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## Review

# Changes in Acetylcholine Extracellular Levels During Cognitive Processes

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Measuring the changes in neurotransmitter extracellular levels in discrete brain areas is considered a tool for identifying the neuronal systems involved in specific behavioral responses or cognitive processes. Acetylcholine (ACh) is the first neurotransmitter whose diffusion from the central nervous system was investigated and whose extracellular levels variations were correlated to changes in neuronal activity. This was done initially by means of the cup technique and then by the microdialysis technique. The latter, notwithstanding some technical limitations, makes it possible to detect variations in extracellular levels of ACh in unrestrained, behaving animals. This review summarizes and discusses the results obtained investigating the changes in ACh release during performance of operant tasks, exposition to novel stimuli, locomotor activity, and the performance of spatial memory tasks, working memory, and place preference memory tasks. Activation of the forebrain cholinergic system has been demonstrated in many tasks and conditions in which the environment requires the animal to analyze novel stimuli that may represent a threat or offer a reward. The sustained cholinergic activation, demonstrated by high levels of extracellular ACh observed during the behavioral paradigms, indicates that many behaviors occur within or require the facilitation provided by the cholinergic system to the operation of pertinent neuronal pathways.

Acetylcholine (ACh) is the first neurotransmitter whose diffusion from the central nervous system was investigated and whose extracellular levels variations were correlated to changes in neuronal activity. ACh outflow from the spinal cord was detected by Bulbring and Burn (1941) during nerve stimulation, and the first attempts to demonstrate ACh outflow from the intact cortex were made 50 years ago. The purpose was to demonstrate whether ACh had a role in the CNS, and the theoretical approach was the same used in the previous decade for demonstrating the neurotransmitter role of ACh in the peripheral nervous system (Burn 1968). Using the cup technique and the leech bioassay, MacIntosh and Oboring (1955) demonstrated in the dog that ACh release was related to the spontaneous electrical activity of the cortex. The cup was formed by a small cylinder exerting a slight pressure over the meninges, filled with Ringer solution containing a cholinesterase inhibitor. No ACh could be detected in the absence of the inhibitor. The method was perfected by Mitchell (1953), and a description of the procedure with an analysis of the early literature reporting the changes in ACh release induced by stimulation of peripheral nerves, specific brain areas, and drugs can be found in reviews by Pepeu (1973), and Moroni and Pepeu (1984).

The first question asked was whether ACh diffusing from the brain into the cortical cup originated from cholinergic nerve endings and whether changes in its extracellular levels were expressions of changes in the activity of the cholinergic nerve endings under the cup. The answer was positive because ACh release was higher from an activated than from a synchronized cortex (Bartolini and Pepeu 1967), and an increase in ACh release from the sensorimotor and parietal cortex could be elicited by forepaw stimulation (Mitchell 1953), visual stimulation (Phillis 1968), stimulation of the mesencephalic reticular formation (Kanai and Szerb 1965), and of the nucleus basalis (Casamenti et al. 1986). Moreover, ACh output from the brain was calcium-dependent

(Randic and Padjen 1967) and, to some extent, frequency-dependent (Szerb 1967).

These experiments allowed us to conclude that activation of the cholinergic neurons innervating the cerebral cortex was a component of the arousal mechanism.

## The Cup Technique in Freely Moving Animals

Beani et al. (1968) were the first to implant the cortical cup semi-permanently in unanaesthetized rabbits. Beani demonstrated that behavioral and EEG depression was associated with a marked decrease in ACh output. Conversely, in freely moving guinea pigs, a DL-DOPA-induced increase in motility was accompanied by an increase in ACh release (Beani and Bianchi 1970). Casamenti et al. (1980) investigated ACh release from the cerebral cortex in morphine-dependent, freely moving rats, demonstrating no changes in tolerant rats but a marked increase during the excitation accompanying naloxone-induced withdrawal syndrome. However, the difficulties of sample collection, the need for long collection periods given the low sensitivity of the bioassays, and the positioning of the cup limited to the cortex allowed only the investigation of cortical ACh release in relation with gross behavioral changes such as sedation and excitation and prevented the study of ACh release during more complex spontaneous and acquired behaviors.

