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ACETYLCHOLINE

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Acetylcholine (ACh) is critical for communication between neurons and muscle at the neuromuscular junction, is involved in direct neurotransmission in autonomic ganglia, and has been implicated in cognitive processing, arousal, and attention in the brain (1). Cholinergic transmission can occur through muscarinic (G protein-coupled) or nicotinic (ionotropic) receptors and is terminated by the action of cholinesterases. Seventeen different subunits of the nicotinic ACh receptor (nAChR) (2) and five different subtypes of the muscarinic receptor (3) have been cloned to date, and a majority of those are known to be expressed in the brain. Although the anatomic locations of cholinergic cell bodies and their projections have been known for some time (Fig. 1.1), recent studies using specific cholinotoxins, electrophysiology, or molecular genetics have altered our view of the functional role of the cholinergic system in the brain. The anatomic, pharmacologic, and biochemical complexity of the cholinergic system indicates an intricate involvement in nervous system function, and new advances in this field are discussed here.

KNOCKOUT OF MUSCARINIC- AND NICOTINIC-RECEPTOR SUBUNITS

A particularly useful tool in identifying the role of individual molecules in the physiologic and behavioral functions of the cholinergic system are transgenic animals that lack specific subunits or subtypes of muscarinic receptors or nAChRs. These animals, termed *"knockout" mice,* can be generated by means of genetic engineering techniques and have been extremely useful in determining the functional role of many proteins that have been identified through molecular cloning (see refs. 4 and 5 for a review of this technology). Mice lacking the α3 (6), α4 (7), α5 (8), α7 (9), α9 (10), β2 (11), β3 (12), or β4 (13) subunit of the nAChR have been reported. In addition, mice lacking the M1 (14), M2 (15), and M4 (16) subtypes of the muscarinic receptor have been generated. These mice have already been used to demonstrate the role of particular receptor subtypes in the physiologic effects of ACh in muscle, the peripheral ganglia, and the central nervous system (Table 1.1).

The function of ACh has been best studied at the neuromuscular junction, where signaling occurs through the muscle form of the nAChR. In the embryo, the nAChR at the neuromuscular junction is a pentamer made up of two α, one β, one γ, and one δ subunit. After birth, the γ subunit is replaced by the ϵ subunit, so that the physiologic properties of the receptor are altered. In mice in which the ϵ subunit has been knocked out, the neuromuscular junction nAChRs remain in the embryonic form; the consequence is survival past birth with progressive muscle degeneration and lethality by 2 to 3 months of age (17). These experiments demonstrate that maturation of the neuromuscular junction nAChR is necessary for muscle cell function and survival and imply that the kinetics of ACh neurotransmission are critical for the health of muscle fibers in adulthood.

Cholinergic neurotransmission within the sympathetic ganglia occurs through several receptor subtypes. In the peripheral nervous system, the issue of which ACh-receptor subtypes are involved in cholinergic neurotransmission has been addressed both by knocking out muscarinic and nicotinic subunits and by treating sympathetic neurons from isolated chick sympathetic ganglia with antisense oligonucleotides (short stretches of DNA that can inhibit the translation of a particular protein of interest) to decrease the expression of α3, α4, and α7 nAChR subunits. Antisense experiments have indicated that the α3 nAChR subunit plays a primary role in nicotinic transmission in sympathetic ganglia and that the α7 subunit also contributes to the observed currents (18,19). These data are in agreement with electrophysiologic and immunoprecipitation studies of nAChR subunits from ganglionic neurons (20). Although the results of studies using this powerful technique are com-

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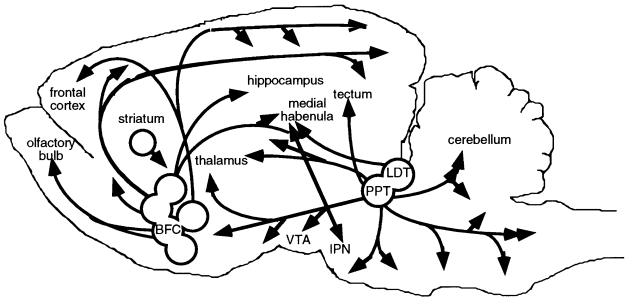

FIGURE 1.1. Anatomy of major cholinergic pathways in the brain. The principal source of cholinergic input to the cortex and hippocampus is the basal forebrain complex, whereas the pedunculopontine and laterodorsal tegmental areas innervate brain stem and midbrain targets preferentially. Cholinergic interneurons are found in the olfactory tubercle, striatum, nucleus accumbens, and islands of Calleja. BFC, basal forebrain complex; VTA, ventral tegmental area; IPN, interpeduncular nucleus; PPT, pedunculopontine tegmental nucleus; LDT, laterodorsal tegmental nucleus.

pelling, some problems have been noted with antisense approaches, including issues of specificity, so that it is useful to complement these studies with other techniques that can be used to manipulate levels of nAChR subunits.

In studies of knockout mice, disruptions of two nicotinic-receptor subunits expressed in sympathetic ganglia, α7 (9) and β2 (11), do not grossly alter ganglionic function. In contrast, if the β2 and the β4 nAChR-subunit mutations are combined (13), or if the α3 nAChR subunit is knocked out (6), mutant mice die perinatally of severe autonomic failure. These experiments suggest that a nicotinic cholinergic receptor composed of the α3/β4 or β2 subunit, or both, is responsible for mediating direct neurotransmission by ACh between ganglionic neurons.

