to this study, glutamatergic synapses supply 40–50% of all synapses to cholinergic neurons in the SI/EA. Cholinergic cells in the basal forebrain of mice express NMDA receptors (De Souza Silva et al., 2006) and glutamatergic stimulation in the basal forebrain results in cortical ACh release and EEG cortical activation (Cape and Jones, 2000; Fournier et al., 2004a; Wigren et al., 2007). The precise source of glutamatergic afferents to basal forebrain cholinergic neurons remains to be elucidated. GABAergic synapses on cholinergic neurons are very rich in the VP and SI/EA (Chang et al., 1995; Ingham et al., 1988; Zaborszky et al., 1986b) and their number could equal that of glutamatergic synapses. Part of the GABAergic input to cholinergic neurons in the VP originates in the nucleus accumbens (Zaborszky and Cullinan, 1992). Additionally, substance P (Bolam et al., 1986), enkephalin (Chang et al., 1987; Martinez-Murillo et al., 1988), somatostatin (Zaborszky, 1989b) and NPY synapses (Zaborszky and Duque, 2000) have been described on cholinergic neurons.

Intrinsic Connections

Using a combination of juxtacellular filling and subsequent chemical identification and morphological reconstruction, several locally arborizing neurons were identified that contained NPY (Duque et al., 2007). Using electron microscopy and double immunolabeling, 40 synapses of local axon terminals of an electrophysiologically identified NPY neuron were reconstructed: 30% of these synapses were with cholinergic neurons, the rest with unlabeled dendritic shafts and spines (Zaborszky et al., 2009). Fig. 28.7B shows a synapse on a cholinergic dendrite originating from an electrophysiologically and morphologically identified NPY neuron. Using a transgenic mouse line that expresses Renilla GFP in NPY neurons, we observed that NPY boutons often synapse with NPY dendrites and soma (Fig. 28.7C). Another population of locally arborizing neurons in rats contains somatostatin (Zaborszky, 1989b; Zaborszky and Duque, 2000). Somatostatin-containing terminals were observed on cholinergic neurons (Zaborszky, 1989b and unpublished observation) and on small dendritic branches and spines of unidentified neurons (Gyengesi and Zaborszky, unpublished). Cholinergic projection neurons posses extensive local collaterals (Duque et al., 2007), however, their postsynaptic target has not been identified. Parvalbumin-containing basal forebrain neurons in rats possess few collaterals (Duque and Zaborszky, 2006), and some of these synapse on cholinergic dendrites (Zaborszky and Duque, 2000 and Fig. 28.7D). Glutamatergic neurons containing vGluT2 are abundant in basal forebrain areas rich in cholinergic neurons in rats (Hur and Zaborszky, 2005) and mice. Based on lesion studies, vGluT2 neurons in the septum innervate parvalbumin-containing neurons in rats (Hajszan et al., 2004). Since cholinergic neurons receive vGluT2 input in the basal forebrain (Hur et al., 2009), it is possible that some of this glutamatergic input originates in locally arborizing vGluT2 neurons.

Organization of Cholinergic Neurons

3D reconstructions suggest that cholinergic neurons and the three classes of non-cholinergic, calcium-binding protein-containing neurons (parvalbumin, calretinin and calbindin) in rats show large-scale association in the entire basal forebrain (Zaborszky et al., 1999). By applying density and relational constraints to cell populations combined in a common 3D coordinate system, we showed that cholinergic and non-cholinergic neurons show small-scale associations in the form of regionally specific cell clusters in the entire cholinergic basal forebrain space, i.e. the space occupied by the cortically projecting cholinergic cell bodies (Zaborszky et al., 2005). Although the existence of cell aggregates in the cholinergic forebrain has been known for more than 20 years, the development and use of new visualization and analytical tools (Nadasdy et al., 2010) have recently enabled the quantitative assessment of these cell clusters and for the first time, specific questions can be addressed relating to the organizational principles of the basal forebrain. Cholinergic cell clusters can also be recognized in mice (Fig. 28.8). A preliminary analysis in the rat has been done of the spatial relationship between cholinergic cell clusters and various neuronal populations whose cortical targets have been defined (Zaborszky et al., 2008). This analysis suggests that cell clusters in the rat basal forebrain may serve an associational function that involves transmitting information from specific locations in the basal forebrain to a small subset of cortical areas that most likely are interconnected. These findings point beyond the general notion that the cholinergic system is a topographically organized projection system: this mechanism may support interactions between cortical and subcortical attentional networks (Parikh and Sarter, 2008; Sarter et al., 2009; see also concluding remarks). In the light of the availability of transgenic mice expressing ChAT neurons, it would be worth determining whether specific cholinergic clusters also project to associated cortical areas in mice.