## The Microdialysis Technique

A fundamental progress in the study of neurotransmitter release in unrestrained, behaving animals was made with the development of the microdialysis technique some 20 years ago (Ungerstedt 1984; Westerink 1995). The microdialysis technique is based on the assumption that the extracellular neurotransmitter levels equilibrate with the solution flowing through dialysis tubings implanted in discrete brain areas. It allows the monitoring of neurotransmitter released not only from the cortex but also from subcortical structures, which were previously reachable only with the push-pull cannula, a cumbersome and difficult technique (Gaddum 1961). The microdialysis, coupled to high performance liquid chromatography (HPLC), makes it possible to detect extra-

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cellular levels of neurotransmitters including ACh, amines, adenosine, NO, peptides, amino acids, and other endogenous molecules. The experience derived from the cortical cup experiments was transferred to microdialysis, and it has never been questioned that ACh detected in the dialysate originates from cholinergic nerve endings and its levels change in accordance to the activity of the cholinergic neurons. Moreover, addition of tetrodotoxin to the superfusate, by blocking nerve ending activity, strongly reduces ACh release (Damsma et al. 1988; Mark et al. 1992; Giovannini et al. 2001).

According to the canonical description of the cholinergic synapse, ACh, stored in the synaptic vesicles, undergoes quantal release that depends on the intensity of the depolarization (Deutch and Roth 1999). Once released in the synaptic cleft, ACh diffuses, binds to pre- and postsynaptic muscarinic and nicotinic receptors, and is hydrolyzed by cholinesterase. In spite of cholinesterase efficiency (Silver 1974), small amounts of ACh can be detected in the extracellular fluid even in the absence of cholinesterase inhibitors. Under these conditions, Scali et al. (1997) found, in the effluent from the microdialysis probe in young rats at rest, ACh levels of  $5.5 \pm 1.0$  and  $5.0 \pm 1.0$  fmole/ $\mu$ L of perfusate (mean of 11–12 experiments  $\pm$  SEM) in the cerebral cortex and in the hippocampus, respectively. However, to facilitate ACh quantification and reduce the sample collection duration, a cholinesterase inhibitor is commonly added to the Ringer solution perfused through the microdialysis probe. With 7  $\mu$ M eserine in the perfusate, the cortical ACh level was  $54.5 \pm 9.2$  fmole/ $\mu$ L (mean of six experiments  $\pm$  SEM; Giovannini et al. 1998).

The microdialysis technique was initially used for investigating drug modulation of ACh release (Bertorelli and Consolo 1990) and age-associated differences in the activity of the cholinergic system. It was found (Wu et al. 1988) that ACh release from the cerebral cortex, hippocampus, and striatum is significantly lower in 18–20-month-old than in 3-month-old rats.

A caveat in the interpretation of the results obtained with microdialysis is that stressor stimuli, such as prolonged handling (Nilsson et al. 1990; Rosenblad and Nilsson 1993), restraint (Imperato et al. 1991), and fear (Acquas et al. 1996), strongly activate the cholinergic system. Therefore, before associating a behavioral response to variations in ACh release, it is necessary to exclude the possible interference of stressors.

### Lesions of the Cholinergic Pathways Decrease ACh Release and Induce Memory Deficits

Experiments with lesions of the forebrain cholinergic neurons made by different neurotoxins have helped to enlighten the role of ACh in learning and memory and have demonstrated that the decrease in ACh extracellular levels is accompanied by specific behavioral deficits. Excitotoxic lesions of the NB induce a long-lasting, significant decrease in cortical ACh release both at rest and under  $K^+$  depolarization, paralleled by disruption of a passive avoidance conditioned response (Casamenti et al. 1988) and working memory (Bartolini et al. 1996; Casamenti et al. 1998). Disruption of the septo-hippocampal projections impairs choice accuracy in short-term memory (Flicker et al. 1983) and results in deficits in a T-maze performance (Rawlins and Olton 1982).