Muscarinic function has also been studied in the autonomic ganglia with knockout technology. Mutation of the M1 muscarinic receptor is sufficient to abolish the M current, a muscarine-mediated potassium current, in the sympathetic ganglia, but M1 mutation does not significantly perturb ganglionic function (14). In contrast, mice lacking

TABLE 1.1. KNOCKOUTS OF MUSCARINIC AND NICOTINIC SUBUNITS

Subunit	Knockout	Knockout Phenotype	References
Muscarinic receptors			
M1	Viable	Disruption of M current and muscarinic seizures	(14,21)
M2	Viable	Disruption of muscarinic receptor-dependent movement and temperature control and antinociception	(15,21)
M4	Viable	Enhancement of D1 receptor-mediated locomotor stimulation	(16,21)
Nicotinic subunits			
α3	High mortality rate before and after weaning	Impaired growth, megalocystis (inflamed urinary bladder) and mydriasis (widely dilated ocular pupils)	(6)
α4	Viable	Reduced antinociception	(7)
α5	Viable	Not yet reported	(8)
α7	Viable	Largely normal; lack MLA-sensitive nicotine response in hippocampal interneurons; may have slightly decreased anxiety response	(9,22)
α9	Viable	Involved in cochlear efferent innervation development and function	(10)
β2	Viable	Lack nicotine-induced increases in passive avoidance, reinforcement, antinociception; show increased neurodegeneration during aging	(7,11,24,31,32)
β3	Viable	Altered locomotor activity	(12)
β4	Viable	Viable, but lethal when combined with β2 subunit knockout	(13)

MLA, methyl-lyaconitine, a β7 antagonist.

the M2 muscarinic-receptor subtype do not show carbachol-induced bradycardia, confirming that the effect of ACh on sympathetic control of heart rate is mediated through the M2 receptor (15). In addition, knockout animals have been very useful in determining which subtypes of muscarinic receptors are responsible for the effects of ACh on modulation of calcium channels in sympathetic neurons (21). A slow, voltage-independent modulation is mediated by M1 receptors, whereas a fast, voltage-dependent modulation is mediated through M2, and neither is affected in M4 knockout mice.

The function of ACh in the brain has also been examined in electrophysiologic experiments with mice lacking cholinergic-receptor subtypes. For example, a rapidly desensitizing nicotinic current in the hippocampus is mediated through an α7-containing receptor (9). Mice lacking the α7 subunit appear grossly normal in behavioral experiments (22), but future experiments should determine whether these currents contribute to nicotine-induced improvements in cognitive function or to nicotine-induced seizure activity. Antisense experiments have also demonstrated that the α5 nAChR subunit can alter the electrophysiologic properties of nAChRs containing the α4 and β2 subunits *in vivo* (23). Mice lacking the β2 subunit have been used to characterize four classes of nAChR in the brain by means of pharmacologic and electrophysiologic techniques (24) and to extend the existing pharmacologic characterization of nicotinic-receptor subtypes (Fig. 1.2). Future experiments using mice lacking individual nAChR α subunits should allow a finer definition of these receptor classes.

A significant development in thinking about nicotinic-receptor function has been the idea that nicotine exerts many of its functions in brain through the regulation of neurotransmitter release, at least partly through terminal and preterminal nAChRs (25,26). Experiments on synaptosomes (nerve terminals) isolated from mice lacking the β2 subunit of the nAChR have shown that presynaptic regulation of GABA release by nicotine is mediated through β2 subunit-containing receptors in most brain areas (27). This is also likely to be the case for other neurotransmitters because the efflux of rubidium, a radioactive tracer that serves as a marker of neurotransmitter vesicle fusion, is mediated through β2 subunit-containing receptors in most brain areas (28). More recently, dopamine release from striatal synaptosomes has been shown to be disrupted in β2-subunit knockout mice, while ACh release in the interpeduncular nucleus is preserved (29). This suggests that a distinct nAChR subtype, most likely containing the β4 subunit, mediates nicotine-elicited ACh release in the interpeduncular nucleus.

Systems-level function and behavioral effects of ACh have also been examined in knockout mice. Muscarinic agonist-induced seizures are dependent on the presence of the M1 receptor because M1 knockout mice are resistant to pilocarpine-induced seizure activity (14). Interestingly, although the M1 receptor has been implicated in the modula-

