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Review

Acetylcholine as a Neuromodulator: Cholinergic Signaling Shapes Nervous System Function and Behavior

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Acetylcholine in the brain alters neuronal excitability, influences synaptic transmission, induces synaptic plasticity, and coordinates firing of groups of neurons. As a result, it changes the state of neuronal networks throughout the brain and modifies their response to internal and external inputs: the classical role of a neuromodulator. Here, we identify actions of cholinergic signaling on cellular and synaptic properties of neurons in several brain areas and discuss consequences of this signaling on behaviors related to drug abuse, attention, food intake, and affect. The diverse effects of acetylcholine depend on site of release, receptor subtypes, and target neuronal population; however, a common theme is that acetylcholine potentiates behaviors that are adaptive to environmental stimuli and decreases responses to ongoing stimuli that do not require immediate action. The ability of acetylcholine to coordinate the response of neuronal networks in many brain areas makes cholinergic modulation an essential mechanism underlying complex behaviors.

Acetylcholine (ACh) is a fast-acting, point-to-point neurotransmitter at the neuromuscular junction and in the autonomic ganglia; however, there are fewer demonstrations of similar actions in the brain (Changeux, 2010). Instead, central cholinergic neurotransmission predominantly changes neuronal excitability, alters presynaptic release of neurotransmitters, and coordinates the firing of groups of neurons (Kawai et al., 2007; Rice and Cragg, 2004; Wonnacott, 1997; Zhang and Sulzer, 2004). As a result, ACh appears to act as a neuromodulator in the brain, despite its role as the primary excitatory neurotransmitter in the periphery.

The definition of a neuromodulator is flexible, but has evolved to describe any kind of neurotransmission that is not directly excitatory (mediated through ionotropic glutamate receptors) or inhibitory (mediated through ionotropic gamma-aminobutyric acid [GABA] receptors) (Ito and Schuman, 2008; Siggins, 1979). Neuromodulation can be thought of as a change in the state of a neuron or group of neurons that alters its response to subsequent stimulation. A number of models have been proposed to explain the actions of ACh in the central nervous system (CNS). For example, ACh has been suggested to be critical for the response to uncertainty, such that an increase in cholinergic tone predicts the unreliability of predictive cues in a known context and improves the signal-to-noise ratio in a learning environment (Yu and Dayan, 2005). Another model has suggested that ACh reinforces neuronal loops and cortical dynamics during learning by enhancing the influence of feed-forward afferent inputs to the cortex carrying sensory information and decreasing excitatory feedback activity mediating retrieval (Hasselmo, 2006). ACh can also alter firing of neurons on a rapid time scale, as in fear-conditioning, when foot-shock results in direct cholinergic activation of interneurons in the auditory cortex that contribute to learning (Letzkus et al., 2011). All these models are consistent with a primary role of ACh as a neuromodulator that changes the state of an ensemble of neurons in response to changing environmental conditions.

In this review, we will provide further support for the idea that cholinergic neurotransmission in the brain is primarily neuromodulatory and is categorically distinct from the actions of ACh at the neuromuscular junction. We propose that the role of ACh as a neuromodulator in the brain is to increase neurotransmitter release in response to other inputs, to promote burst firing, and/or suppress tonic firing, depending upon the system and the neuronal subtypes stimulated. Further, ACh contributes to synaptic plasticity in many brain areas.

Cholinergic Neurons and Ach Receptors

The two primary sources of ACh in the brain include projection neurons that innervate distal areas and local interneurons that are interspersed among their cellular targets. Cholinergic projection neurons are found in nuclei throughout the brain, such as the pedunculopontine and laterodorsal tegmental areas (PPTg and LDTg), the medial habenula (MHb) (Ren et al., 2011), and the basal forebrain (BF) complex (Mesulam, 1995; Zaborszky, 2002; Zaborszky et al., 2008), including the medial septum. These cholinergic neurons project widely and diffusely, innervating neurons throughout the CNS. Cholinergic interneurons are typified by the tonically active ACh neurons of the striatum and nucleus accumbens, and there is some indication from anatomical studies that cholinergic interneurons are present in the rodent and human neocortex, but not the nonhuman primate cortex (Benagiano et al., 2003; Mesulam, 1995; von Engelhardt

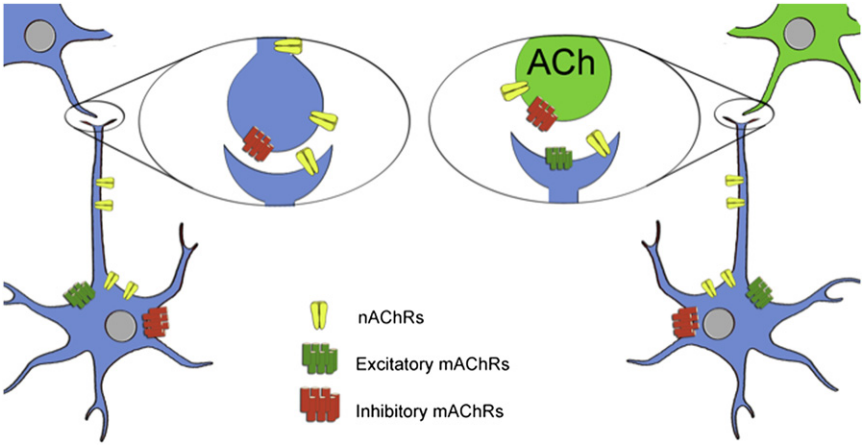

Figure 1. Sites of Action for Nicotinic and Muscarinic Acetylcholine Receptors
Nicotinic (nAChR) and muscarinic (mAChR) acetylcholine receptors are localized both pre- and postsynaptically. Presynaptic mAChRs (M2, M4) are largely inhibitory and act as inhibitory autoreceptors on cholinergic terminals, with M2 the predominant autoreceptor in the hippocampus and cerebral cortex and M4 predominant in striatum (Wess, 2003b; Wess et al., 2003). Postsynaptic mAChRs can be either inhibitory (M2, M4) or excitatory (M1, M3, M5) (Wess, 2003b; Wess et al., 2003). Presynaptic nAChRs induce release of a number of neurotransmitters, including GABA, glutamate, dopamine, serotonin, norepinephrine, and acetylcholine (McGehee et al., 1995; Wonnacott, 1997). Postsynaptic nAChRs depolarize neurons, increase their firing rate, and can contribute to long-term potentiation (Bucher and Goaillard, 2011; Ge and Dani, 2005; Ji et al., 2001; Kawai et al., 2007; Mansvelder and McGehee, 2000; Picciotto et al., 1995, 1998; Radcliffe and Dani, 1998; Wooltorton et al., 2003).

et al., 2007). The actions of ACh released from both populations of cholinergic cells are mediated through pre- and postsynaptic receptors on a large variety of neuronal subtypes throughout the brain, and it should be noted that cholinergic inputs contribute to cortical and hippocampal function across phylogeny.