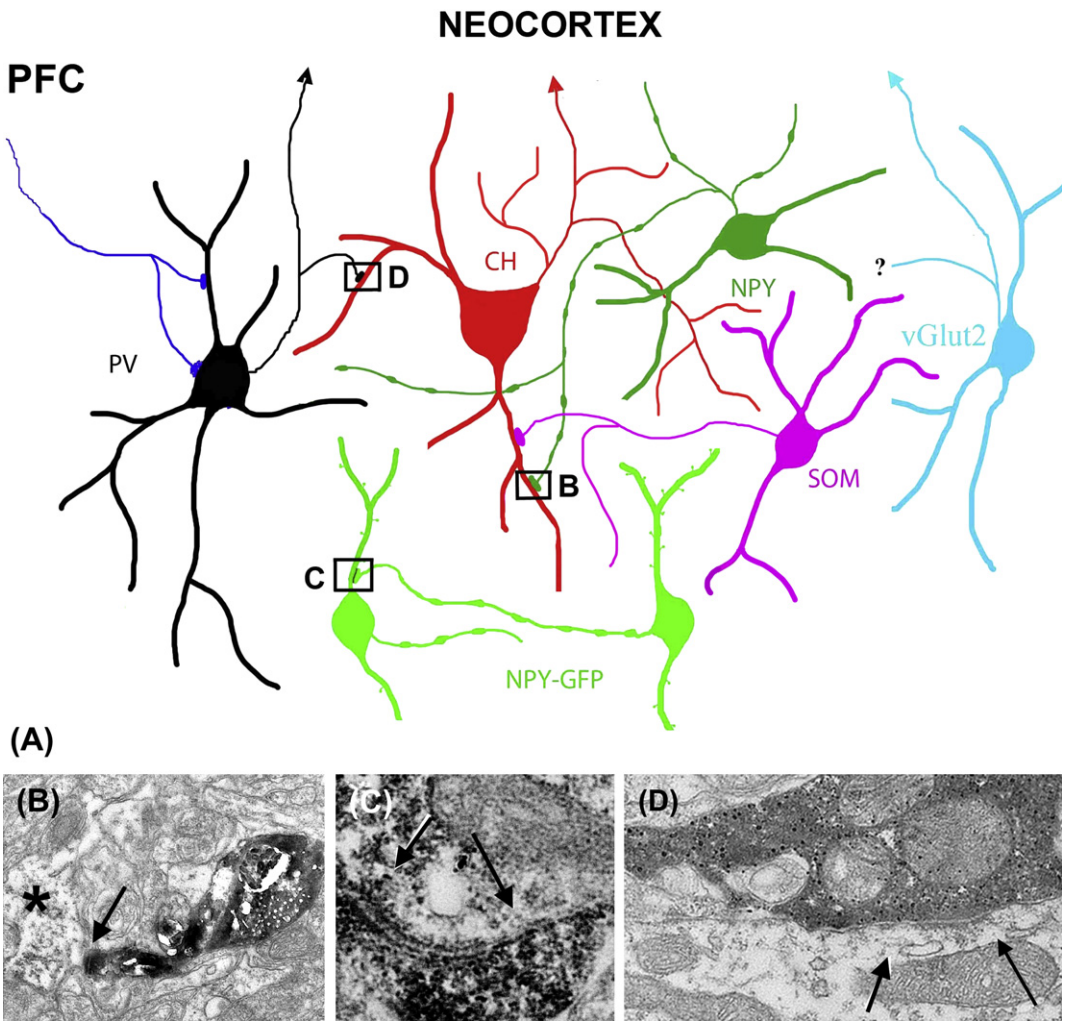

FIGURE 28.7 Intrinsic connections. (A) Schematic diagram illustrating putative interconnections among various cell types in the basal forebrain. Individual cell types are labeled with different colors. Black neuron: A parvalbumin-containing GABAergic neuron receives glutamatergic input from the prefrontal cortex (PFC); its main axon projects to the neocortex that gives rise to a local collateral synapsing with a cholinergic dendrite shown in panel (D). From the material of Zaborszky and Duque, 2000. Red neuron: a cholinergic (CH) projection neuron; it receives synaptic input from a local NPY neuron (green); an example of an NPY-cholinergic synapse from an electrophysiologically identified local NPY neuron is shown in panel (B). The cholinergic dendrite receives another synapse from a locally arborizing somatostatin-containing neuron (SOM, purple). Green neurons symbolize local NPY neurons. The lower 2 NPY neurons from NPY-GFP transgenic mice are interconnected: the synapse is displayed in panel (C). (B) The cholinergic profile (asterisk) is labeled with DAB that is distinctly different from the NPY bouton that is reacted with NiDAB. Note the presence of clear vesicles in the NPY-terminal. Arrow points the synaptic cleft. (C) An NPY-dendrite receives a synapse from an NPY-axon, both profiles are labeled with DAB. Arrows point to the postsynaptic side (Gyengesi and Zaborszky, unpublished observation). (D) An electrophysiologically and chemically identified parvalbumin neuron (a 3D reconstruction of this neuron has been published in Zaborszky and Duque, 2000) with its local axon-collateral that synapses with a lightly-labeled cholinergic dendrite. The parvalbumin neuron was labeled with NiDAB and the cholinergic one with DAB. Arrows point to the postsynaptic thickening.

DEVELOPMENT AND MOLECULAR SPECIFICATION OF BASAL FOREBRAIN CHOLINERGIC PROJECTION NEURONS

Progenitor Domains of the Subpallium

Along the dorso-ventral axis the telecephalon becomes subdivided into the pallium (the cortical anlage) and the subpallium. The subpallium (or subcortical telencephalon), is relatively complex in terms of the structures that are formed from this area, which include the strio-pallidal system, parts of the amygdala, and the septum as well as all cholinergic neurons, including basal forebrain projection and striatal interneurons and cortical interneurons that emigrate tangentially from the subpallium (Puelles et al., 2000). The invaginated mouse subpallium is divided into several progenitor domains, including the lateral (LGE) and medial (MGE) ganglionic eminence, the origin of the striatum and pallidum, respectively. The non-invaginated

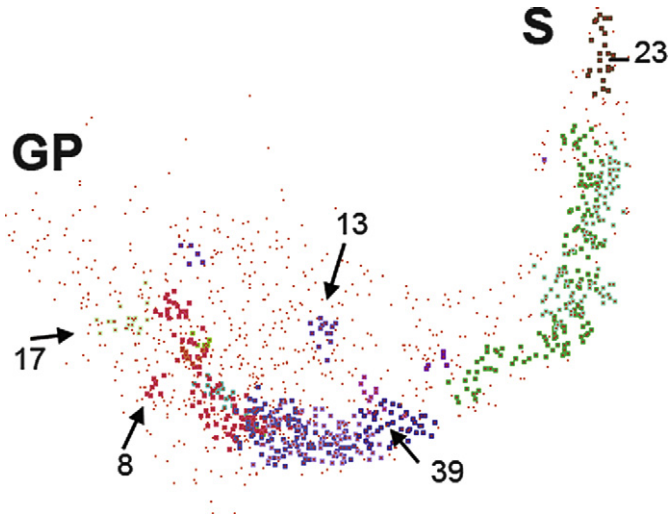

FIGURE 28.8 **Clustered organization of cholinergic neurons in mice.** Composite map of cholinergic cell bodies (small red dots) mapped from 10 sections 300 μm apart with the Neurolucida^R system from an NPY-GFP mice (same as shown in Figs. 1–3). For better visualization outlines of sections were removed. Cholinergic cells constitute a continuous collection of cells from rostral (S, septum, right aspect of the diagram) to caudal towards the globus pallidus (GP, left side of the diagram). Anterior view. The distribution of cholinergic cell bodies (n=1372) is not homogeneous, dense clusters of cells are interrupted by regions of low cellular density. Using a cluster program (Nadasdy et al., 2010) that identifies cell clusters based upon cell density (n) around each neuron within a given diameter (d). Based upon scanning the parameter space, we choose cluster parameters n=7; d=250 μm, thus in this analysis, in each cluster around each cell (seed) within a 250 μm diameter there are at least 7 cells. See details in the original publication. Square symbols with different colors label seeds within the same non-overlapping cluster; some of them are labeled with their seed numbers. Single cholinergic cells that are not assigned to any cluster remained labeled with small red dot. According to preliminary analysis in rat, each cluster project in a specific combination only to a few specific cortical targets that seem to be interconnected.

telencephalon includes the anterior peduncular area (AEP), the commissural septo-preoptic area (POC), the preoptic area (POA) and the preoptic hypothalamic region. These anatomically defined regions contain several progenitor subdomains that are uniquely defined by the combinatorial expression of basic helix-loop helix (bHLH) and homeobox transcription factors (Flames et al., 2007; Moreno et al., 2009; Puelles et al., 2004). Using knockout mouse lines devoid of various transcription factors that are expressed normally in the subpallium, basal forebrain cholinergic neurons may originate in the POC/AEP, MGE and septal ventricular zone (Ashbreuk et al., 2002; Elshatory and Gan, 2008; Fragkouli et al., 2005; Furusho et al., 2006; Marin and Rubinstein, 2001; Mori et al., 2004; Schambra et al., 1989; Zhao et al., 2003).

More recently, Garcia-Lopez et al. (2008) redefined the subpallial progenitor domains and suggested that the entire population of corticopetal cholinergic cells originates from the POC. This recently defined POC contains a domain that was previously described as part of AEP. The AEP topographically relates to the telencephalic stalk, where the internal capsule/cerebral peduncle enters/exits the telencephalon and corresponds to a distinct radial domain, sandwiched between the MGE (pallidum proper) and the POC. The AEP domain expresses Dlx5, Lhx6 and moderate Nkx2.1 and Lhx7/8 genes and lacks sonic hedgehog (Shh). As best appreciated in horizontal sections (e.g. Fig. 5 in Garcia-Lopez et al., 2008), this domain extends from the ventricular zone at the rostral septum, sandwiched between the prospective HDB and VP through the path of the stria terminalis into the bed nucleus of the stria terminalis and below the developing globus pallidus into medial regions of the amygdala. This cell corridor appears to produce somatostatin, calbindin and NPY neurons and largely corresponds to the sublenticular extended amygdala (EA) and basal part of the SI. The POC domain, encompassing an area at the base of the septum related to the anterior commissure, dorsolateral and lateral preoptic areas, express Nkx2.1, Lhx6, Lhx7/8, Gbx1 and Shh at E12.5. Fig. 28.9 schematically depicts the embryonic telencephalon with the various subpallial progenitor domains, indicating the putative location of specific basal forebrain cholinergic precursor lines.

Transcription Factors Determining Cholinergic Fate

Progenitor cycling, cell cycle exit, migration, differentiation and survival depend on the complex interaction of a hierarchy of genes in the subpallium that is similar to the one described in the spinal cord (e.g. Lee et al., 2008). Whereas much is known about the specification of cortical interneurons and projection neurons (Merot et al., 2009; Suter et al., 2007; Wonders and Anderson, 2006; Xu et al., 2004), data are only slowly emerging relating to the basal forebrain cholinergic system. Such information is important for understanding how developmental neuropsychiatric disorders could be associated with dysfunctions of the basal forebrain cholinergic system, and may help to design strategies to rebuild the diseased basalo-cortical network to alleviate the devastating consequences of cholinergic loss in AD and related disorders.