The most selective procedure for the disruption of the cholinergic neurons is the use of 192IgG-saporin, which, injected intracerebrally (Heckers et al. 1994), causes an almost complete cholinergic deafferentation to the cortex and hippocampus and a significant decrease in ACh release from these structures (Rossner et al. 1995). All behavioral studies performed in rats with i.c.v. injections of 192IgG-saporin indicate that only very extensive lesions involving >90% of cholinergic neurons result reliably in severely impaired performances (for review, see Wrenn and

Wiley 1998). Impairments in water-maze acquisition (Leanza et al. 1995), delayed matching (Leanza et al. 1996), and nonmatching to position task (McDonald et al. 1997), as well as acquisition, but not retention, of an object discrimination (Vnek et al. 1996) or of spatial working memory (Shen et al. 1996) have been reported in the rat. Ballmaier et al. (2002) demonstrated in rats that bilateral 192IgG-saporin lesions of the nucleus basalis reduce cortical ACh release below detection limits and abolish prepulse inhibition. Restoration of ACh levels to normal levels by a cholinesterase inhibitor was associated with reappearance of prepulse inhibition, a finding indicating a role of the forebrain cholinergic system in the sensory motor gating.

### Acetylcholine and Attention

Studies in rodents and monkeys indicate that the cholinergic projection neurons may be required not for learning per se, but may be important for specific aspects of attention (Sarter and Bruno 2000). Microdialysis experiments indicate that cortical ACh increases during performance of simple operant tasks are limited to early acquisition stages, when demands on attentional processing are high (Muir 1996). Similarly, Orsetti et al. (1996) observed a large increase in cortical and hippocampal ACh release during acquisition of a rewarded operant behavior, but not during its recall. Cortical ACh release increases in rats performing a visual attentional task (Dalley et al. 2001) and is directly correlated with the attentional effort during an operant task designed to assess sustained attention (Himmelheber et al. 2000). Activation of basalocortical cholinergic afferents may foster the attentional processing that is central to the memory-related aspects of anxiety caused by threat-related stimuli and associations (Berntson et al. 1998). Intraparenchymal injections of 192IgG-saporin have been used to study the effects of basal forebrain cholinergic lesions on attentional processing (Stoehr et al. 1997). Most of the studies report disrupted attentional processing in NB- or MS-injected animals (McGaughy et al. 1996; Wrenn and Wiley 1998), thus confirming the role of the cholinergic system in attention. However, a correlation between attentional effort, required by the task difficulties, and ACh release has not always been found (Passetti et al. 2000).

### ACh Release and Motor Activity

Most of the tests used to study cognitive responses in animals involve locomotor activation that appears to be associated with an increase in ACh release. However, attempts to establish a direct correlation between motor activity and ACh release provided contradictory results. Watanabe et al. (1990) demonstrated a direct correlation between spontaneous motility and ACh release from the striatum under conditions minimizing the effect of arousal and novelty. A relationship between ACh release from the cerebral cortex, hippocampus, and striatum and locomotor activity, considered a measure of behavioral arousal, was reported by Day et al. (1991), and a similar correlation was also found by Mizuno et al. (1991). However, Day and Fibiger (1992), Moore et al. (1992), and Thiel et al. (1998b) did not confirm this correlation. It is therefore important to define whether changes in ACh release from the cerebral cortex and hippocampus are always associated with, and in some way related to, motor activity or conditions exist in which increases in ACh release unrelated to motor activity take place.

Giovannini et al. (2001) found no correlation between ACh release and motor activity in the first exposure to a novel environment but only in a second exposure to the same environment after 60 min, when habituation was setting in. This finding indicates that ACh release from the frontal cortex and hippocampus has several components, one of which is motor activity.