ACh signals through two classes of receptors: metabotropic muscarinic receptors (mAChRs) and ionotropic nicotinic receptors (nAChRs) (reviewed in Picciotto et al., 2000 and Wess, 2003a). Muscarinic receptors are coupled either to G_q proteins (M1, M3, and M5 subtypes) that activate phospholipase C or $G_{i/o}$ proteins (M2 and M4 subtypes) that negatively couple to adenylate cyclase (reviewed in Wess, 2003a), linking ACh activity to a variety of biochemical signaling cascades. Moreover, mAChRs are located both pre- and postsynaptically throughout the brain, producing diverse consequences for brain activity (Figure 1). As examples of the heterogeneous effects of mAChR stimulation, presynaptic M2/M4 mAChRs can act as inhibitory autoreceptors on cholinergic terminals (Douglas et al., 2002; Raiteri et al., 1984) and reduce glutamate release from corticocortical and corticostriatal synapses (Higley et al., 2009, Gil et al., 1997). In contrast, M1/M5 receptors can stimulate dopamine (DA) release from striatal synaptosomes (Zhang et al., 2002) and postsynaptic M1/M5 receptors can increase excitability of cortical pyramidal neurons (Douglas et al., 2002; McCormick and Prince, 1985).

Nicotinic receptors function as nonselective, excitatory cation channels (Changeux et al., 1998; Picciotto et al., 2001) and occur as homomeric or heteromeric assemblies of a large family of α- and β-subunits (α2-α7 and β2-β4; reviewed in Picciotto et al., 2000). While neuromodulators are typically associated with metabotropic signaling, the role of the ionotropic nAChRs in the brain appears to be largely modulatory as well (Picciotto, 2003). For example, nAChRs are not clustered at postsynaptic membranes apposed to sites of ACh release, but are rather dispersed along the surface (and intracellular compartments) of neurons, including presynaptic terminals (McGehee et al., 1995; Vidal and Changeux, 1993), cell bodies, and even axons (Arroyo-Jiménez et al., 1999; Hill et al., 1993; Kawai et al., 2007). In addition, stimulation of nAChRs can increase the release of glutamate, GABA, DA, ACh, norepinephrine, and serotonin (McGehee et al., 1995; Wonnacott, 1997) (Figure 1). Nicotinic modulation of neurotransmitter release is often subtype-specific, and this specificity can vary across brain areas, with distinct nAChRs coupling to release of glutamate (α7) versus GABA (α4β2) (Mansvelder et al., 2002) in the ventral tegmental area (VTA), while β2-containing nAChRs can modulate the release of glutamate from thalamocortical projections (Parikh et al., 2010). Similarly, different nAChR subtypes mediate the release of DA (α4/α6β2) versus ACh (α3β4) (Grady et al., 2001). Presynaptic effects of nAChRs contribute to synaptic plasticity in the VTA (Mansvelder and McGehee, 2000; Wooltorton et al., 2003), hippocampus (Ge and Dani, 2005; Ji et al., 2001; Radcliffe and Dani, 1998), and prefrontal cortex (Couey et al., 2007). In addition, nAChRs may also be important for synchronizing neuronal activity. For example, nicotine is reported to coordinate firing of thalamocortical fibers through effects on nAChRs in white matter (Bucher and Goaillard, 2011; Kawai et al., 2007). Despite the clear effects of presynaptic nAChRs in electrophysiological studies, their relationship to the behavioral consequences of nicotine administration is not completely understood. For example, nicotine stimulates the firing of DA neurons through actions in the VTA and increases release of DA from the midbrain projections to the nucleus accumbens (NAc) through actions on terminal nAChRs, but local infusion of nicotine into the VTA has much greater effects on locomotion and self-administration than local infusion into the NAc (Ferrari et al., 2002; Ikemoto et al., 2006). Recent studies have, however, suggested that nAChRs in the NAc are important for the motivational effects of nicotine (association between stimulus and drug intake), rather than the primary reinforcing effects of the drug (desire for drug) (Brunzell et al., 2010). In addition, it is clear that cholinergic interneurons and their regulation of muscarinic receptor signaling are also critical components in striatum-dependent decision making (see, e.g., Goldberg et al., 2012).

While presynaptic effects of nAChRs have been the focus of a great deal of work, effects of nicotinic stimulation are clearly not exclusively presynaptic (Figure 1). Exogenous application of nicotine can induce significant inward currents in neurons in a number of brain areas (Léna and Changeux, 1999; Picciotto

et al., 1995, 1998), and there have been several examples of direct postsynaptic effects of ACh in the brain (Alkondon et al., 1998; Jones et al., 1999). Notably, recent studies using optogenetic techniques demonstrated that ACh can mediate postsynaptic responses through nAChRs in the hippocampus (Bell et al., 2011; Gu and Yakel, 2011) and cortex (Arroyo et al., 2012).