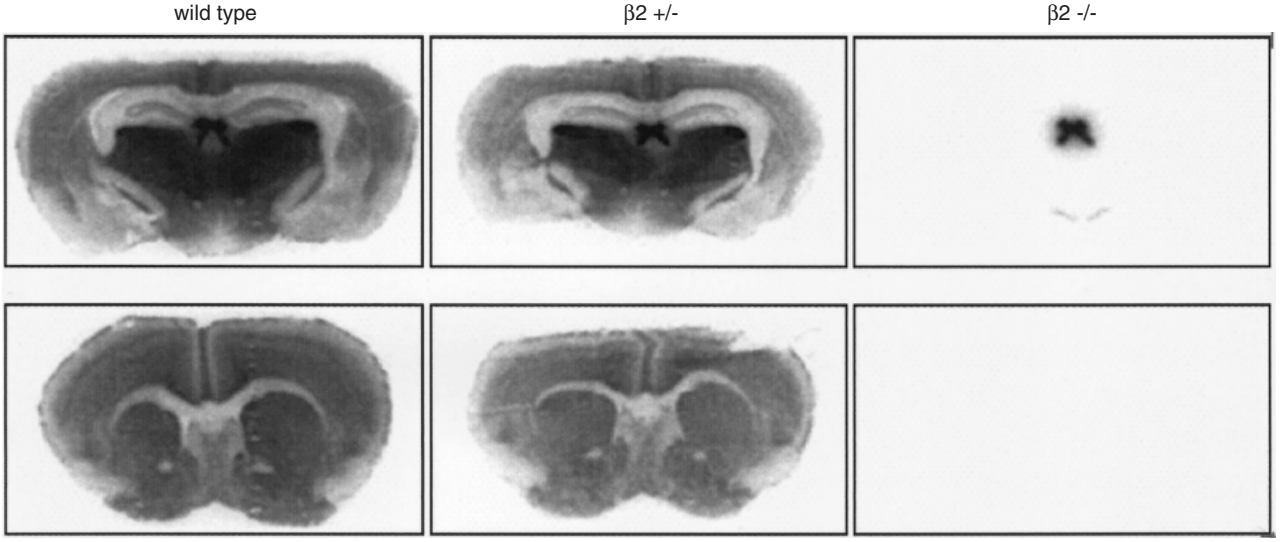

FIGURE 1.2. Nicotinic ligand binding in brain slices from wild-type and β2-subunit knockout mice. Mice lacking individual subunits of the nicotinic acetylcholine receptor (nAChR) can be used to distinguish between subclasses of receptors. For example, although β2-subunit knockout mice lack the highest-affinity subclass of nicotine binding sites, the frog toxin epibatidine, shown here, still reveals β4 subunit-containing nAChRs in the medial habenula (remaining binding shown in panel at *top, far right*). Binding of epibatidine in brain slices through thalamus *(top)* or striatum *(bottom)* is shown in wild-type heterozygous (β2 +/−) and homozygous (β2 −/−) β2-subunit knockout mice.

tion of potassium channels in the hippocampus in pharmacologic experiments, muscarinic modulation of potassium channels is unchanged in the hippocampus of M1 knockout mice (30). In contrast, the pharmacologic effects of muscarinic agonists on movement, temperature control, and antinociception appear to be mediated through the M2 receptor because these responses are absent in M2 knockout mice (15). M4 receptors are also involved in locomotion; these knockout animals exhibit increased basal locomotor activity and a potentiated locomotor response to D1-selective dopaminergic agonists (16).

Like the M2 receptors, the α4/β2 subtype of nAChR is implicated in antinociceptive cholinergic pathways. Mice lacking either of these subunits show decreased nicotine-induced analgesia (7). In behavioral experiments, the β2 nicotinic subunit mediates the ability of nicotine to improve avoidance learning and may also be involved in the circuitry underlying this form of associative learning in wild-type mice (11). In addition, this subunit appears to be necessary for the mouse to experience the reinforcing properties of nicotine because animals without the β2 subunit will not self-administer nicotine (31). Extensions of these experiments to mice lacking other subunits of the nicotinic receptor should allow identification of the receptor subtypes that are activated by smoking in humans and result in tobacco addiction. An interesting effect of ACh on neuronal survival was demonstrated in mice lacking the β2 nAChR subunit (32). Mice that lack this cholinergic-receptor subtype show progressive neuronal loss with age in cortical and hippocampal brain areas, which appears to lead to age-related impairments in spatial learning. These experiments demonstrate that the effects of ACh on cognition, antinociception, locomotion, and overall neuronal activity are differentially mediated through the various subtypes of muscarinic and nicotinic receptors, and that the various roles of ACh may be separated pharmacologically, suggesting new targets for rational drug design.

ROLE FOR CHOLINERGIC NEURONS IN AROUSAL AND SLEEP

Traditionally, the basal forebrain complex, the primary source of cholinergic innervation to the telencephalon (Fig. 1.1), was thought to be involved in arousal or sleep regulation. Either lesions or electric stimulation of subregions of the basal forebrain can facilitate sleep and synchronize the EEG, and cholinergic drugs regulate EEG synchrony (33). Moreover, a correlation between cortical ACh release and the state of behavioral activation or sleep has been observed in rodents. Thus, it was hypothesized that cholinergic input to the neocortex from the basal forebrain is critical for regulating arousal (see ref. 34 for review).

The pontomesencephalic tegmentum is also critical for the sleep–wake cycle. These neurons largely do not innervate the neocortex but project to the diencephalon (thalamus) and the basal forebrain complex. Stimulation of tegmental brainstem cholinergic neurons can evoke cortical ACh release and EEG desynchrony, and these effects are blocked by reversibly decreasing the activity of the basal forebrain (35). Moreover, application of cholinergic agonists to the basal forebrain produces behavioral activation and EEG desynchrony (33). Although the brainstem cholinergic projections to the thalamus undoubtedly also contribute to EEG regulation (36), these findings suggest that cholinergic projections to the basal forebrain from the pontomesencephalic tegmentum regulate behavioral arousal.

It was subsequently noted that cholinergic tegmental projections largely formed connections with noncholinergic neurons within the basal forebrain (37). This finding is critical because it could explain why stimulation of the horizontal diagonal band, preoptic area, and substantia innominata, but not of the septal nucleus and nucleus basalis, produces sleep in the cat (33). The ratio of cholinergic to noncholinergic neurons in the horizontal diagonal band, preoptic area, and substantia innominata is significantly lower than in the septum and nucleus basalis. This observation has led to the hypothesis that activation of primarily noncholinergic neurons is responsible for producing sleep after basal forebrain stimulation (33). These noncholinergic neurons are believed to be GABAergic and achieve their effects through inhibition of cholinergic basal forebrain neurons and neurons within the brainstem reticular formation. In contrast, stimulation of the nucleus basalis or septal nucleus produces behavioral activation and cortical ACh release, and this is consistent with the notion that basal forebrain cholinergic neurons are involved in behavioral arousal (activation), whereas noncholinergic basal forebrain neurons are involved in regulating the sleep state. These two effects are related (sleep vs. arousal), but the qualitative contributions of the GABA and cholinergic systems to sleep and arousal are opposed.