Other components could be attention, arousal, anxiety, and stress. The presence of several functions associated with or depending on ACh release may explain the contradictions between the results reported in this section. The relationship between motor activity and ACh release may depend on the region investigated, the different levels of arousal and attention, and the type of behavior. As already mentioned, Watanabe et al. (1990) demonstrated a relationship between motor activity and ACh from the striatum, a structure involved in motor activity control. The same amount of motor activity is needed for the acquisition of an operant behavior, during which attention is required and a large increase in cortical and hippocampal ACh release occurs, as is required for recall, which is not associated with an increased release and requires a low level of attention (Orsetti et al. 1996). Further evidence that ACh release and motor activity are not necessarily related comes from the work of Ragozzino et al. (1996). They showed that glucose administration was followed by an increase in ACh release from the hippocampus and an improvement of spontaneous alternation in a four-arm maze, with no increase in the number of arms explored.

Hippocampal ACh release shows a circadian variation (Mizuno et al. 1991, 1994; Mitsushima et al. 1998), increasing at the start of the dark period in nocturnal animals such as rats, which corresponds to the active phase. Indeed, it has recently been demonstrated (Sei et al. 2003) that in *clock* mutant mice, which show ~2-h delayed circadian profiles in body temperature, activity, and sleep-wake rhythm, the increase in hippocampal ACh release in the first 2 h of the active period is suppressed, thus providing evidence that hippocampal cholinergic function, and presumably arousal, are strongly affected by clock-related molecular mechanisms.

### ACh Release and Novelty

Inglis and Fibiger (1995) observed that visual, auditory, olfactory, and tactile stimuli increased ACh release in the cerebral cortex and hippocampus and elicited different behaviors including signs of fear, in response to noise and stimulation, exploratory behavior after a visual stimulus, and sniffing and consummatory behavior after olfactory stimulation. All stimuli produced an ACh increase of the same size in the hippocampus, whereas in the cortex, the tactile stimulation produced a larger increase than the other stimuli. It appears therefore that the cholinergic system responds with an activation to all types of external inputs, irrespective of the behavioral response. The relationship between stimulation and cholinergic response was further investigated by Acquas et al. (1996). It was observed that a paired tone and light stimulus significantly increased ACh release in the frontal cortex and hippocampus when it was presented for the first time or was the conditioned stimulus of a conditioned fear paradigm. However, no increase in ACh release and behavioral response occurred if the tone and noise stimuli were presented repeatedly over an 8-d period leading to habituation development.

A novel environment represents a stressful condition, and the first exposure to it causes pronounced behavioral activation (Aloisi et al. 1997; Ceccarelli et al. 1999), which provides one of the most elementary forms of learning. Rats placed in novel environments, either an arena with objects or a Y maze, showed a 150%–200% increase in ACh release from the cerebral cortex (Giovannini et al. 1998). If the rats were placed in the arena for only 2 min, no habituation occurred. However, if the rats were left for 30 min in the arena, habituation developed, as demonstrated by a much smaller increase in motor activity and ACh release when the rats were placed again in the arena 60 min later, in comparison with the first exposure (Giovannini et al. 2001). The maximum increase was 64% in the cortex and 200% in the

hippocampus in the first exploration of the arena and 37% and 51%, respectively, in the second exploration. Under similar experimental conditions, the increase in aspartate, glutamate, GABA, and ACh, in the ventral hippocampus, associated with exploratory activity in a novel environment, also tends to be much smaller in the second period of exposure to the arena than in the first, indicating the development of habituation (Bianchi et al. 2003).

The increase in ACh release from the frontal cortex and the hippocampus confirms the activation of the forebrain cholinergic neurons by novelty previously demonstrated by electrophysiological techniques (Gray and McNaughton 1983). The presence of hippocampal theta rhythm during exploratory activity (Whishaw and Vanderwolf 1973) and attention (Green and Arduini 1954) is further evidence of cholinergic activation because theta rhythm depends on the septo-hippocampal cholinergic pathway (Stewart and Fox 1990).