Modes of Cholinergic Neuromodulation

Although there is considerable evidence for the actions of ACh on target neurons, the mode of cholinergic transmission has remained controversial. The debate has focused on whether cholinergic signaling occurs via traditional synapses (cellular specializations comprising closely apposed pre- and postsynaptic membranes with associated release/receptor machinery) or via volume transmission (actions of a neurotransmitter that occur at a distance from its site of release, mediated by diffusion through the extracellular space (Zoli et al., 1999). Accumulating evidence indicates that ACh can act through volume transmission in the brain. The relatively diffuse nature of brain cholinergic innervation further reinforces this idea. There is an anatomical mismatch between the sites of ACh release (Houser, 1990; Wainer et al., 1984a, 1984b) and the location of cholinergic receptors (Arroyo-Jiménez et al., 1999; Hill et al., 1993; Kawai et al., 2007). There is also evidence that extracellular levels of ACh fluctuate in a manner that is not consistent with localized clearance of a synaptic transmitter (Hajnal et al., 1998; Laplante et al., 2004; Mark et al., 1996; Parikh et al., 2004; Reid et al., 1998). However, contrasting observations, including the role of ACh in fast synaptic transmission at the neuromuscular junction and the high level of expression of ACh esterase (AChE; a highly efficient degradative enzyme responsible for clearing ACh from the extracellular space) have limited the acceptance of this idea. Ultimately, it is difficult to know how far ACh can diffuse from its site of release and whether volume transmission would allow for rapid transfer of information, suggesting that this is not the only mechanism through which ACh influences neuronal function in the brain. Anatomical studies have identified cortical cholinergic synapses that are structurally similar to those of other point-to-point neurotransmitters in both rats (Turrini et al., 2001) and humans (Smiley et al., 1997). Effects of ACh on a rapid time-scale likely underlie its role in stimulus-response tasks in which subsecond reactivity is required for appropriate behavioral responses, as in prefrontal cortex-dependent cue detection (Parikh et al., 2007a) or auditory discrimination (Letzkus et al., 2011). The data indicate that differences in sites of receptor expression, affinity of ACh at both mAChRs and nAChRs, rates of synaptic clearance by [AChE]) and local concentration of ACh in and outside the synapse are critical for the control and specificity of cholinergic signaling. In addition, differences in the time-scale of release at the local microcircuit level further refine the action of ACh in complex behaviors (reviewed in Hasselmo and Giocomo, 2006; Sarter et al., 2009; and Yu and Dayan, 2005).

Role of Ach in Synaptic Plasticity and Neuronal Development

An important role for both nAChRs and mAChRs has been defined in hippocampal synaptic plasticity (reviewed in Giocomo and Hasselmo, 2007 and McKay et al., 2007), and these effects are mediated through intracellular signaling pathways downstream of mAChRs and nAChRs (reviewed in Berg and Conroy, 2002; Cancela, 2001; Lanzafame et al., 2003; and Rathouz et al., 1996). Recent studies suggest that the timing of ACh release and the subtype of receptor is critical for the type of plasticity induced (Gu and Yakel, 2011); however, it is clear that nAChRs and mAChRs on both GABAergic and glutamatergic neurons in the hippocampus can alter the subsequent response to excitatory inputs (Drever et al., 2011). Similarly, stimulation of nAChRs on glutamatergic terminals in the VTA can induce long-term potentiation (LTP) of excitatory inputs onto DA neurons (Mansvelder and McGehee, 2000), whereas differential time-scales of effects of nAChRs on glutamatergic and GABAergic terminals in this area appears to be important for changes in dopaminergic firing following prolonged exposure to nicotine (Mansvelder et al., 2002; Wooltorton et al., 2003).

The ability of ACh to influence synaptic plasticity and dynamics of local circuits can also occur through astrocytic control of synaptic Ca^{2+} concentration following nAChR stimulation (Takata et al., 2011). Astrocytic signaling can lead to LTP as a result of the temporal coincidence of the postsynaptic activity and the astrocyte Ca^{2+} signal simultaneously evoked by cholinergic stimulation (Navarrete et al., 2012).

In contrast to the ability of nAChR stimulation to promote LTP in a number of brain areas, nAChR-mediated facilitation of GABA release reduces calcium levels in prefrontocortical dendrites (Couey et al., 2007). In addition, activation of nAChRs can also decrease subsequent stimulation of calcium entry into cortical neurons in response to glutamate (Stevens et al., 2003). The decrease in glutamate-mediated calcium entry is mediated through activation of high affinity nAChRs, subsequent activation of the protein phosphatase calcineurin, and inactivation of L-type calcium channels. If this mechanism is also recruited as a result of ACh signaling in vivo, it would suggest that one consequence of cholinergic activity in cortical neurons would be a significant decrease in subsequent calcium-mediated glutamate responses.

Finally, in addition to the ability of ACh to modulate neuronal activity acutely in adulthood, ACh can also alter a number of processes in neuronal development, and the molecular basis for a number of these developmental effects of ACh signaling have been elucidated recently. For example, one fundamental role for ACh signaling through nAChRs is to regulate the timing of expression of the chloride transporter that is necessary for the ability of GABA to hyperpolarize, and therefore inhibit, central neurons (Liu et al., 2006). Disrupting nAChR signaling delays the switch from GABA-mediated excitation to inhibition. Recent studies have also shown that nAChRs contribute to the maturation of GABAergic (Kawai et al., 2002; Zago et al., 2006) and glutamatergic (Lozada et al., 2012a, b) synapses, highlighting an important role for ACh signaling in synaptic development, as well as neuronal pathfinding and target selection (reviewed in Role and Berg, 1996). In addition, signaling through nAChRs is also important for establishing critical periods for activity-dependent shaping of visual cortical function (Morishita et al., 2010) and maturation of thalamocortical (Aramakis and Metherate, 1998; Aramakis et al., 2000; Hsieh et al., 2002) and

corticothalamic (Heath et al., 2010; Horst et al., 2012; King et al., 2003; Picciotto et al., 1995) glutamatergic synapses. It appears likely that ACh release, potentially in response to salient stimuli, potentiates glutamatergic synapses during development through an LTP-like mechanism (Aramakis and Metherate, 1998), highlighting another important role for cholinergic signaling in synaptic plasticity. Several neurotrophic factors are also involved in the development and maturation of cholinergic neurons, but the dependence on neurotrophins is not homogenous throughout the CNS (for reviews, see Angelucci et al., 2005 and Schindowski et al., 2008). Although a comprehensive review of the developmental effects of ACh is beyond the scope of this article, it is important to note that various developmental processes can be affected by ACh signaling (for more comprehensive reviews, see Heath and Picciotto, 2009; Liu et al., 2007; Metherate and Hsieh, 2003; and Role and Berg, 1996).

Brain Systems Modulated by Ach Signaling

Mesolimbic DA System, Addiction and Reward

A great deal of research has focused on the effects of cholinergic agents on the mesolimbic DA system and its short- and long-term modulation (for reviews, see Fagen et al., 2003; and Mansvelder et al., 2003), particularly because the addictive effects of nicotine are mediated primarily through stimulation of nAChRs in the VTA (Drenan et al., 2008; Maskos et al., 2005; McGranahan et al., 2011; Picciotto et al., 1998). Cholinergic input from the PPTg and LDTg acting through both mAChRs and nAChRs is critical for modulating the function of the VTA. Stimulation of nAChRs and M5-type mAChRs increases the tonic excitability of these DA neurons (Corrigall et al., 2002; Miller and Blaha, 2005; Yeomans and Baptista, 1997). ACh released in the VTA would potentiate glutamatergic synaptic transmission onto DA neurons through $\alpha 7$ nAChRs and therefore increase the likelihood of burst firing of these neurons (Grenhoff et al., 1986; Maskos, 2008; McGehee et al., 1995).