Nkx2.1 is one of the earliest (E9–9.5) genes expressed in the medial neural plate which overlies the *Shh* secreting axial mesoderm (Puelles et al., 2000). Nearly all proliferating cells in the MGE and the more ventrally located preoptic region express NKx2.1 and all cholinergic projection neurons of the basal forebrain express Nkx2.1 at P25 (Xu et al., 2008). Nkx2.1 is crucial for the

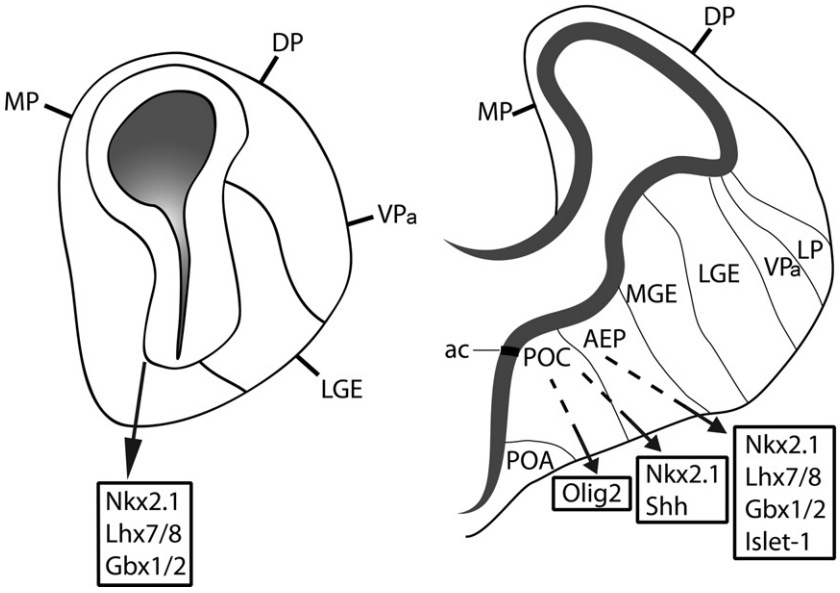

FIGURE 28.9 Schematic drawings of two coronal sections (left rostral, right caudal) through the embryonic telencephalon of the mouse (at about E14.5) to show the various ventricular domains. The subdivisions of the subpallium are according to Garcia-Lopez et al., (2008). The molecular specification of basal forebrain cholinergic projection (basal forebrain cholinergic) neurons based on several studies listed in this chapter suggests that various subpopulation of basal forebrain cholinergic neurons may originate from different progenitor domains. AEP, anterior peduncular area; ac, anterior commissure; DP, dorsal pallium; LGE, lateral ganglionic eminence; LP, lateral pallium; MGE, medial ganglionic eminence; MP, medial pallium; POA, preoptic area; POC, commissural septo-preoptic area; VPa, ventral pallium.

genesis of striatal and basal forebrain cholinergic projection neurons, supported by findings that Nkx2.1$^{-/-}$ mutants at birth show a complete absence of the high affinity NGF receptor (TrkA) expressing cells, a marker for basal forebrain cholinergic neurons (Marin et al., 2000; Sussel et al., 1999). Nkx2.1 is also expressed in cells positive for GAD, thus this gene is not sufficient to specify cholinergic identity.

Lhx7 (also known as L3/Lhx8 or Lhx8). Cholinergic projection neurons in the MS/VDB are reduced by 70% and in more posterior areas of the basal forebrain by 90% whereas cholinergic neurons in the nucleus accumbens and olfactory tubercle are spared in mice with a null mutation of the gene Lhx7 (Fragkouli et al., 2005; Mori et al., 2004; Zhao et al., 2003).

Islet1, a LIM homeodomain transcription factor, is richly expressed deep in the MGE, LGE and in the primordial septum at E15 in rat and in the developing striatum at E18–20. As early as E20, striatal Islet-1 cells co-express TrkA, and in the P7-P14 striatum, the majority of Islet-1 cells co-expressed ChAT as well (Wang and Liu, 2001). The co-localization of Islet-1 and ChAT has been recently shown in cells of the magnocellular preoptic area and in the septum in adult mouse brain (Elshatory and Gan, 2008). Conditional deletion of Islet-1 results in depletion of cholinergic interneurons in the striatum and cholinergic projection neurons in the nucleus basalis, without significantly affecting cholinergic projection neurons in the septum (Elshatroy and Gan, 2008).

Gbx1/Gbx2, homeobox genes are expressed in the mantle zone of the MGE/POA during development and are present in the basal forebrain cholinergic neurons of adult mice (Assinmacopoulos et al., 2000; Asbreuk et al., 2002). Islet-1 and Gbx2 expression are reduced in the proliferative and mantle region of the preoptic area in Lhx7-0 mutants (Zhao et al., 2003).

Shh (Sonic hedgehog) secretion from the axial mesoderm is required for ventral specification of the entire neuraxis from the spinal cord to the basal telencephalon (Ericson et al., 1995; Shimamura and Rubinstein, 1997). *Shh* expression begins in the ventral MGE and POA shortly after Nkx2.1 and fate mapping analysis of the Nkx2.1$^{+/-}$;ShhCre$^{/+}$ mice at PO shows that many cells in the diagonal band, VP and preoptic region derive from the *Shh* lineage, with the majority also expressing Nkx2.1 (Flandin et al., 2010). *Shh* and its receptor, Ptc-1, are expressed by cholinergic neurons in the septum in adult mice (Reilly et al., 2002). In basal forebrain culture *Shh* and NGF show synergistic effect: by 8 days *in vitro* the number of ChAT-positive cells increased over and above the effects of NGF alone (Reilly et al., 2002).

Olig2, a basic helix-loop-helix transcription factor, is expressed in the MGE, and AEP/POA area. About 5% of Olig2 lineage cells express ChAT and the number of cholinergic cells was reduced by 40% in the Olig2 knockout mouse in the caudate putamen and the caudal part of the basal forebrain magnocellular complex, including the MCPO, and SI, but not in the globus pallidus, diagonal band and medial septal nucleus (Furusho et al., 2006). The expression pattern of Nkx2.1, and Lhx8 transcription factors did not change in Olig2 knockout mice.

BMPs (bone morphogenetic proteins), members of the transforming growth factor-β (TGF- β) superfamily of growth and differentiation factors play roles in the dorsoventral patterning of the neural tube vis-à-vis

Shh. BMP-9 is highly expressed in the septum and spinal cord at E14 in mouse and, in primary cell cultures, upregulates ACh synthesis (Lopez-Covilla et al., 2000). Moreover, BMP-9 induced/enhanced the expression of several genes that belong to the basal forebrain cholinergic transcriptome indicating a potential role of BMP-9 in specification and maintenance of the cholinergic phenotype (Lopez-Coviella et al., 2005).