Not only environmental novelty, but also novel tastes are associated with an increase in ACh release in the insular cortex, which is involved in the mnemonic gustatory representation of tastes (Miranda et al. 2000). These investigators demonstrated a doubling of ACh release when the rats received saccharine in their drinking fluid, the disappearance of the increase at the third saccharine administration, and a new sharp increase when saccharine was substituted with quinine. In this experiment, the involvement of motor activity in the cholinergic activation can be excluded. Unfortunately, it has not been investigated whether a specific novel gustatory stimulus also activates ACh release in the hippocampus and in other cortical areas or only in the gustatory area. The role of the cholinergic system of the insular cortex in the encoding of taste information has been demonstrated by Naor and Dudai (1996), who disrupted the encoding by blocking the muscarinic receptors by local administration of scopolamine.

### Spatial Memory

Hippocampal ACh release increases during performance of a learned spatial memory task (Ragozzino et al. 1999; Stancampiano et al. 1999), and, interestingly, the improvement in radial arm maze performance is positively correlated to the increase in ACh release during 12 d of task learning (Fadda et al. 2000). These results show that the learning of the spatial task modifies the function of cholinergic neurons projecting to the hippocampus, which become progressively more active. In a behavioral paradigm investigating spatial orientation, Van der Zee (1995) showed that spatial discrimination learning selectively increases muscarinic ACh receptor immunoreactivity in cell bodies of CA1–CA2 pyramidal neurons. Changes were also observed in the neocortex, but not in the amygdala (Van der Zee and Luiten 1999). During the habituation, that is, the learning period, exploration-associated synaptic changes are likely to occur, and variations in ACh release accompanied by alterations in mAChRs density might reflect these changes.

Memory processes are mediated, in the intact brain, by parallel, sometimes interdependent, but other times independent and even competing, neural systems (White and McDonald 2002). It has recently been demonstrated that hippocampal ACh release increases both when rats are tested in a hippocampal-dependent spontaneous alternation task and in an amygdala-dependent conditioned place preference (CPP) task (McIntyre et al. 2002). Interestingly, the magnitude of hippocampal ACh release is negatively correlated with good performance in the CPP task, indicating not only a competition between the two structures in this type of memory, but also that activation of the cholinergic hippocampal system adversely affects the expression

of an amygdala-dependent type of memory (McIntyre et al. 2002). However, competition is not the only interaction between the hippocampus and the amygdala, because it has been demonstrated (McIntyre et al. 2003) that ACh release in the amygdala is positively correlated with performance in a hippocampal spatial working memory task. The two structures seem to have a nonreciprocal interaction in that the hippocampus competes with the amygdala, whereas the amygdala cooperates with the hippocampus during learning.

Furthermore, in a cross maze task, the time courses of ACh release in the hippocampus and striatum were different during training (Chang and Gold 2003). Whereas the hippocampal cholinergic system was activated first during training and was involved in the acquisition of a "place solution," depending on spatial memory, the striatal system was activated later in training, when the hippocampus still remains activated, when the rats shifted to a "response solution" to solve the maze (Chang and Gold 2003). According to these investigators, the shift of the cholinergic activation from hippocampus to striatum is a marker of the transition from declarative to procedural learning.

The experiments summarized in the above paragraph demonstrate that simultaneous monitoring of ACh release from different brain areas makes it possible to tease out the specific roles of the components of the central cholinergic system.

## Conclusions

As shown in Table 1, activation of the forebrain cholinergic system has been demonstrated in many tasks and conditions in which the environment requires the analysis of novel stimuli that may represent a threat or offer a reward. The question is how the increase in extracellular ACh release resulting from the activation of the cholinergic neurons contributes to the learning and memory processes.