Extracellular ACh levels are increased in the VTA during drug self-administration (You et al., 2008), which could result from an increase in ACh release from PPTgg and LDTg afferents (Futami et al., 1995; Omelchenko and Sesack, 2006). Cholinergic neurons within PPT do not exhibit burst firing, and they are more active during wakefulness and rapid eye movement (REM) sleep versus slow wave sleep (Datta and Siwek, 2002); however, there is currently no evidence that VTA DA neurons show circadian variations in activity, suggesting that the diurnally regulated neurons may not project to VTA. In addition, PPTg neurons change their firing rate in response to both locomotion and acquisition of reward (Datta and Siwek, 2002). These observations have led to the idea that the PPTg acts as a gate for salient sensory information associated with reward and/or requiring movement (Norton et al., 2011).

In contrast to the increased firing rate of cholinergic neurons in the PPTg in response to contextual information related to reward, tonically active cholinergic interneurons in the striatum pause their firing following exposure to cues associated with reward (Goldberg and Reynolds, 2011). The pause is thought to be mediated by interactions between the cells' intrinsic membrane properties and strong feed-forward excitation from the thalamus (Ding et al., 2010). These cholinergic interneurons

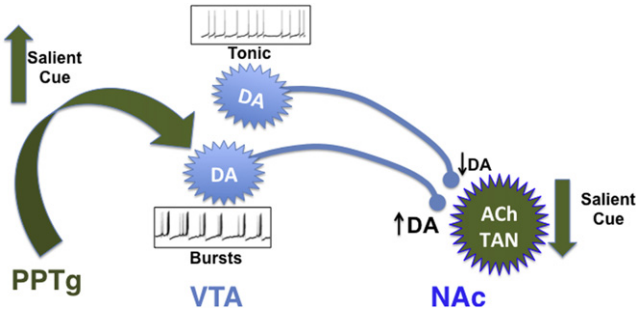

Figure 2. Effects of Acetylcholine on Activity of Dopamine Neurons in the Mesolimbic Circuit

Salient cues associated with primary reward increase activity of PPTg neurons, inducing acetylcholine release in the VTA (Futami et al., 1995; Omelchenko and Sesack, 2006). Acetylcholine increases firing of DA neurons in the VTA and is likely to be important for burst firing of these neurons (Maskos, 2008). Salient cues associated with reward also induce a pause in firing of tonically active cholinergic neurons in the NAc (Goldberg and Reynolds, 2011). Decreased release of ACh onto terminals in NAc attenuates DA release due to tonic firing of DA neurons, while preserving DA release in response to phasic firing (Exley and Cragg, 2008).

can regulate the duration, magnitude, and spatial pattern of activity of striatal neurons, potentially creating an attentional gate that facilitates movement toward salient stimuli (Oldenburg and Ding, 2011). The function of striatal cholinergic interneurons is also impaired in patients with movement disorders involve the dopaminergic system, such as Parkinson's and Huntington's disease and in animal models of these diseases (Ding et al., 2011). Cholinergic signaling in striatum and NAc is also thought to be critical for mediating the association between drugs of abuse and cues in the environment that drive drug craving and relapse to drug use after abstinence (Exley and Cragg, 2008). The effects of striatal ACh are mediated in part through activation of nAChRs on dopaminergic terminals, leading to tonic, low level DA release when cholinergic interneurons are firing. The pause results in decreased tonic DA release, but maintained phasic DA release (Exley and Cragg, 2008). In contrast, mAChRs reduce the probability of glutamate release from excitatory afferents to the striatum, negatively regulating the ability of these inputs to drive striatal activity (Barral et al., 1999; Higley et al., 2009; Pakhotin and Bracci, 2007). Reduced concentration of glutamate in the synaptic cleft results in diminished activation of voltage-dependent N-methyl-D-aspartate (NMDA)-type glutamate receptors, shortening excitatory response duration and limiting temporal integration of inputs (Higley et al. 2009). Thus, the pause in cholinergic interneuron firing would be predicted to enhance the efficacy and summation of glutamatergic inputs arriving during this period.

These findings suggest that salient sensory stimuli in the environment, such as those associated with reward or drugs of abuse, would increase activity of PPTg cholinergic neurons, leading to increased phasic firing of DA neurons in the VTA (Maskos, 2008), while at the same time decreasing the firing of tonically active cholinergic neurons in the NAc and striatum, leading to a larger differential in DA release in response to phasic firing as compared to tonic firing (Exley and Cragg, 2008) (Figure 2). At the behavioral level, this conclusion is consistent with the finding

that disruption of PPTg activity decreases the rewarding and locomotor effects of drugs of abuse, such as cocaine and nicotine (Champtiaux et al., 2006; Corrigall et al., 1994, 2002), while lesion of NAc cholinergic neurons increases cocaine self-administration, as might be expected if a pause in cholinergic interneuron firing in NAc signals salience (Smith et al., 2004).

The behavioral role of individual ACh receptor subtypes in NAc is more complex, however. Consistent with a role for the pause in NAc cholinergic neurons in behaviors related to drug reward, antagonism of α7-type nAChRs in NAc increases motivation to lever press for nicotine (Brunzell and McIntosh, 2012). Less intuitively, blockade of mAChRs using scopolamine decreases reinstatement of cocaine seeking (Yee et al., 2011), but this may be due to increased ACh release through blockade of inhibitory autoreceptors (Douglas et al., 2001). Consistent with the fact that neuromodulation can be complex, it has also been shown that antagonism of the α6/β2 class of nAChRs expressed on DA terminals in NAc decreases the breakpoint for progressive ratio responding in rats self-administering nicotine, suggesting that there is also a role for ACh signaling through this class of receptors for mediating the motivational value of nicotine (Brunzell et al., 2010).

A number of studies have focused on the ability of the habenula, particularly the MHb, to oppose the behavioral processes mediated through the VTA (for reviews, see Fowler and Kenny, 2012 and Hikosaka, 2010). The MHb-interpeduncular pathway is cholinergic, and it has been proposed that its effects on VTA neuron firing are mediated indirectly through inhibition of the PPTg (Maskos, 2008). Decreasing the expression of nAChRs containing the α5 subunit in the MHb results in increased nicotine self-administration (Fowler et al., 2011), suggesting that this cholinergic system normally acts as a brake on drug reward.