In summary, the differentiation of the various basal forebrain cholinergic projection neurons is not uniform: apparently, the differentiation of rostrally located cholinergic projection neurons (septal, Ch1-2) is Islet-1 independent. On the other hand, the co-operation of Lhx7 and Islet-1 is necessary to promote cholinergic differentiation in more caudally located basal forebrainC neurons (Fragkouli et al., 2009). In addition, a proportion of basal forebrain cholinergic neurons derive from Olig2 lineage cells that seem to be independent from the Lhx8 and Nkx2.1 lineage (Furusho et al., 2006). Several other homeodomain or bHLH transcription factors, including *Dlx (*distal-less), *Mash-1* (mammalian achaete-schute homolog), *Gsh1/2* and *Vax1* are expressed in subpallial areas from where cholinergic neurons originate (Anderson et al., 1997; Long et al., 2007; 2009; Poitras et al., 2007; Soria et al., 2004; Taglialatela et al., 2004; Yun et al., 2003). However, no data suggests their direct involvement in cholinergic differentiation. If indeed basal forebrain cholinergic neurons do originate from several progenitor domains, it will be interesting to determine whether the twisted bundle arrangement of cholinergic projection neurons along with the global and local configuration of basal forebrain clusters as suggested for rats (Zaborszky, 2002; Zaborszky et al., 2005) are determined by the special temporal and spatial expression pattern of various transcription factors. It is also unresolved whether or not the various progenitor populations arise at the same time or according to a caudal to rostral gradient – as indicated in the rat using tritiated thymidine autoradiography (Bayer and Altman, 2004; Semba and Fibiger, 1988).

In mice, cholinergic neurons in the basal forebrain arise between E11–E15 (Schambra et al., 1989; Sweeney et al., 1989). In rats, using immunostaining for the low-affinity NGF receptor $p75^{NTR}$, it is found that cholinergic neurons are first visible in the ventrolateral telencephalic wall at E13 and their axons accumulate in the intermediate zone beneath the subplate before entering the cortical plate at about the time of birth (Koh and Loy, 1989). ChAT activity can first be measured at P6 in BALB/c mice and reach adult values by seven weeks (Hohmann and Ebner, 1985). Perikaryal areas in forebrain regions undergo an initial stage of progressive soma and proximal dendrite hypertrophy which peaks during the third postnatal week, followed by a decrease of soma that stabilizes around P35 (Gould and Butcher, 1989). These morphological measures are paralleled by changing levels of ChAT- and $p75^{NTR}$-mRNA in rat basal forebrain cells (Koh and Higgins, 1991). ChAT activity in rat cortical areas reaches adult levels at P35 (Armstrong et al., 1987; Dori and Parnavelas, 1989; McDonald et al., 1987). An excellent review of phylogenetic data and ontogentic maturation of the basal forebrain cholinergic system is given by Semba (2004).

TRANSGENIC MOUSE MODELS OF NEURODEGENERATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS

General Characteristics of AD

Transgenic animals are extensively used to study *in vivo* gene function, to model human neurodegenerative diseases, and to monitor therapeutic strategies for these diseases (e.g. Biscaro et al., 2009; Caccamo et al., 2006; Gotz and Ittner, 2008; Jankowsky et al., 2005). The reader is referred to recent reviews on commonly used techniques for producing transgenic mice for modeling AD (Elder et al., 2010; Gama Sosa et al., 2010; Garringer et al., 2010). We will not attempt an exhaustive review of the vast body of literature on transgenic mouse modeling of neurodegeneration of basal forebrain cholinergic neurons. Table 28.1 summarizes some of the transgenic lines that are linked to cholinergic deficits in AD. Many transgenic mouse lines are available at http://jaxmice.jax.org/.

Human AD occurs in middle or late life and is characterized by a progressive dementia. Typically, cognitive impairments appear insidiously, with impairments in memory, language, attention, visuo-spatial perception, judgment, and behavior becoming progressively more severe. The neuropathology of AD includes the formation of extracellular neuritic amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFT) along with neuronal and synapse loss in selected brain areas, including the entorhinal cortex, the hippocampus, association cortices and subcortical structures such as the amygdala and the basal forebrain cholinergic system (for a more detailed review on pathology, see Gotz and Ittner, 2008; Crews et al., 2010).

Massive cell death in the nucleus basalis was originally suggested to be one of the major hallmarks of AD (up to 90% cell loss: Whitehouse et al., 1981; 20–60%: Iraizoz et al., 1991; Lehericy et al., 1993; Cullen and Halliday, 1998) and the resulting ACh deficits in cortical and hippocampal regions have been correlated with the severity of dementia (Davis et al., 1999; Shinotoh et al., 2000). However, more recent studies

TABLE 28.1

	Author	Type of transgene	Cholinergic deficit
APP	Sturchler-Pierrat et al., 1997	$hAPP_{751}$ is expressed with the Swedis double mutation at positions 670/671 alone or in conjuction with the London mutation (V717I). Thy-1 promoter	Dystrophic putative cholinergic fibers (stained with acetylcholinesterase=AChE) in the plaque vicinity in the hippocampus
APP	Bronfman et al., 2000	Transgenic mice carrying the APP (695 isoform) London (V642I) mutation	Extensive AChE-positive fiber depletion of the subiculum with reduction of cholinergic cell size in the MS, but not NBM in mice of 17-22 months old. Increased cholinergic fiber density in CA1 and dentate gyrus
APP	Boncristiano et al., 2002	APP23 Mice express mutant APPswe under the control of Thy1 promoter.	In aged (24 months) heterozygote APP23 mice reveal modest (11%) decrease in ChAT activity and disruption and decrease in cholinergic (AChE) fiber density compared with age-matched wild-type mice. No loss of BFC (ChAT) neurons, but the volume of cholinergic neurons in the MS/VDB at 8 and 23 months showed significant reduction. 3-8 months after electrolytic lesioning NBM, cortical ChAT activity decreases 38% with significant cholinergic fiber loss. Disruption of BFC system does not promote cortical amyloidosis
APP	German et al., 2003	Homozygous PDAPP, mice express a hAPP cDNA with the Indiana mutation (V717V-F). Platelet-derived growth factor β promoter	There was an age-related reduction in the density of cholinergic nerve terminals (stained with ChAT) in the cerebral ctx and hippocampus. The most prominent loss occurs between ages 2 and 4 months and the cholinergic degenerative changes occur before the deposition of Aβ plaques. Dystrophic cholinergic fibers in the vicinity of plaques. At age 2 years there was no difference in the number or size of BFC somata compared with 2-month old PDAPP mice
APP	Aucoin et al., 2005	Transgenic mice carrying familial AD-linked mutations ($hAPP_{SWE,IND}$)	Dystrophic ChAT axons near Aβ plaques. Significant (24-26%) decreases of ChAT innervation density in the hippocampus at 12/14 month and in parietal ctx at 18 month
$APP/BACE1^{null}$	Ohno et al., 2004	BACE1 knockout mice overexpress hAPP ($BACE^{-/-}$,$Tg2576^{+}$)	Impaired cholinergic (AHP in response to depolarizing current before and after carbachol) regulation of CA1 neuronal excitability found in Tg2576 AD model is ameliorated in these bigenic mice.
$APP/KLC1^{null}$	Stokin et al., 2005	$TgswAPP^{Prp}$;$KLC1^{wt}/KLC1^{null}$	Cholinergic and non-cholinergic axonal swellings in the BF increase with reduction of the kinesin light chain; increased accumulation of amyloid and plaques in sensory and entorhinal cortices with increasing age.
APP/α7 $nAChR^{null}$	Hernandez et al., 2010	A7KO-APP: Tg2576 (mice express APPswe) X mice heterozygous for the null mutation of nAChR (A7KO)	Increased reduction of ChAT protein and activity in the hippocampus and more severe hippocampal memory deficit in 5-month-old doubly transgene mice than the wt, A7KO or APP mice. There is a reduction of ChAT protein and activity in the BF, but similar ChAT activity is also reduced in A7KO animals.