ROLE FOR CHOLINERGIC NEURONS IN MOTIVATION AND REWARD

Cholinergic neurons have also been implicated in motivation and reward. The strongest evidence for the hypothesis that nAChRs are involved in motivation and reward is that nicotine is abused by humans and is reinforcing in animals (see ref. 38 for review). The effects of nicotine on tests of reinforcement and behavioral sensitization are primarily mediated through the mesolimbic dopamine system (39). Indeed, the ventral tegmental area (VTA) may be sufficient to mediate the reinforcing properties of nicotine, as local injection of nicotine or nicotinic agonists into the VTA can result in increased locomotion (40) or conditioned place preference (41).

Basal forebrain cholinergic neurons may also be involved in modulating cortical processing of stimuli with conditioned or unconditioned rewarding properties because these neurons are more responsive to stimuli with a high incentive value. Novel stimuli that typically elicit orienting responses and attention in animals increase cortical ACh release, but this effect is diminished with repeated exposure if the stimulus has no contingent incentive valence. In contrast, if the stimulus is repeatedly paired with an incentive stimulus (e.g., food or foot shock), the (now-conditioned) stimulus can evoke ACh release even after multiple exposures (42). These sorts of changes are reflected in the firing of ''reinforcement-related'' neurons within the primate basal forebrain (43). Pontomesencephalic cholinergic neurons are also involved in motivation and reward, although these effects are likely mediated, in part, by projections to the dopamine neurons within the VTA (44,45).

Stimulation of the VTA by the pedunculopontine tegmental nucleus (PPT) enhances mesostriatal dopamine transmission (45,46). While a significant proportion of the PPT neurons that project to the tegmental dopamine neurons are noncholinergic (44), the cholinergic input *per se* appears to stimulate dopamine neurons (47). Thus, ascending projections from the PPT to the dopamine cells may regulate the ability of mesostriatal dopamine neurons to affect incentive/motivational processes.

This innervation of dopamine cells by cholinergic neurons may explain the finding that lesions of the PPT can modulate the rewarding qualities of addictive drugs. Lesions of the PPT reduce the self-administration of nicotine (48) and opiates (49). Moreover, conditioned place preference for food, opiates (50), morphine (51), and amphetamine (52) is blocked or reduced by PPT lesions, whereas cocaine-induced reward is unaffected (53). Although the mesolimbic dopamine pathway is known to be involved in drug reward (see ref. 54 for review), it is not yet known whether the influence of the PPT on drug reinforcement is through cholinergic projections. It is also not known whether the effect of PPT lesions on these processes is mediated through projections to areas other than the dopamine cell groups within the VTA.

The PPT may have another, more critical, role in motivation and reward via afferent inputs from the striatum (55). Excitotoxic lesions of the PPT (that equivalently destroy both cholinergic and noncholinergic neurons) disrupt responding for conditioned reinforcement and augment stimulant-induced orofacial stereotypy, yet no difference is observed in stimulant-induced locomotion or other measures of food consumption (42,56). These data may implicate the PPT (and its innervation from the striatum) in response selection when discrimination is involved because the disruption of responding for conditioned reinforcement resulted from decreased discrimination of response between a lever associated with reinforcement and an inactive lever (56). However, a recent study found that although PPT lesions increased sucrose consumption, similar lesions did not affect discrimination or contrast effects (57). Nevertheless, the hypothesis of Winn (58) is that lesions of the PPT affect responding for rewarding stimuli similarly to lesions of the frontal cortex, so that the role of the PPT, like that of the basal forebrain, is expanded into higher-order cognitive processes.

ROLE FOR CHOLINERGIC NEURONS IN COGNITIVE PROCESSES

Lesion Studies

The hypothesis of cholinergic involvement in learning and memory processes arose from several findings. Both destruction of the basal forebrain complex and the administration of cholinergic antagonists produce profound deficits in a variety of forms of cognition, including learning and memory (59,60).

The original finding that lesions of the basal forebrain could produce deficits in a variety of cognitive tasks suggested a role for ACh in cognitive function. Electrolytic, radiofrequency, or nonspecific excitotoxic lesions of cholinergic subnuclei within the basal forebrain (particularly the medial septum/diagonal band) profoundly impair performance on a variety of tests of learning, memory, and attention, particularly the Morris water maze and passive avoidance learning (see ref. 59 for review). These deficits appeared to be reversed following regeneration of cholinergic projections across a bridging graft (61) or after grafting of ACh-producing cells in the hippocampus (62). These findings have been interpreted as support for the hypothesis of cholinergic involvement in cognitive functions; however (as with arousal and sleep), noncholinergic neurons within the basal forebrain may likewise be involved in these effects, and more specific approaches must be employed to address these issues.