Taken together, these studies suggest that point-to-point ACh signaling could have opposing behavioral consequences, depending on the receptor subtypes, neuronal populations, and brain areas stimulated, and that effects of ACh mediated through volume transmission could be distinct from those mediated locally.

Cortex and Attention

Numerous studies indicate that ACh plays an important role in the regulation of cortical activity over multiple timescales. The precise function of ACh on any given circuit also depends on the specific expression pattern of nAChRs and mAChRs, as well as the temporal dynamics of ACh concentration in the extracellular space. Neocortical ACh function has been linked to control of circuits underlying attention, cue detection, and memory (Hasselmo and Sarter, 2011). The primary cholinergic input to the cerebral cortex comes from the BF complex including the substantia innominata the nucleus basalis of Meynert (Mesulam, 1995), though the latter remains debated (Zaborszky et al., 1999). Cholinergic terminals are distributed throughout the cortex, with more dense projections in superficial layers (Mesulam, 1995).

The cellular mechanisms underlying the effects of ACh on cortical circuits have been investigated at many levels. Seminal studies revealed that ACh can produce biphasic changes in the activity of pyramidal neurons, the principal excitatory cells in the neocortex, comprising fast inhibition followed by a slow depolarization (McCormick and Prince, 1985, 1986). The fast

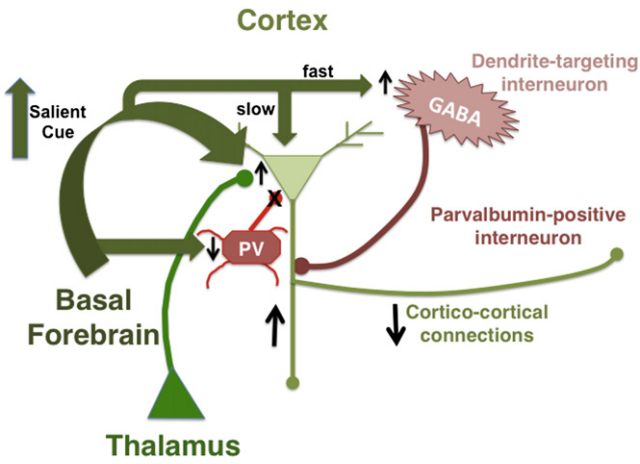

Figure 3. Effects of Acetylcholine on Activity of Cortical Neurons
Salient cues induce acetylcholine release onto interneurons targeting the apical dendrites of cortical pyramidal neurons, resulting in rapid inhibition of pyramidal cells (Arroyo et al., 2012; Couey et al., 2007; Fanselow et al., 2008; Férézou et al., 2002; Gulledge et al., 2007; Kawaguchi and Kubota, 1997). Acetylcholine subsequently depolarizes pyramidal neurons through M1 mAChRs (Delmas and Brown, 2005; McCormick and Prince, 1985, 1986). Acetylcholine also activates stimulatory α4β2 nAChRs on glutamatergic thalamocortical terminals (Gil et al., 1997; Lambe et al., 2003; Oldford and Castro-Alamancos, 2003) and inhibitory M2 mAChRs on GABAergic terminals of parvalbumin-expressing (PV) interneurons (Kruglikov and Rudy, 2008). Activation of PV interneurons enhances stimulation of pyramidal neuron firing by thalamocortical inputs (Gabernet et al., 2005; Higley and Contreras, 2006; Kruglikov and Rudy, 2008). Acetylcholine also suppresses corticocortical transmission through inhibitory M2 mAChRs on pyramidal cell axon terminals (Gil et al., 1997; Hsieh et al., 2000; Kimura and Baughman, 1997; Oldford and Castro-Alamancos, 2003), reducing intracortical communication while preserving responses to thalamic inputs (Kimura et al., 1999).

inhibition is at least partially mediated by the actions of both nAChRs and mAChRs that increase the excitability and firing rates of dendrite-targeting GABAergic interneurons (Arroyo et al., 2012; Couey et al., 2007; Fanselow et al., 2008; Férézou et al., 2002; Gulledge et al., 2007; Kawaguchi and Kubota, 1997). The slow depolarization is mediated by M1 mAChR-mediated closure of M-type (KCNQ) potassium channels in pyramidal neurons (Delmas and Brown, 2005), enhancing their excitability and reducing their spike frequency adaptation (Gulledge et al., 2007; Hasselmo and Giocomo, 2006). In addition, nAChRs expressed in deep layer pyramidal neurons may contribute to direct excitation of these cells (Bailey et al., 2010; Kassam et al., 2008; Poorthuis et al., 2012).

ACh also modulates synaptic transmission in cortical circuits (Figure 3). Activation of α4β2 nAChRs on thalamocortical terminals enhances glutamate release in both sensory and association cortex (Gil et al., 1997; Lambe et al., 2003; Oldford and Castro-Alamancos, 2003), whereas activation of mAChRs on terminals of parvalbumin-expressing interneurons decreases the probability of GABA release onto the perisynaptic compartment of pyramidal neurons and therefore reduces postsynaptic inhibition of pyramidal neurons (Kruglikov and Rudy, 2008). These interneurons normally decrease the response of cortical neurons to feed-forward excitation (Gabernet et al., 2005; Higley and Contreras, 2006), and the reduction of GABA release from these interneurons by ACh therefore enhances the ability of

thalamocortical inputs to stimulate pyramidal neuron firing (Kruglikov and Rudy, 2008).

In contrast, mAChRs located on pyramidal cell axon terminals suppress corticocortical transmission (Gil et al., 1997; Hsieh et al., 2000; Kimura and Baughman, 1997; Oldford and Castro-Alamancos, 2003). Moreover, the ACh-mediated increased excitability of dendrite-targeting interneurons described above likely contributes to reduced efficacy of intracortical communication. The simultaneous enhancement of feed-forward inputs from the thalamus through cholinergic actions on parvalbumin-positive interneurons and suppression of intracortical feedback inputs through effects on dendrite-targeting interneurons may increase the "signal-to-noise" ratio in cortical networks, making neurons more sensitive to external stimuli. In keeping with this view, mAChR activation strongly suppresses the spread of intracortical activity, leaving responses to thalamic inputs relatively intact (Kimura et al., 1999). Intriguingly, in the prefrontal cortex, the expression of nAChRs in deep pyramidal cells may produce layer-specific cholinergic modulation, selectively enhancing activity of output neurons (Poorthuis et al., 2012).