(*Continued*)

TABLE 28.1—cont'd

	Author	Type of transgene	Cholinergic deficit
APP/M_1KO	Davis et al., 2010	APPswe/ind x M_1KO	Loss of M_1 mAChRs increases amyloidogenic APP processing in neurons, as evidenced by decreased carbachol-regulated shedding of the neuroprotective APP ectodomain APPsα and increased production of toxic Aβ peptides. Expression of M1 mAChRs on the M_1KO background rescued this phenotype.
APP/$p75^{NTR-/-}$	Knowles et al., 2009	Th1-hAPP $^{London/Swe}$ x $p75^{NTR-/-}$	Doubly transgenic mice exhibited significantly diminished hippocampal neuritic dystrophy and complete reversal of BFC neurite degeneration relative to those expressing wild-type $p75^{NTR}$
PS1/APP	Wong et al., 1999	Doubly transgenic mice ($APP_{K670N,M671L}$ + $PS1_{M146L}$, Holcomb et al., 1998)	Prominent diminution in the density and size of cholinergic (VAChT, ChAT) varicosities in the the frontal ctx and hippocampus. No significant changes in the size of BFC neurons at 8 months. Overexpression of PS1 $_{M146L}$ alone did not induce cholinergic pathology. Singly transgenic APP mice show increased density of cholinergic varicosities in the frontal and parietal cortices
PS1/APP	Jaffar et al., 2001	Doubly transgenic mice ($PS1_{M146L}$ + APPswe)	$p75^{NTR}$-IR fibers in the hippocampus and cortex were more pronounced in the APPswe and PS1 mice than the doubly transgenic mice. Dystrophic $p75^{NTR}$-IR fibers around plaques in the cortex and hippocampus. No change in BF cell size/number at 12 months of age. In the singly transgenic APP or PS1 mice the total number of $p75^{NTR}$-IR neurons in the medial septum increased. No NFT pathology.
PS1/APP	Savonenko et al., 2005	APPswe/PS1ΔE9	Strongest correlation between deficit in episodic-like memory task and total Aβ loads in the brain at 18 months of age. Mild decrease of cholinergic markers in the cortex and hippocampus
PS1/APP	Wang et al., 2006	PS1 M146V knock-in allele is expressed on wild-type PS1 ($PS1^{M146V/+}$) or PSI null ($PS1^{M146V/-}$) background and crossed with the Tg2576 APP mice	Introduction of the PS1 M146V mutation on Tg2576 background resulted in earlier onset of plaque pathology. Removing the wild-type PS1 in the presence of the PS1 M146V mutation greatly exacerbated the amyloid burden, indicating a protective role of the wild-type PS1 against the FAD mutation-induced amyloid pathology
PS1/APP	Perez et al., 2007	Heterozygous transgenic mice harboring mutant APPswe/PS1ΔE9	Dystrophic cholinergic (ChAT) neurites in the cortex and hippocampus appear as early as 2-3 month. Significant reduction in the density of cholinergic fibers in aged (16 mo) mice with reduced ChAT activity in the cortex and hippocampus. Occasional cholinergic dystrophic neuritis were seen in the vicinity of Aβ-IR plaques in the oldest mice in the BF. Cholinergic neuron number remained unchanged at 10-16 months. ChAT-IR neurons in the BF were enlarged in the oldest (12-16mo) mice compared to age-matched non-tg mice

Tau	Lewis et al., 2000	JNLP3 mice expressing 4R taus with the P301L mutation identified in familial cases of FTPD-17, mPrP promoter	First transgenic model with marked tangle pathology and cell loss in various forebrain and hindbrain regions. Mentioning of NFT lesion in the septal nuclei without description of the location or type of neurons affected
hTau	Andorfer et al., 2005; Polydoro et al., 2010	Crossing mice that express a tau transgene derived from a human PAC, H1 haplotype, termed 8c mice with tau knock-out mice that have a targeted disruption of exon 1 of tau	Aged mice expressing nonmutant human tau in the absence of mouse tau developed NFTs and extensive cell death in the piriform cortex, neocortex and hippocampus with spatial memory deficits. No cholinergic deficit is reported
Tau	Kohler et al., 2010	pR5 mouse strain that overexpres the longest human tau isoform (2+3+4R) together with the P301L mutation under the control of mThy1.2 promoter (Gotz et al., 2001)	pR5 mice develop widespread neurofibrillary lesion (hippocampus, amygdala, somatosensory cortex), but BFC neuron did not express the human tau , nor they show differences (number or mean are of profiles) with ChAT staining as compared to non-tg littermates at 20 months of age
Tau/APP	Casas et al., 2004	APP(SL)PS1KI, carries M233T/L234P knocked-in mutations in PS1 with overexpression of hAPP751 carrying the London (V717I) and Swedish (K670N/M671L) mutations under the control of the Th1 promoter	There is a 50% cell loss of CA1 neurons at 10 month of age. No report on BFC pathology
Tau/APP	Ribe et al., 2005; Perez et al., 2005	Mice expressing double Swedish mutation APPswe (K670N-M671L) and human 4-repeat tau containing a triple mutation (G272V, P301L, R406W)	Accelerated neurofibrillary degeneration and neuronal loss in the hippocampus and entorhinal ctx relative to single transgenic Tau line. No cholinergic deficit reported
3 x TgAD	Robertson et al., 2009	Harboring the $PS1_{M146V}$, hAPPswe and tau_{301L} transgenes (Oddo et al., 2003). The 3 transgenes were subcloned into the Thy1,2 casette.	A special band of Aβ immunoreactivity develops in layer III of the retrosplenial cortex (RSg), reminiscent of cholinergic terminals. Damage to cholinergic afferents results in loss of cholinergic markers and reduction of Aβ-IR. It is suggested that septal cholinergic axons transport Aβ or APP to RSg
3xTg-AD	Mastrangelo and Bowers, 2008	Triple transgenic model of Oddo et al., 2003	Documentation of the evolution of transgene expression, amyloid deposition, tau phophorylation in the hippocampus, entorhinal ctx, primary motor ctx and amygdala over a 26 month period in male 3xTg-AD mice.
^{triple}AD	Rhein et al., 2009; Grueninger et al., 2010	Cross breeding of $APPswePS2^{N1411}$ double transgenic mice with P301L tau transgenic pR5 mice (pR5/APP/PS2)	This new triple transgenic model shows age-dependent accumulation of Aβ plaques and NFTs in the cortex, hippocampus and amygdala with no measurable cell loss at 16 months. The BFC system apparently has not been investigated.

emphasize neuronal atrophy rather than cell death, suggested by the use of more rigorous stereological criteria. For example, Vogels et al. (1990), found an overall cell loss of only 15%. The reasons for such discrepancies in findings could relate to differences in sampling strategy, staining protocol, patient selection criteria or different stages of the disease (Allen et al., 1988; Gilmor et al., 1999; Iraizoz et al., 1991; Lehericy et al., 1993; Vogels et al., 1990). Similarly controversial are data regarding neuronal loss in nucleus basalis/SI during normal aging: ranging from 23% to 50% cell loss to no neuronal loss at all (Chui et al., 1984; De Lacalle et al., 1991; Whitehouse et al., 1981). In addition to AD, there are structural changes in the basal forebrain found occasionally in Parkinson's disease, Rett syndrome, progressive supranuclear palsy, Parkinson dementia complex of Guam, dementia pugilistica, Pick's disease, Korsakoff's syndrome, Down syndrome, Wernicke's encephalopathy, and Cretuzfeldt-Jacob disease (reviewed in Swaab, 2003).

Neurofibrillary changes emerge early and the pathology in the nucleus basalis parallels the progression of the AD-related stages in the cerebral cortex (Mesulam et al., 2004; Sassin et al., 2000). However, much controversy remains: whether or not the neuropathological changes are primary or secondary to cortical pathology; and what is the time course of cholinergic deficit (Mesulam, 2004). Postmortem studies have shown that mild AD is associated with preserved cortical ChAT activity (DeKosky et al., 2002). In fact, ChAT activity is increased in the hippocampus of patients with mild cognitive impairment (MCI), and counts of ChAT-positive cells revealed a similar number of cholinergic neurons in the nucleus basalis in MCI, early AD patients and non-demented healthy elderly controls (Gilmor et al., 1999). However, comparable amounts of basal forebrain cholinergic cells do not necessarily reflect an intact and fully functional cholinergic system, since shrinkage of cholinergic neurons has also been observed in AD patients (Vogels et al., 1990). These unresolved issues are at least in part due to the fact that neuropathological examinations are restricted to postmortem cases. Recent studies, using voxel-based morphometry (Hall et al., 2008) and probabilistic 3D maps of the nucleus basalis (Groethe et al., 2010; Zaborszky et al., 2008) suggest that the basal forebrain cholinergic space displays volume reduction and this is correlated with cortical gray matter atrophy and cognitive decline in MCI patients. These findings establish, for the first time, a link between degeneration of specific cholinergic compartments of the basal forebrain cholinergic system and cognitive-related deficits in subjects at high risk of developing AD (Groethe et al., 2010). A significant reduction of the SI volume in early stages of AD was recently reported by George et al., (2009).