Novel approaches for selectively destroying cholinergic neurons depend on the differential sensitivity of basal forebrain neurons to excitotoxins and new types of immunotoxins. Systematic studies have demonstrated that cholinergic and noncholinergic neurons within the basal forebrain are differentially sensitive to excitotoxic amino acids such as quisqualate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (Fig. 1.3), kainate, and *N*-methyl-D-aspartate (NMDA) (59). Based on the results of these studies, new methods for preferentially destroying cholinergic neurons have been described (63). Moreover, an IgG–saporin toxin has been developed that takes advantage of the fact that basal forebrain cholinergic neurons are particularly enriched with low-affinity receptors for nerve growth factor (64). The toxin selectively binds to the receptor for nerve growth factor and then kills the neuron expressing the receptor. More excitingly, recent studies suggest that IgG–saporin can be used to destroy the cholinergic innervation of

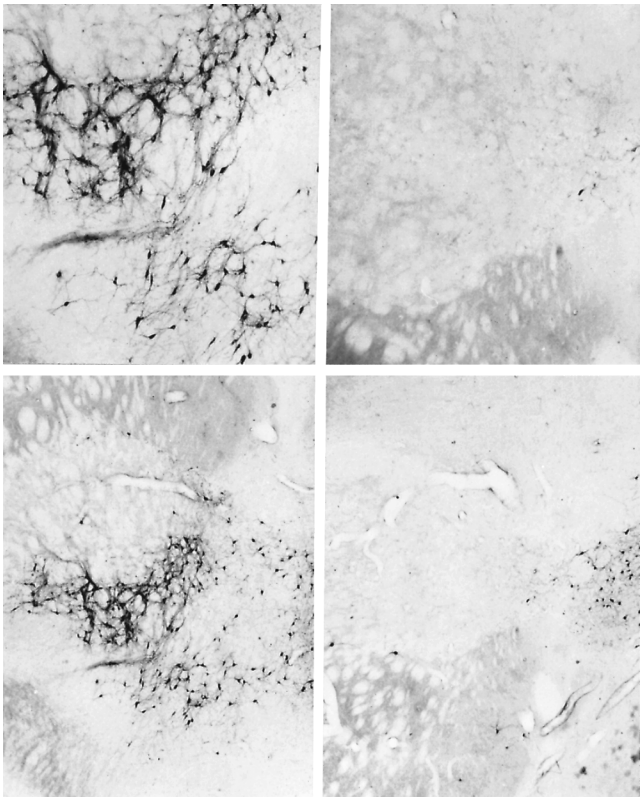

FIGURE 1.3. Acetylcholinesterase staining of the nucleus basalis magnocellularis after infusion of saline solution or AMPA to destroy cholinergic neurons preferentially. Low concentrations of the glutamatergic agonist AMPA selectively destroy cholinergic neurons (measured by acetylcholinesterase staining) and spare γ-aminobutyric acid (GABA) neurons *(left).* In contrast, control sections show robust acetylcholinesterase staining after infusion of saline solution *(right).* This process allows more specific cholinergic lesions to be generated, so that the function of the neurons in behavioral processes can be clarified. (Courtesy of Professor Barry J. Everitt, University of Cambridge.)

terminal regions into which the toxin is injected (65). These methods have been applied to studies of learning and memory in an attempt to qualify earlier findings.

Based on either excitotoxic or saporin lesions of the basal forebrain, the hypothesis for cholinergic function has been revised considerably. Essentially, selective damage to cholinergic neurons of the basal forebrain has failed to produce the retrograde or anterograde amnesia or deficits in learning that have been reported to result from nonspecific lesions of the basal forebrain (59,66). Previously, the medial septal/diagonal band nuclei and their projections to posterior cortical regions were thought to be critical for spatial learning and contextual conditioning (59). By means of saporin lesions, however, cholinergic depletion within the hippocampus or posterior parietal cortex has been shown to result in impairments in latent inhibition or unblocking (65), with sparing of spatial learning (67) and spatial working memory (68). Moreover, selective excitotoxic lesions of the medial septum/diagonal band produce enhancements in contextual conditioning but impairments in discrete cue (trace) conditioning (69). Both sets of data may suggest that the attentional processing of discrete stimuli is disrupted following cholinergic depletion from posterior cortical regions. It is possible, however, that the depletion of ACh from caudal or rostral cortical regions alone may be insufficient to impair performance of some tasks, whereas combined depletions may have more than additive effects (70).

Other investigators have further argued that the cholinergic innervation of rostral (e.g., frontal) cortex from the nucleus basalis is also involved in attentional functions, such as vigilance or sustained, divided attention (59,71). Direct pharmacologic manipulation of basal forebrain neurons has been used to alter activated cholinergic efflux in the frontal cortex and performance of tasks related to stimulus processing or detection (72). Selective excitotoxic lesions or pharmacologic manipulation of the nucleus basalis has also been reported to impair performance in a five-choice serial reaction task that requires animals to detect and respond to brief visual stimuli (73). Interestingly, the observation that appetitive pavlovian learning for a discrete cue is enhanced after nucleus basalis lesions (74) suggests that attentional processing of discrete cues may not be affected by depletion of ACh from the rostral neocortex except when divided attention is required. The findings of these latter studies are also bolstered by advances in the measurement of ACh transmission *in vivo,* which allows investigators to quantify directly the extent of the lesions produced by the toxins for the first time (75). Taken together, the available data seem to suggest that basal forebrain cholinergic neurons are capable of regulating the cortical processing of sensory stimuli within a variety of domains, which may be explained by a role for basal forebrain ACh in the regulation of cortical processing.