Although the cellular and synaptic effects of ACh described above provide a potential mechanism for the ability of ACh to increase signal detection and modulate sensory attention, a number of observations suggest that this simple model is incomplete. ACh, acting via M4 mAChRs, directly inhibits spiny stellate cells in somatosensory cortex receiving thalamic input (Eggermann and Feldmeyer, 2009). Furthermore, activation of M1 mAChRs hyperpolarizes pyramidal neurons via a mechanism dependent on fully loaded internal calcium stores that occurs more quickly than the closure of M-type potassium channels (Gulledge et al., 2007; Gulledge and Stuart, 2005). Thus, the effect of ACh on the activity of pyramidal neurons depends critically on the state of the neuron and the timing of ACh release. Neurons with depleted calcium stores would be more susceptible to indirect ACh-induced depolarization via M4 mAChRs, whereas rapid, direct inhibitory effects of ACh through M1 mAChRs would dominate in neurons with fully replenished stores. Furthermore, studies showing that mAChR activation reduces cortico-cortical transmission have relied on electrical stimulation to evoke glutamate release, leaving the identity of the activated presynaptic terminals ambiguous. It is possible that distinct populations of intracortical synapses, such as those comprising local recurrent networks versus long-range intra-areal projections, might be differentially modulated by ACh. Indeed, in the CA1 region of the hippocampus, long-range perforant inputs from the entorhinal cortex are less inhibited by ACh than the Schaeffer collaterals arising from CA3 (Hasselmo and Schnell, 1994). The advent of optogenetic tools for selectively targeted difference populations of excitatory inputs (Gradinaru et al., 2007) will be a key development for elucidating the precise role of ACh on various circuit elements.

ACh also modulates cortical circuits over longer time scales by influencing neuronal plasticity. In the auditory cortex, pairing sensory stimulation with stimulation of the basal forebrain results in long-term reorganization of cortical receptive field structure, including a persistent shift in the receptive field toward the paired stimulus (Froemke et al., 2007). In the visual system, ACh facilitates ocular dominance plasticity in kittens via M1 mAChRs (Gu and Singer, 1993), and in rodents, the protein Lynx1 suppresses nicotinic signaling in primary visual cortex, and its removal promotes ocular dominance plasticity in older animals (Morishita et al., 2010).

At the cellular level, cholinergic agonists enhance LTP of glutamatergic association fibers in the piriform cortex and Schaeffer collaterals in the CA1 region of the hippocampus (Huerta and Lisman, 1993). In contrast, M3 mAChRs facilitate long-term depression of synapses in the monocular area of the superficial visual cortex (Kirkwood et al., 1999; McCoy and McMahon, 2007). Surprisingly, the same authors observed enhanced LTP in the binocular cortex (McCoy et al., 2008). These regional differences indicate that cell-type specific expression of different receptor subtypes is critical for the varied actions of ACh.

The pleiotropic effects of ACh on cortical circuits described above are likely to underlie its ability to modulate cognitive behaviors. In rodents, lesions of cholinergic inputs to the cortex impair tests of sustained attention, particularly across sensory modalities (McGaughy et al., 1996, 2002; Turchi and Sarter, 1997). In addition, stimulation of $\alpha4\beta2$ nAChRs in the medial prefrontal cortex enhances performance in a visual attention task (Howe et al., 2010), while genetic deletion of these receptors in the medial PFC impairs visual attention (Guillem et al., 2011) and auditory discrimination (Horst et al., 2012). Notably, transient rises in prefrontal ACh are significantly correlated with cue detection, suggesting that the temporal dynamics of cholinergic signaling are also critical for normal behavior (Parikh et al., 2007b). In primates, locally applied ACh enhances the attentional modulation of neuronal activity in the primary visual cortex, while the muscarinic antagonist scopolamine reduces the effects of attention (Herrero et al., 2008). Taken together, these findings suggest that cholinergic actions across both ionotropic and metabotropic receptors and diverse brain areas contribute to cognitive processing.

Hypothalamus and Food Intake

The role of ACh in control of autonomic functions is well known, but it is likely that actions of ACh in the brain also modulate adaptive responses to environmental and metabolic conditions. Cholinergic signaling can alter thermoregulation (Myers and Waller, 1973), sleep patterns (Steriade, 2004), food intake (Grunberg et al., 1988; Mineur et al., 2011), and endocrine functions, such as pancreatic release of insulin and glucagon (Ishikawa et al., 1982). The hypothalamus is essential for homeostatic responses regulating metabolism, and consequently, modulation of hypothalamic function by ACh is likely to be an important component of adaptation to peripheral autonomic signals to the brain.

A small number of studies have investigated the role of ACh signaling in the hypothalamus, which receives input from the PPTg and LDTg (Hallanger and Wainer, 1988; Jones and Beaudet, 1987). Activity in both these areas adapts quickly to environmental changes (Majkutewicz et al., 2010; Woolf, 1991) and is linked to peripheral control of feeding behavior (Phillis, 2005). There are also intrinsic neurons within the hypothalamus that express cholinergic markers (Tago et al., 1987) along with the pro-opiomelanocortin (POMC) peptide (Meister et al., 2006), and nAChRs in the hypothalamus are critical for feeding behavior (Jo et al., 2002). It has also been suggested that neurons in the median eminence could project to the hypothalamus (Schäfer

et al., 1998). Corticotropin-releasing hormone-expressing neurons in this area can affect metabolism. In nonhuman primates, neurons in the substantia innominata and lateral hypothalamus (LH), most of which express cholinergic markers, were activated in response to presentation of food when the animals were hungry (Rolls et al., 1979). Consistent with a potential role for ACh in coordinating caloric need with food-seeking behaviors, long-term maintenance on a high-fat/high-sugar diet significantly downregulated levels of AChE in a number of brain areas that was particularly pronounced in the hypothalamus (Kaizer et al., 2004). One possibility is that the role of ACh in the hypothalamus is to integrate the interoceptive cues related to hunger with exteroceptive cues of food availability, threat, or other salient conditions (Craig, 2002, 2003), but this remains to be tested.