Amyloid Precursor Protein

One of the most prominent features of AD is the presence of amyloid plaques consisting of dystrophic neurites and a central core of amyloid-β peptide (Aβ) that is derived from the amyloid precursor protein (APP) by proteolytic cleavage. APP is a single transmembrane domain protein with multiple alternate transcripts, which are expressed ubiquitously and present in dendrites, cell bodies and axons. APP is coded by a gene located on the long arm of human chromosome 21. The normal metabolic processing of APP by three proteases generates both amyloidogenic (amyloid-β peptide: $A\beta_{42}$) and non-amyloidogenic products ($A\beta_{40}$). The non-amyloidogenic cleavage is mediated by *α-secretases* (ADAM family of metalloproteases). Cleavage by α-secretase occurs within the Aβ domain, thereby preventing the generation and release of the Aβ peptide. The cleavage results in two fragments: the large amino (N) terminal ectodomain (sAPPα) which is secreted into the extracellular space and the smaller intramembranous 10–11kDa carboxy-terminal fragment (C83). APP molecules that are not cleaved by the α-secretase pathway become a substrate for *β-secretase* (β-site APP-cleaving enzyme 1; BACE1), releasing an ectodomain (sAPPβ), and retaining the last 99 amino acids of APP (known as C99) within the membrane, containing the whole Aβ sequence. The β-secretase is a transmembrane protein belonging to the pepsin family of aspartyl proteases. C99 is subsequently cleaved between residues 39–43 amino acids from the amino terminus to release Aβ, by the gama-secretase complex. This cleavage predominantly produces $A\beta_{40}$, and the more amyloidogenic $A\beta_{42}$ at a ratio of 10:1. The gamma-secretase is an enzyme complex integrated in the cell membrane and is required for Aβ formation and consists of four proteins; presenilin (PS1 or PS2), and three others – nicastrin; anterior pharynx defective [APH-1], and presenilin enhancer 2 [PEN-2]. Presenilin, APH1 and nicastrin first form a stable complex. After association with PEN-2 and cleavage of presenilin, the complex becomes active gamma-secretase and clips its substrate, APP-C99 to generate toxic Aβ species.

The most widely used transgenic models for AD involve targeted transgenic insertion of mutant human APP under various promoters, including the mouse thymus cell antigen (Thy1), platelet derived growth factor-β (PDGF) or prion protein promoter (PrP) (Gotz and Ittner, 2008; McGowan et al., 2006). Mice over-expressing APP in various models (ref. see German and Eisch, 2004) revealed contradictory results in terms of cholinergic neurons in the basal forebrain or loss of cholinergic function. Cholinergic nerve terminal

abnormalities and reduction of the density of cholinergic terminals are common in the hippocampus and frontal cortex of APP mouse models (Bronfman et al., 2000; Boncristiano et al., 2002; Games et al., 1995). On the other hand, they found no loss of cholinergic basal forebrain neurons in 2-year-old transgenic animals (Boncristiano et al., 2002; German et al., 2003). In contradiction, Li and Shen (2000) reported that mice over-expressing human APP770 showed loss of cholinergic neurons, starting at 9 months, with a further decrease in the number of nucleus basalis and medial septal neurons in 10-month-old mice. However, the number of neurons in the cerebral cortex and hippocampal area CA1 remained unchanged in these mice (Li and Shen, 2000). Unilateral nucleus basalis lesions in adult APP23 mice resulted in an additional reduction in ChAT activity and cortical cholinergic fiber loss (Boncristiano et al., 2002). These observations suggest that the severe cholinergic deficit in AD may be caused by both the loss of cholinergic basal forebrain neurons and locally by cerebral amyloidosis in the neocortex.

Tau Transgenic Mouse Models

Neurofibrillary tangles (NFT), composed primarily of a hyperphosphorylated form of microtubule-associated protein (MAPT) tau, accumulate intracellularly in AD. Neurons containing NFTs eventually degenerate in AD (Braak and Braak, 1997). The increase of CSF tau/β-Amyloid$_{42}$ ratio is suggested to be a biomarker that can predict future dementia of an AD type (Fagan et al., 2007). Tau is encoded by a single gene on chromosome 17 and is expressed in six isoforms in the adult human brain, by alternative splicing of the MAPT gene. These isoforms differ in that they contain three (3Rtau) or four (4Rtau) microtubule binding repeats (R) in the carboxy-terminus and one (1N), two (2N) or zero (NO) amino terminal inserts in the amino-terminal region. Tau protein – when differentially phosphorylated – promotes the assembly, disassembly and reassembly of microtubules, as needed by the cell. Under pathological conditions, tau becomes hyperphosphorylated and dissociates from microtubules, causing them to depolymerize, while tau is deposited in aggregates such as NFTs (Gotz and Ittner, 2008). Mutations in the MAPT gene have been linked to NFT formation in frontotemporal dementia (FDTP-17; Iqbal et al., 2009). However, no tau mutations occur in AD, although this disease is also characterized by NFT formation and cell death. There are several transgenic mouse models with taupathies that differ in the type of expressed human mutation, the insertion site of the transgene, the promoter used to drive transgene expression, the level of expression of the transgene and/or mouse genetic background (for ref see Adams et al., 2009; Gotz and Ittner, 2008; McGowan et al., 2006). For example, in the JNPL3 model the transgene P301L mutation in exon 10 is driven under PrP and the NFTs are mainly present in the hindbrain and spinal cord (Lewis et al., 2000), with sporadic NFTs in the cortex, hippocampus and basal ganglia. In the rTg4510 mouse the transgenic tauP301L is driven by the Ca2+/calmodulin kinase II (CaMKII) promoter system designed for specific expression in the forebrain (Ramsden et al., 2005). In this latter model the tau pathology is evident from 2.5 months of age, and in 10-month-old mice severe neuronal degeneration can be observed in the hippocampus and neocortex with age-dependent development of cognitive impairments. The mTau mice, overexpressing genomic wild-type mouse tau using a BAC derived transgene, show a progressive increase in hyperphosphorylated tau pathology beginning in the entorhinal cortex and then spreading to other regions of the cortex and hippocampus with ages up to 15–18 months (Adams et al., 2009). Aged mice expressing nonmutant human tau in the absence of mouse tau (Htau mice) developed NFTs and extensive cell death in the piriform cortex and hippocampus (Andorfer et al., 2005). The pR5 mouse strain, overexpressing the longest human tau isoform (htau40) with the P301L mutation under the control of the mThy1,2 promoter, develop widespread neurofibrillary lesions in the hippocampus, amygdala and somatosensory cortex (Gotz et al., 2001; Kohler et al., 2010).

None of the single or double tau transgenic mice models report pathology in the basal forebrain cholinergic system (Perez et al., 2005; Ribe et al., 2005). The mechanism of neuron death in taupathies is unclear. but single cell expression profile analysis of basal forebrain cholinergic neurons from AD brains suggest that there is a shift in the ratio of 3R tau to 4R tau (Ginsberg et al., 2006a). The re-expression of cell-cycle proteins and DNA synthesis in htau mice indicates that tau pathology and neurodegeneration may be linked via abnormal, incomplete cell-cycle re-entry (Andorfer et al., 2005; Lopes et al., 2009; Nagy et al., 1999). Various cell cycle proteins have been shown to be expressed in the nucleus basalis of AD patients, suggesting that this mechanism may be indeed involved in the demise of basal forebrain cholinergic neurons (Yang et al., 2003).