Tegmental cholinergic neurons have also been implicated in cognitive processes (58,76). Although some of the effects of PPT lesions on learning and memory may be related to generalized anxiety (76), PPT lesions also produce a set of behavioral deficits that are consistent with executive dysfunction and impairments in frontal lobe functioning (58). In particular, PPT lesions result in deficits of behavioral inhibition and motor perseveration. Notably, working memory performance does not seem to be affected by destruction of the PPT (77). The position of the PPT as a modulator of dopaminergic systems (which affect frontal cortex function), in addition to the influence of the frontal cortex on the PPT (mediated through the striatum), suggests that this nucleus is in an excellent position to affect the functions of the frontostriatal system. Further research that attempts to control for the extent and selectivity of PPT lesions is necessary.

Muscarinic Mechanisms

Although lesions of cholinergic nuclei have implicated ACh in various behavioral processes, it is also of interest to deter-

mine which cholinergic-receptor subtypes mediate these responses to ACh. Systemic infusions of the muscarinic-receptor antagonists atropine and scopolamine produce an amnesic syndrome in humans (78), monkeys (79), and rats (80). Several lines of evidence suggest that multiple central nervous system structures, including the medial septum/diagonal band region, are critical in mediating the effects of muscarinic drugs on mnemonic functions (80). Infusions of muscarinic-receptor antagonists into a variety of cortical regions, including the hippocampus, prefrontal cortex, and amygdala, can impair the cognitive functions associated with these respective regions (81). Similarly, the effects of systemic muscarinic antagonists are attenuated by intraseptal injections of muscarinic agonists, and intraseptal applications of muscarinic antagonists mimic the amnesic effects of systemic treatment with muscarinic antagonists in experimental animals (82). These results suggest that activation of muscarinic receptors by ACh at multiple forebrain sites, including within the somatodendritic regions of the cholinergic neurons, may be involved in the behavioral dysfunction produced by muscarinic cholinergic antagonists.

Figure 1.4 presents the results of an experiment aimed at determining the relationship between *in vivo* cortical cholinergic transmission and the cognitive effects of muscarinic-receptor antagonists. Scopolamine was administered systemically to rats performing a test of working memory, the spatial delayed alternation task, both alone and in combination with FG7142, an anxiogenic β-carboline that acts as an inverse agonist of the benzodiazepine site of the $GABA_A$ receptor. Consistent with previous findings, scopolamine produced dose-dependent performance impairments when administered 45 minutes before testing on the delayed alternation task, suggesting that decrements in cholinergic stimulation of muscarinic receptors result in cognitive dysfunction. FG7142 (20 mg/kg) significantly elevated prefrontal cortical ACh release *in vivo* (measured in parallel studies), and FG7142 on its own impaired delayed alternation performance. Interestingly, the fact that coadministration of FG7142 and scopolamine did not affect the slope of the dose–response curve for scopolamine suggests that these two drugs act on different mechanisms to impair delayed alternation performance. The additivity of these effects indicates that supranormal ACh transmission produced by FG7142 likely does not contribute to the working memory deficits produced by this drug; moreover, the data indicate that the impairments produced by scopolamine are independent of the level of ongoing cortical cholinergic transmission. Thus, it is possible that the cognitive effects of muscarinic antagonists may not be solely the consequence of changes in cortical cholinergic transmission.

The septohippocampal pathway was first believed to convey only cholinergic fibers to the hippocampus (83); the noncholinergic, GABAergic component was discovered almost two decades later (84). Work focusing on the GABA limb of the septohippocampal GABA pathway has suggested that the septohippocampal GABA and cholinergic pathways may both be critical for the effects of septal efferents on cognitive functioning (85). In support of this hypothesis, agents that increase impulse flow in the septohippocampal GABA pathway, including muscarinic agonists, augment

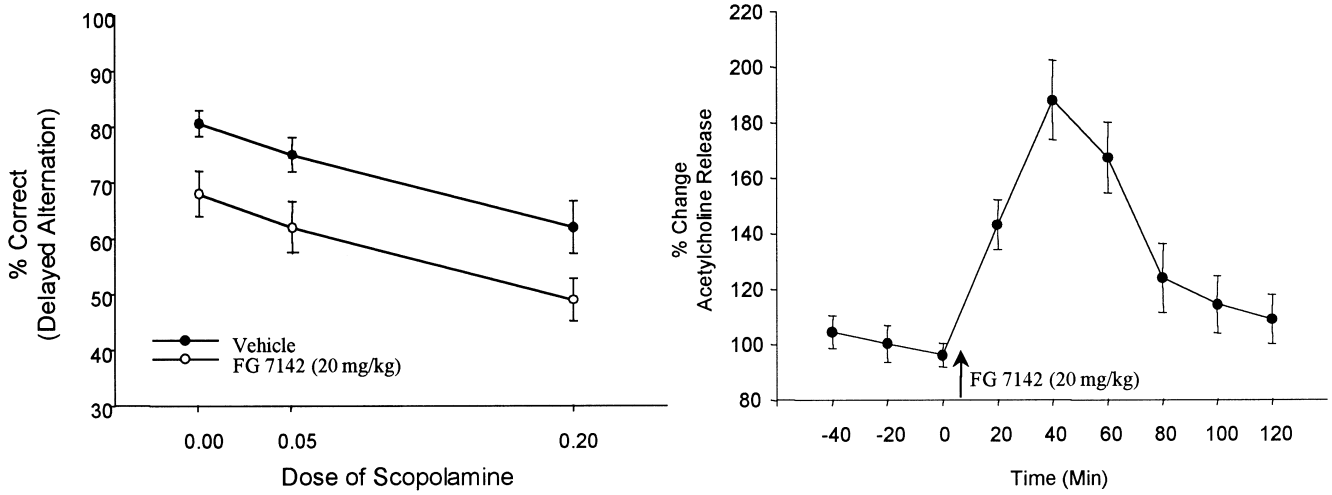

FIGURE 1.4. The cognitive effects of scopolamine administration are insensitive to phasic changes in cortical acetylcholine (ACh) release. Scopolamine dose-dependently impairs performance on a test of spatial working memory, the delayed alternation task, in control rats and rats treated with FG7142, an inverse agonist of the benzodiazepine site of the γ-aminobutyric acid subtype A ($GABA_A$) receptor *(left).* Although FG7142 increases prefrontal cortical ACh release *in vivo (right)* and produces performance deficits on its own *(left),* it does not alter the slope of the dose–response curve for scopolamine.