At the cellular level, stimulation of nAChRs and mAChRs on LH neurons increases and decreases GABA release, respectively (Jo and Role, 2002). The data suggest that nAChRs and mAChRs may be localized to different populations of GABAergic terminals, but from these studies, it is difficult to determine what the effects of synaptically evoked ACh on LH GABA release might be. Optogenetic stimulation of cholinergic transmission in the LH and hypothalamus will be useful in identifying the source of ACh input to these areas, the role of intrinsic ACh in hypothalamic function, and the differential role of mAChRs and nAChRs in shaping responses to ACh in these brain regions. In the arcuate nucleus of the hypothalamus, nicotine increases the firing rate of both POMC- and neuropeptide Y (NPY)-positive neurons, although the increase in POMC neuron activity predominates in vitro due to more rapid desensitization of nAChR responses in NPY neurons, and in vivo, as evidenced by an increase in c-*fos* immunoreactivity predominantly in POMC-positive cells (Huang et al., 2011; Mineur et al., 2011). Thus, as in the mesolimbic system and the cortex, distinct actions of ACh appear to converge through effects on receptor populations with different electrophysiological properties expressed on distinct subsets of neurons to promote a coordinated output, in this case, activation of POMC neurons.

ACh also regulates glutamatergic transmission in other neuronal subtypes involved in food intake. Stimulation of nAChRs on orexin-positive neurons in the LH induces concurrent release of glutamate and ACh, which could lead to feed-forward stimulation of this circuit once activated (Pasumarthi and Fadel, 2010). There is also some indication from studies of hypothalamic neurons in culture that ACh signaling can be upregulated to compensate for prolonged blockade of glutamatergic signaling (Belousov et al., 2001). Thus, ACh acting through nAChRs may also potentiate glutamate signaling in particular neuronal subtypes of the hypothalamus, although the functional consequences of this regulation are not yet known.

As might be expected from the complex regulation of hypothalamic neuronal activity by ACh, cholinergic modulation of feeding behavior is multifactorial and state-dependent. In rats, the mAChR competitive antagonist atropine modestly altered the frequency and choice of meals, but not their size (Nissenbaum and Sclafani, 1988). Consistent with the ability of nicotine in tobacco smoke to decrease body weight in humans and food intake in rats (Grunberg et al., 1988), β4-containing nAChRs on POMC neurons are critical for the ability of nicotine to reduce food intake in mice (Mineur et al., 2011). These observations underscore a potential role for ACh in metabolic regulation involving POMC neurons; however, very little is known about the role of endogenous ACh-mediated modulation of the arcuate nucleus.

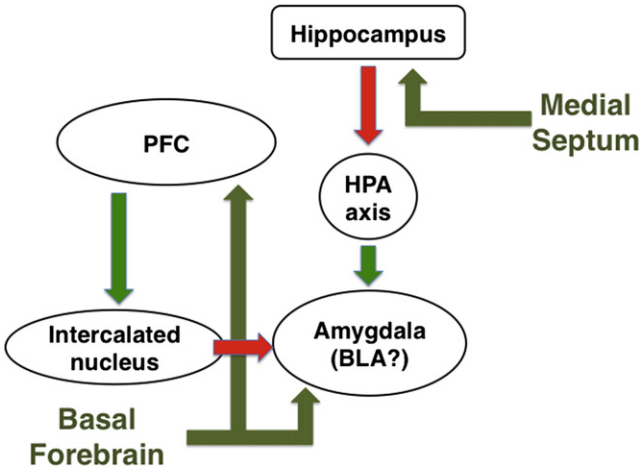

Figure 4. Effects of Acetylcholine on Hippocampal-Amygdala Stress Response

Stress increases acetylcholine release in the hippocampus and frontal cortex (Mark et al., 1996) and impairs signaling in the PFC (Arnsten, 2009). The hippocampus provides inhibitory feedback to the amygdala through inhibition of the HPA axis (Tasker and Herman, 2011), whereas the PFC can normally decrease basolateral amygdala activity through projections to the intercalated nucleus (Mańko et al., 2011; Pinard et al., 2012). The effects of stress-induced acetylcholine release on output of the hippocampus and cortex is unknown, but cholinergic modulation of cortico-amygdala glutamatergic connections strengthens associations between environmental stimuli and stressful events (Mansvelder et al., 2009).

ACH and Stress-Related Systems

Increasing evidence suggests that ACh signaling in a number of brain areas is important for stress responses (Figure 4). In addition to the well-documented role of the hippocampus in learning and memory, the amygdala in mediating fear responses and the prefrontal cortex (PFC) in attention, these brain areas are critical nodes in adaptation and responses to stress (Belujon and Grace, 2011; Gozzi et al., 2010; McGaugh, 2004; Sapolsky, 2000; Tottenham and Sheridan, 2009). Dysfunction in the activity of these regions is strongly implicated in major depressive disorder (Sheline et al., 1998; Videbech and Ravnkilde, 2004). The hippocampus, amygdala, and PFC receive a very high level of cholinergic input that comes from the BF complex and, in particular, from the medial septum and nucleus basalis, respectively (Mesulam, 1995). Several studies have shown that stress increases ACh release in a brain region-specific manner (Mark et al., 1996). For instance, hippocampal and cortical ACh levels can increase following restraint stress in rats, while ACh levels in the amygdala are unchanged, although an increase in amygdalar cholinergic tone can also reduce basolateral amygdala (BLA) activity though activation of mAChRs (Power and Sah, 2008). Conversely, acute activation of presynaptic α7 nAChRs in the BLA can also favor the release of glutamate from impinging cortical projections, which is critical for aversive memory and

fear (Klein and Yakel, 2006). Stimulation of this pathway during development blunts paired facilitation due to subsequent stimulation, however, which would be expected to decrease BLA reactivity (Jiang and Role, 2008), further highlighting the role of cholinergic signaling in plasticity of this system. The hippocampus provides inhibitory feedback to the amygdala through inhibition of the hypothalamic-pituitary-adrenal (HPA) axis (Tasker and Herman, 2011). Interestingly, relief from stress leads to an increase in cholinergic signaling in the amygdala and PFC (Mark et al., 1996), indicating that the valence of ACh varies by brain area. The effect of increased cortical ACh levels on amygdala signaling has not been studied, but stress impairs PFC output (Arnsten, 2009), and PFC can normally decrease basolateral amygdala activity through projections to the intercalated nucleus (Mańko et al., 2011; Pinard et al., 2012).