Presenilins

The presenilin genes (PS1 and PS2) are two homologous genes encoding polytopic 8 transmembrane proteins. So far, more than 190 mutations (http://www.molgen.ua.ac.be/ADMutations), mainly involving the conserved transmembrane domains or a region adjacent to a large intracytoplasmic loop, have been identified. Mutations in the presenilin genes are thought to account

for about 20–25% of all familial AD cases. Presenilins are part of the gamma-secretase complex that cleaves APP to produce toxic $A\beta_{42}$ species. Mutations in PS1 and PS2 cause misfolding of APP that in turn, might be what causes APP to be cut in the wrong place by gamma- and β-secretases, thereby releasing extra $A\beta_{42}$. It is interesting to note that a double transgenic mouse (PS/APP) that overexpresses mutated PS1 and APP genes showed cholinergic dystrophic neurites and decreases in ChAT enzyme activity in the cerebral cortex and hippocampus implying diminished function of the cholinergic system. Nonetheless, no significant changes in basal forebrain cholinergic neurons were noticed in these transgenic animals (Perez et al., 2007; Wong et al., 1999).

α,-β-secretases, Retromer Sorting

Reducing the activity of the β-secretase BACE1 by crossing APP transgenic mice onto a *BACE−/−* background reduced amyloid formation and deposition and rescued these mice from Aβ dependent hippocampal memory deficits (Ohno et al., 2004). Furthermore, impaired *in vitro* hippocampal cholinergic regulation of neuronal excitability found in the Tg2576 APP model is ameliorated in BACE1−/−Tg2576+ bigenic mice. Expression of the α-secretase ADAM10 in APP transgenic mice also reduced amyloid formation, ameliorated hippocampal behavioural deficits and LTP impairment, providing *in vivo* evidence for ADAM10 as a functional α-secretase (Postina et al., 2004).

The retromer sorting pathway is made up of multimeric coat complex which transports a transmembrane retromer-binding receptor, and it is involved in sorting APP and/or BACE along the endosome-trans-Golgi network trafficking pathway. Studies in both animal models and cell culture have shown that deficiencies in the complex and sorting receptor (SORL1) cause an elevation in Aβ and Aβ aggregates. Retromer deficiency causes hippocampal-dependent memory and synaptic dysfunction; cholinergic deficits yet to be reported in these models (Small and Duff, 2008).

APP Transgene with α7nAChR or mAChR Receptor Knock-Outs

The α7nAChR is highly expressed in human postmortem basal forebrain areas. mRNAs for this receptor are colocalized within rat basal forebrain cholinergic neurons (Breese et al., 1997; Azam et al., 2003). Furthermore, soluble Aβ has been shown to bind with high affinity to nAChR and this leads to inhibition of ACh release and causes cell death *in vitro* (Wang et al., 2000), suggesting that the interaction of α7nAChR and $A\beta_{42}$ may be involved in the pathophysiology of AD. Activation of α7nAChR has been shown to maintain septohippocampal cholinergic neurons *in vivo* (Ren et al., 2007).

In the study of Hernandez et al. (2010), the Tg2576 mice transgenic for the Swedish APP mutation were crossed with α7nAChR knock-out mice (A7KO). Double transgenic mice showed accelerated hippocampal dependent memory deficits with enhanced accumulation of soluble Aβ. ChAT activity decreased in both the hippocampus and basal forebrain, however, ChAT activity in the basal forebrain decreased in the A7KO mice as well. In a bigenic line where the human APP is expressed with the Indiana mutation, the deletion of the α7nAChR improved cognitive deficits (Dziewczapolski et al., 2009), indicating the complexity of interpretation involved in studying combinations of transgenes.

By crossing transgenic mice with the Swedish and Indiana mutations of APP with M1 knock-out mice, Davis et al. (2010) have shown that M1 receptor deletion exacerbates production of toxic Aβ peptides and amyloid plaques in both the hippocampus and cortex both of which are targets of cholinergic terminals from the basal forebrain.

Axonal Transport and ApoE Models

Axonal transport of APP in neurons is mediated by the direct binding of APP to the kinesin light chain subunit of kinesin-1 (KLC1), a microtubule motor protein (Hirokawa and Takemura, 2005; Kamal et al., 2001). Reduction of KLC1 in APP transgenic mice (Stokin et al., 2005) increased cholinergic axonal swelling in the nucleus basalis and increased amyloid-β peptide levels and amyloid deposition in cortical areas, implicating axonal transport deficits in the pathogenesis of AD.

In humans, ApoE is a single gene located on chromosome 19 with three major allelic variants (e2, e3 and e4). Individuals with one or two copies of ApoEe4 allele typically develop the disease at a younger age and display a greater risk of developing AD. Crossing APP transgenic PDAPP (platelet derived growth factor promoter-expressing APP) mice onto an *ApoE−/−* background strongly reduced Aβ levels and deposition in the brain (Bales et al., 1997). No observation is reported in relation to cholinergic deficit.

Summary of Mouse Models of Human Disease Related to Basal Forebrain

The combination of familial AD mutations in genetically altered mice resulted in various lineages with double and triple transgenic animals, which proved to be potent models in the area of AD research (Oddo

et al., 2003; Jankowsky et al., 2005; McGowan et al., 2006; Gotz and Ittner, 2008). The various mouse models firmly established the role of $A\beta_{42}$ in the development of amyloid plaques, deciphered the intracellular production, metabolism, trafficking and pathological effect of Aβ (LaFerle et al., 2007). Moreover, these transgenic models identified the toxic Aβ and tau species, and the tentative relationship between Aβ and tau pathology, lending support to the amyloid cascade hypothesis (Berger et al., 2007; Hardy and Selkoe, 2002; Santa-Cruz et al., 2005). In AD, loss of cortical and hippocampal ChAT activity correlates well with the severity of dementia (DeKosky et al., 1992; Perry et al., 1978). However, cognitive impairment in some of these models is better correlated with Aβ concentrations than cholinergic deficits in cortex and hippocampus (Savonenko et al., 2005). Also, the lack of robust cholinergic cell loss in the basal forebrain suggest that the current mouse models of AD using expression of various familial mutations are not a full replication of the sporadic disease induced pathology, at least in terms of selective vulnerability of basal forebrain cholinergic neurons.

TROPHIC FACTOR MAINTENANCE AND THE P75 NEUROTROPHIN RECEPTOR

Neurotrophins can enhance survival and function of both developing and mature basal forebrain cholinergic neurons. The neutrotrophins, Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF) are synthesized by hippocampal and cortical neurons, which are the target cells of basal forebrain cholinergic neurons. NGF is taken up by the terminals of basal forebrain cholinergic neurons and is retrogradely transported to the cell body region, affecting various functions, including expression of ChAT (Hatanaka et al., 1988) and the vesicular acetylcholine transporter, VACht (Berse et al., 1999; Pongrac and Rylett, 1998). NGF increases cholinergic neuron number *in vitro* (Hatanaka et al., 1988) and supports survival of postnatal basal forebrain neurons (Nonomura and Hatanaka, 1992; Nonomura et al., 1995; Ward and Hagg, 2000). Two distinct receptor types have been distinguished for neutrotrophin actions, Trks and the p75 neurotrophin receptor ($p75^{NTR}$). The Trks are receptor tyrosine kinases that utilize a complex set of substrates and adaptor proteins to activate signaling cascades required for neurotrophin actions on neuronal differentiation, plasticity and survival. TrkA−/− mice have reduced numbers of cholinergic septal neurons at P25 suggesting that TrkA signaling is required for the normal maturation and possibly survival of basal forebrain cholinergic neurons (Fagan et al., 1997). NGF acts via the TrkA receptor on ChAT and VAChT protein in contextual memory consolidation (Woolf et al., 2001). The $p75^{NTR}$ is a transmembrane glycoprotein and is a member of the TNF receptor/Fas/CD40 superfamily. Cholinergic basal forebrain neurons express both TrkA and $p75^{NTR}$ receptors (Hartikka and Hefti, 1988; Heckers et al., 1994). The $p75^{NTR}$ appears to modify TrkA signaling when the two receptor types are co-expressed and *in vitro* data suggest that proneutrophins (proNGF) can mediate apoptosis through $p75^{NTR}$ (Friedman and Greene, 1999; Volosin et al., 2006).