learning and memory (86), whereas agents that impair learning and memory decrease impulse flow in the GABA pathway (87). Interestingly, impulse flow in the septohippocampal GABA pathway is maintained by ACh released via the *tonic* firing activity of septohippocampal cholinergic neurons. This release occurs via local axon collaterals of septohippocampal neurons, which then synapse on septohippocampal GABA neurons within the medial septum/diagonal band (Fig. 1.5). Thus, interaction between the septohippocampal GABA pathway and muscarinic mechanisms within the medial septum/diagonal band may be crucial for learning and memory (86).

Cholinergic neurons, the primary source of ACh input to the hippocampus, innervate both the hippocampal pyramidal neurons and subpopulations of GABAergic interneurons (88). In contrast, septohippocampal GABA neurons are very selective in their innervation pattern, do not innervate the pyramidal cells at all, but innervate almost every type of hippocampal interneuron (89). Septohippocampal GABA neurons are able to produce a powerful disinhibitory effect on pyramidal cells via this connectivity and so enhance their excitability (90). Loss of cholinergic neurons severely disables the septohippocampal pathway by reducing both the direct excitatory cholinergic drive and the indirect disinhibitory GABA drive to the hippocampus via locally released ACh. A restoration of cholinergic function within the medial septum/diagonal band, not just in the hippocampus, could therefore be critical for the treatment of cognitive deficits associated with the septohippocampal pathway.

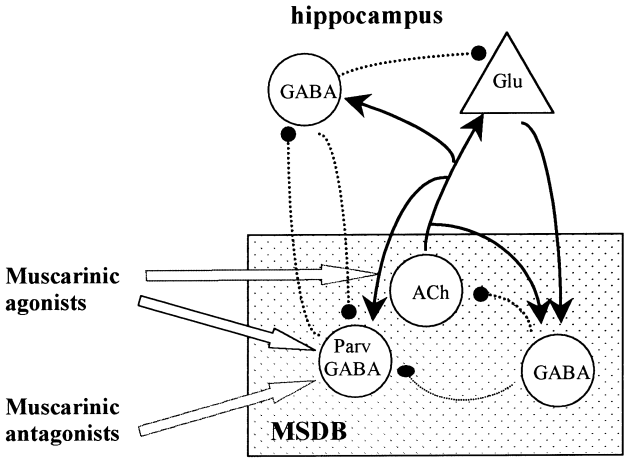

FIGURE 1.5. Schematic representation of the septohippocampal pathway. The medial/septum diagonal band region is composed primarily of cholinergic and GABAergic neurons, and the activity of both neuronal populations is regulated by locally released γ-aminobutyric acid (GABA). The cholinergic neurons and a subpopulation of GABA neurons, containing the calcium-binding protein parvalbumin (parv), project to the hippocampus via the fimbria/fornix. Muscarinic agonists may not increase hippocampal acetylcholine release directly, but rather activate septohippocampal GABA neurons via M3 (and possibly M5) receptors. Similarly, muscarinic antagonists disrupt impulse flow in the septohippocampal GABA pathway.

At a molecular level, the excitatory effects of ACh on hippocampal pyramidal cells were at first thought to be mediated via the M1 subtype of muscarinic receptor, partly as a result of closing of M-type potassium channels, so that specific M1-receptor agonists were developed. However, M1-receptor agonists were found to be of limited use in improving cognition. This might not be surprising because studies of knockout mice lacking M1 receptors show no change in muscarinic enhancement of potassium currents in the hippocampus (30). The finding that non-M1 receptors (M3 and possibly M5) mediate the effects of ACh in the medial septum/diagonal band may further explain the limited effectiveness of M1 agonists in improving learning and memory functions and supports the need for M3-selective agonists (85).

Nicotinic Mechanisms

Nicotinic systems are also involved in several important aspects of cognitive function, including attention, learning, and memory (60). Nicotinic ACh receptors are expressed throughout the brain, including areas involved in cognitive function, such as the hippocampus and frontal cortex (91). Nicotinic agonists improve performance on a variety of memory tasks, particularly following lesions or aging, whereas nicotinic antagonists such as mecamylamine impair working memory function (60). The nAChR subtypes involved in cognitive function are under investigation, and different subtypes may be involved in the performance of different cognitive tasks. As mentioned above, experiments on knockout mice have implicated nAChRs containing the β2 subunit in both passive avoidance learning (11) and maintenance of spatial learning during aging (32). Although the cellular basis for the effects of nicotine are likely to be diverse, one site of action for nicotine, excitation of hippocampal GABAergic interneurons through both α7 and non-α7 subtypes of the nAChR, has been demonstrated by several groups (see ref. 92 for review). Further, although theta rhythm in the hippocampus, a mechanism that appears to facilitate the induction of synaptic plasticity, is abolished by atropine (93), it is converted to burst-mode activity by nicotinic antagonists (94).