At the cellular level, neuronal activity in the hippocampus is strongly modulated by both nAChRs and mAChRs. Cholinergic inputs to the hippocampus from the medial septum and the diagonal band of Broca impinge on both glutamatergic and GABAergic neurons throughout the structure, and a comprehensive review of the effects of ACh on synaptic plasticity in the hippocampus has been published recently (Drever et al., 2011). The ability of ACh to induce synaptic plasticity through actions on pre- and postsynaptic nAChRs and mAChRs is likely to modulate learning and memory, including memory of stressful events (Nijholt et al., 2004), and a role for ACh in regulation of hippocampal excitability through presynaptic release of glutamate and GABA has also been well-characterized (Alkondon et al., 1997; Freund et al., 1988; Radcliffe et al., 1999). Stress also induces alternative splicing of the AChE messenger RNA (mRNA) in the hippocampus, leading to altered ACh signaling in this structure (Nijholt et al., 2004). There is currently no consensus on how these cholinergic actions converge to regulate the output of the hippocampus in response to stress, although one possibility is that ACh is critical for regulating theta oscillations, and the concurrent effects of mAChRs and nAChRs on excitatory and inhibitory transmission serve to regulate rhythmic activity (Drever et al., 2011; Fisahn et al., 1998). Although theta rhythms are thought to be critical for memory encoding, disturbance of hippocampal rhythms may also contribute to mood disorders (Femenía et al., 2012).

The amygdala also receives cholinergic inputs from the basal forebrain complex (Mesulam, 1995) and is consistently hyperactivated in fMRI studies of patients with mood disorders (Drevets, 2001). In rodents, decreasing ACh signaling through nAChRs depresses neuronal activity in the basolateral amygdala, as measured by c-*fos* immunoreactivity (Mineur et al., 2007). As discussed above, ACh shapes the output of cortical neurons, and cortico-amygdala glutamatergic connections are also strongly and persistently potentiated by nAChR stimulation (Mansvelder et al., 2009). Thus, ACh release in the amygdala is thought to strengthen associations between environmental stimuli and stressful events, potentially contributing to maladaptive learning underlying affective disorders (Mansvelder et al., 2009).

There is strong evidence that increasing ACh signaling in humans results in increased symptoms of depression (Janowsky et al., 1972; Risch et al., 1980). This has been observed with administration of the AChE blocker physostigmine to patients with a history of depression, individuals with Tourette's syndrome, and normal volunteers (Risch et al., 1980, 1981; Shytle et al., 2000). A similar effect has also been described with organophosphate inhibitors of AChE (Rosenstock et al., 1991). More recently, human imaging and post mortem studies have suggested that there is increased occupancy of nAChRs by ACh that is highest in individuals who are actively depressed and intermediate in those who have a history of depression with no change in overall nAChR number (Saricicek et al., 2012). In rodent studies, the Flinders rat model was selected for its sensitivity to challenge with an AChE inhibitor, and sensitive rats also display a constellation of depression-like endophenotypes, supporting the idea that increasing ACh levels increases symptoms of depression (Overstreet, 1993).

Consistent with an increase in ACh leading to symptoms of depression, antagonism of mAChRs or nAChRs or blockade of ACh signaling through nAChRs with partial agonists can decrease depression-like behavior in rodents (Caldarone et al., 2004; De Pablo et al., 1991; Mineur et al., 2007; Picciotto et al., 2002; Rabenstein et al., 2006). In humans, clinical trials have suggested that blockade of either mAChRs (Furey and Drevets, 2006; Furey et al., 2010) or nAChRs (George et al., 2008; Shytle et al., 2002) can decrease symptoms of depression. While an increase in cholinergic tone appears to be sufficient to induce depression-like symptoms in humans, a recent study has shown that decreasing striatal cholinergic tone in the mouse can lead to depression-like symptoms, likely through interneuron-dependent disinhibition of striatal neurons (Warner-Schmidt et al., 2012), highlighting the fact that ACh can induce heterogeneous effects in different brain areas that appear to have opposite behavioral consequences. The behavioral effect of ACh signaling in vivo likely depends on the baseline conditions in the particular circuit of interest at the time of ACh release and is the result of integration of its sometimes conflicting effects in different circuits. More studies are necessary to determine whether preclinical studies of cholinergic signaling in hippocampus, PFC, and/or amygdala can be linked to the effects of ACh in human subjects and to identify physiological mechanisms that are essential for these effects on behaviors related to mood and affect.

Conclusions

A comprehensive explanation of cholinergic neuromodulation is not yet possible, given the large number of behaviors, circuits, neuronal subtypes, and cholinergic receptors in the brain. Despite that complexity, some unifying themes have emerged. The well-defined temporal association between firing of cholinergic projection neurons in the brain stem and the pause in firing of tonically active cholinergic interneurons in the striatum can facilitate the association of salient rewarding events with cues in the environment, contributing to reward prediction and promoting orienting behaviors toward potentially rewarding stimuli. This likely occurs through coordinated increases in glutamatergic drive that facilitate DA neuron burst firing and decreases in response to subthreshold, tonic signals from DA terminals. Similarly, salient signals that require focused attention for correct performance of behavioral tasks increase feed-forward

activation of principal cortical neurons and decrease inhibition through specific classes of interneurons. The promotion of coordinated firing of adjacent axons and the promotion of rhythmic activity in structures, such as the hippocampus when ACh is released and levels are high, may provide an increase in the baseline excitability of neurons that are then available for robust responses to glutamate, and this state dependent facilitation of neurotransmission in pathways activated in response to ACh release is likely to be maintained due to facilitated neuronal plasticity. This organization is echoed in the hypothalamus, where, despite the ubiquitous expression of nAChRs on multiple neuronal subtypes with reciprocal functions, the kinetics of activation of one set of receptors may bias the output in one direction, based on the starting conditions. This is obviously a gross oversimplification that will be sensitive to the timing, duration, and localization of ACh signaling, but may provide a framework for generation of hypotheses. Finally, increases in ACh signaling appear to contribute to stress-related illnesses, such as major depressive disorder, although the specific neuronal substrates and cellular mechanisms responsible for these effects are only beginning to be studied.

Despite a great deal of progress, there are still critical gaps in our understanding of the dynamics of ACh release from different neuronal populations; how that changes in response to environmental conditions, such as metabolic need or stress; and how far from the site of release ACh can diffuse in different brain areas. While novel tools will allow more precise stimulation of ACh release, the patterns of release will not be optimal unless there is a better understanding of the physiological patterns of firing. The ability to mimic patterns of ACh release in vivo will be critical for identifying the physiological effects of cholinergic neuromodulation and distinguishing the actual from the possible effects of ACh in the brain.

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