Initial studies of mice lacking $p75^{NTR}$ produced conflicting results, reporting either an increased (Van der Zee et al., 1996; Yeo et al., 1997) or decreased number of basal forebrain cholinergic neurons (Peterson et al., 1999) or no change at all in cholinergic cell number (Ward and Hagg, 1999). Other studies reported a small decrease in the number of cholinergic neurons in $p75^{NTR}$ knockout mice with a markedly increased cell size (Greferath et al., 2000). A careful re-analysis of the septal cholinergic neurons using partial ($p75^{exonIII}$) and complete ($p75^{exonIV}$) knockout mice with different genetic background revealed that the null $p75^{exonIV}$ mutation, which prevents expression of both the full-length and the shorter $p75^{NTR}$ isoforms, indeed results in a 28% increase in cholinergic cell number, independent of the background. The discrepant results of previous studies are most likely due to the less rigorous sampling and counting procedures and to the different genetic background. In fact, the effect of genetic background on the number of cholinergic cells is larger than the difference between wild type and $p75^{NTR}$ mutants (Naumann et al., 2002).

The extracellular domain of $p75^{NTR}$, similar to APP, is cleaved by a metalloprotease, generating a transmembrane-linked C-terminal fragment. This is then cleaved by the gamma secretase, liberating a soluble intracellular domain (Jung et al., 2003). Ligand binding can initiate this process, and both fragments of $p75^{NTR}$ have been shown to mediate death signaling (Underwood et al., 2008). Aβ is a ligand for the $p75^{NTR}$ and injection of $A\beta_{42}$ into the hippocampus of adult mice resulted in a significant degeneration of wild-type but not $p75^{NTR}$ deficient basal forebrain cholinergic neurons (Sotthibundhu et al., 2008). Furthermore, Knowles et al. (2009) using doubly transgenic mice expressing the London and Swedish APP familial mutation on $p75^{NTR\ (-/-)}$ null mutant background have shown a complete reversal of basal forebrain cholinergic neurite degeneration relative to those expressing wild-type $p75^{NTR}$. These *in vitro* and *in vivo* experiments suggest that $p75^{NTR}$ is likely to play a significant role in enabling Aβ-induced neurodegeneration. NGF and the receptor proteins TrkA and $p75^{NTR}$ decline as AD progresses (Mufson et al., 2003). Moreover, NGF

retrograde transport or NGF binding to TrkA receptors, or both, are reduced in individuals with AD, which results in dysfunctional trophic support of the cholinergic system (Counts and Mufson, 2005; Ginsberg et al., 2006a, b; Isacson et al., 2002). More recently, it has been suggested that increased levels of proNGF (Volosin et al., 2006) and/or exacerbated degradation of mature NGF via increased activity of matrix metalloproteinase 9 plays a role in NGF-dependent degeneration in AD (Bruno et al., 2009).

CONCLUDING REMARKS

Single unit studies in anesthetized and behaving rats showed that identified cholinergic neurons increase their firing during cortical EEG activation (Duque et al., 2000; Lee et al., 2004; 2005; Manns et al., 2000a, b). Activity of basal forebrain cholinergic neurons is associated with an increase in cortical release of ACh. Cortical ACh release is high during wakefulness and rapid eye movement (REM) sleep and is low during non-REM sleep that is characterized by EEG delta power with periodic oscillations of medium-frequency high amplitude spindles (Douglas et al., 2002 Jasper and Tessier, 1971; Kanai and Szerb, 1965). It was proposed long ago that basal forebrain neurons, as part of the 'diffuse ascending reticular activating system' constitute an extrathalamic route to mediate brainstem and hypothalamic influences to modulate cortical function (Saper, 1987; Sarter and Bruno, 1997).

The early suggestion, using the cortical cup technique (Collier and Mitchell, 1966), that sensory stimulations evoke an increase in release of ACh from sensory cortical areas with some degree of regional specificity, has been confirmed recently with *in vivo* dialysis of ACh combined with HPLC (Fournier et al., 2004b; Kozak et al., 2005; Laplante et al., 2005; Nelson et al., 2005; Rasmusson et al., 2007). Furthermore, lesions and stimulations in the basal forebrain suggest that cortical release of ACh from the basal forebrain cholinergic neurons appears to be essential for a learning-associated enhancement of sensory processing and cortical plasticity (Baskerville et al., 1997; Conner et al., 2003; Juliano et al., 1990; Kilgard and Merzenich, 1998; Metherate and Ashe, 1991; Rasmusson, 2000; Weinberger, 2007). Moreover, recent studies using enzyme-selective microelectrodes in attentional task-performing rats demonstrated that cholinergic signals are manifested at different time-scales in various cortical areas to support specific cognitive operations. For example, selective cholinergic activation in the prefrontal cortex at the scale of seconds is associated with cue detection, while changes at the scale of minutes may occur cortex-wide to support a more general arousal effect of ACh (Parikh et al., 2007).

By simultaneous recording of large basal forebrain populations along with local field potentials from the prefrontal cortex, Lin et al., (2006) identified basal forebrain cell assemblies engaging in transient population synchronization that were accompanied by brief increases in theta and gamma oscillations in the prefrontal cortex. Such neuronal ensemble bursting in basal forebrain by affecting the activity of specific cortical circuits could support top down attention (Lin and Nicolelis, 2008). In another study, Goard and Dan (2009) stimulated the nucleus basalis of urethane-anesthetized rats while recording from V1 with a silicon polytrode. In the control condition (visual stimulus without basal forebrain stimulation), the multiunit activity in the visual cortex was highly correlated among the 27 channels, but poorly time-locked to the stimulus. Following basal forebrain stimulation, the activity was less correlated among channels, but appeared to be more time-locked to the visual stimulus. Application of atropine, a selective muscarinic antagonist, greatly reduced the degree of decorrelation and slightly increased the response reliability induced by basal forebrain stimulation. Both effects were interpreted as improving visual representation in the cortex.

These functional data are consistent with the presence of multiple cholinergic modules in the form of regionally specific cell clusters as described in the cholinergic basal forebrain space of rats (Zaborszky et al., 2005). Our preliminary studies on rats suggest that specific cell clusters project only to a few cortical areas that most likely are interconnected (Zaborszky et al., 2008). Such a mechanism could, for example, mediate the correlation/decorrelation of specific cortical units as observed in the experiments of Goard and Dan (2009).

Against the relatively 'diffuse' termination of the ascending brainstem and hypothalamic axons in the basal forebrain, the restricted input from the prefrontal cortex to basal forebrain neurons (Zaborszky et al., 1997), including specific clusters, might be instrumental in communicating state-related changes from basal forebrain neurons to specific posterior sensory areas to modulate selective cognitive processes (Golmayo et al., 2003). It is unclear, however, if the same basal forebrain neurons that receive state-related brainstem or diencephalic input are the ones that also mediate specific functions, like selective attention and sensory plasticity. Using genetic manipulations to confer light sensitivity on specific groups of neurons for the first time allows the opportunity to stimulate or inhibit specific neurons and record their activity in living animals (Gradinaru et al., 2009). It is likely that introducing optogenetic tools into basal forebrain research will contribute to a better understanding of the functions of specific cholinergic circuits.

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