Another major contributor to the cholinergic hypothesis of cognitive functioning was the discovery in the early 1980s that cholinergic neurons in the basal forebrain degenerate in Alzheimer's disease (95). Since then, loss/atrophy of cholinergic neurons has been reported not only in Alzheimer's disease but also in Parkinson's disease, Lewy body dementia, progressive supranuclear palsy, and several other disorders (96), although not all studies have reported losses in cholinergic neurons (97). In those that have reported losses, the greatest reduction in numbers, of the order of 50% to 65%, has been observed in cholinergic neurons of the nucleus basalis and the medial septal/diagonal band regions of patients pathologically verified as having Alzheimer's disease (96). Loss of high-affinity nAChRs has also been seen in the brains of patients with Alzheimer's disease (98), and

nicotinic agonists have been proposed as potential therapeutic agents to treat the disease (60).

ROLE FOR CHOLINERGIC NEURONS IN STIMULUS PROCESSING

Several lines of evidence suggest that cholinergic neurotransmission through nAChRs can affect stimulus processing. In support of this notion, nicotine has been reported to alleviate some sensory gating deficits in schizophrenic patients (99), and animal studies also suggest that nicotine may act to facilitate sensory inhibition, such as prepulse inhibition of startle in mice (100) and rats (101). In another animal model of sensory processing, latent inhibition, nicotine can either enhance or disrupt sensory habituation, depending on the preexposure parameters (102). Lesions of the nucleus accumbens or the pedunculopontine nucleus have been shown to block prepulse inhibition (103), whereas lesions of the hippocampus, septum, medial raphe, and nucleus accumbens disrupt latent inhibition (104), observations suggesting that nicotine may act in one or more of these brain areas to affect sensory processing. Another brain area that is likely to mediate the effect of nicotine on sensory gating in schizophrenia is the hippocampus. Postmortem studies have shown a reduced number of α-bungarotoxin-sensitive nAChRs (α7-containing nAChRs) in the hippocampus in schizophrenic patients (105). Further, pharmacologic (106) and genetic (107) studies have suggested a role for the α7 nAChR in prepulse inhibition in rodents.

A series of physiologic studies also supports the concept that ACh, acting on muscarinic receptors within the cerebral cortex, promotes cortical responses to exogenous stimuli. ACh can produce a biphasic effect on membrane polarization in cortical neurons: a rapid hyperpolarization followed by a prolonged depolarization (108). The inhibitory component was mediated through ACh-induced activation of GABAergic interneurons that inhibited the pyramidal cells in a feed-forward manner. In contrast, the long-lasting depolarization was mediated through direct effects of ACh on the cortical neuron. Subsequent studies suggested that this effect is mediated by blockade of I_m, a voltage-sensitive rectifying K^+ channel (14). In addition, ACh reduced spike frequency adaptation by blocking the after-hyperpolarization effect.

The net physiologic effect of these changes in cortical cell physiology may be to render pyramidal cells more responsive to afferent input. Because the membrane is more depolarized, neurons are more likely to fire in response to a given excitatory stimulus; also, the response to that stimulus may be prolonged because the after-hyperpolarization has been blocked. Thus, it seems plausible that muscarinic cholinergic effects on cortical pyramidal cells may indeed promote stimulus access to the cortical circuit. Inasmuch as attentional processing may represent the ability of stimuli to be processed actively within the neocortex, these physiologic actions of ACh may be consistent with the reported behavioral effects of cholinergic lesions.

CONCLUSIONS

Recent studies using new physiologic techniques, cholinergic-selective toxins, and molecular genetic techniques have refined our ideas about the role of ACh in the brain. In particular, it is clear that cholinergic and GABAergic pathways are intimately connected in the hippocampus and basal forebrain complex and may combine to exert their effects on cognition, attention, and arousal. Further, the subtypes of cholinergic receptors that mediate these effects of ACh are beginning to be elucidated with the use of knockout mice that lack specific receptor subunits. These techniques have contributed to a minirevolution in our views of how ACh contributes to cognitive processes. Research in this area is moving very quickly, and it is likely that these ideas will continue to be refined as the new techniques are applied to previously described behavioral paradigms. Improvements in existing techniques—for example, through the development of inducible and site-specific mutations in cholinergic-receptor subtypes—will also contribute to further refinements in our view of cholinergic functions in the brain.

SUMMARY

Acetylcholine is critical for communication between neurons and muscle at the neuromuscular junction, is involved in direct neurotransmission in autonomic ganglia, and has been implicated in cognitive processing, arousal, and attention in the brain. The results of recent studies in which specific cholinotoxins, electrophysiology, or molecular genetic techniques were used have altered our view of the functional role of the cholinergic system in the brain. Mice that lack specific subunits or subtypes of muscarinic or nAChRs have recently been generated and used to demonstrate the role of particular receptor subtypes in physiologic effects of ACh in muscle, peripheral ganglia, and the central nervous system. Roles for cholinergic neurons have been found in arousal and sleep, motivation and reward, cognitive processes, and stimulus processing. The evaluation of these functions by means of novel cholinotoxins and new electrophysiologic techniques have refined our ideas about the role of ACh in the brain. The evidence that cholinergic and GABAergic pathways are intimately connected in the hippocampus and basal forebrain complex and may combine to affect cognition, attention, and arousal is reviewed. In addition, the subtypes of cholinergic receptors that mediate these effects of ACh are discussed based on studies of knockout mice that lack specific receptor subunits. Improvements in existing techniques—for example, through the development of inducible and site-specific mutations in

cholinergic-receptor subtypes—will contribute to further refinements in our view of cholinergic functions in the brain.

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