The principal cellular mechanism thought to underlie neuronal plasticity is long-term potentiation (LTP), which is believed to represent the basis of information acquisition and encoding. This event is generally studied in hippocampal slices and only more recently in cortical slices (Castro-Alamancos et al. 1995). Application of ACh to hippocampal slices induces a theta rhythm of neuronal activity (Rowntree and Bland 1986; Huerta

**Table 1.** Spontaneous and Acquired Behaviors Associated With Increased ACh Release in the Cerebral Cortex and Hippocampus of the Rat

Behavioral paradigm	Structure	Increase (%)	Reference
Exploration (novelty)	Cortex	164	Giovannini et al. 2001
	Hippocampus	300	
	Hippocampus	311	Thiel et al. 1998b
	Frontal cortex	375	
	N. acc. core	120	Thiel et al. 1998a
	N. acc. shell	180	
Locomotor activity	Hippocampus	160	Ceccarelli et al. 1999
	Cortex	177	
	Hippocampus	279	Day et al. 1991
	Striatum	158	
	Hippocampus	170	Mizuno et al. 1991
	Parietal cortex	195	
Visual attention	Medial prefrontal cortex	210	Passetti et al. 2000
Arousal/attention	Hippocampus	250	Acquas et al. 1996
	Frontal cortex	275	
Sustained attention	Frontoparietal cortex	140–170	Himmelheber et al. 2000; Arnold et al. 2002
Restraint stress	Hippocampus	177	Mizuno and Kimura 1997
	Dorsal hippocampus	168	
	Prefrontal cortex	161	Mark et al. 1996
	Amygdala	127 (NS)	
	N. acc.	NS	
Chronic stress	Hippocampus	800	Mizoguchi et al. 2001
Working memory	Prefrontal cortex	237	Hironaka et al. 2001
	Hippocampus		
Spatial learning	Hippocampus	245	Fadda et al. 2000
	Hippocampus	236	Stancampiano et al. 1999
Sensory stimulation (visual, tactile, auditory, olfactory)	Hippocampus	130–175	Inglis and Fibiger 1995
	Frontal cortex	155–245	
Tactile stimulation (handling)	Hippocampus	200	Fischer et al. 1991
Tactile stimulation	Frontal cortex	220	Acquas et al. 1998
Novel taste	Insular cortex	287	Miranda et al. 2000
Conditioned place preference	Hippocampus	160	McIntyre et al. 2002
	Hippocampus	150	
Spontaneous alteration	Frontal cortex	270	Giovannini et al. 1998
	Hippocampus	136–160	Ragozzino et al. 1996; Ragozzino et al. 1998
	Striatum	140	Chang and Gold 2003
Cross maze	Hippocampus	160	
	Striatum	140	
Operant behavior	Hippocampus	650	Orsetti et al. 1996
	Parietal cortex	400	
Lever-press extinction training	Medial prefrontal cortex	190	Izaki et al. 2001
Visuospatial attentional task	Medial prefrontal cortex	150–200	Dalley et al. 2001
Aversive stimulation	Hippocampus	156	Thiel et al. 2000
Contextual fear conditioning	Hippocampus	666	Nail-Boucherie et al. 2000

ACh increase is calculated on the basis of the data presented in the single papers, as percent over basal release. (NS) Not significant.

and Lisman 1995), and muscarinic receptor agonists increase LTP in vitro (Blitzer et al. 1990), facilitate LTP in vivo, and restore learning and memory impaired by scopolamine in a passive avoidance task (Iga et al. 1996). It appears that the presence of theta rhythm creates a permissive environment in the hippocampus for the induction of LTP (Huerta and Lisman 1995; Nguyen and Kandel 1997). This finding demonstrates a possible link between the hippocampal cholinergic system, LTP in vivo, and learning (Iga et al. 1996). When ACh is released in the vicinity of neurons depolarized either by glutamate or a sensory stimulus, the responsiveness of the neurons is enhanced for long periods (Tremblay et al. 1990a,b; Metherate 1998). Therefore, activation of basal forebrain cholinergic neurons might create conditions favorable for neuronal plasticity, and the plasticity observed depends on LTP. Indeed, long-term cholinergic enhancement, attributable to disinhibition and increased release of ACh in the cortex during neuronal excitation by other sources (Rasmusson 2000), may be a form of long-term potentiation. The existence of such a mechanism for the control of cortical neuronal plasticity identifies the basal forebrain cholinergic neurons as powerful modulators of long-lasting changes in cortical neuronal excitability. Pairing a cutaneous electrical stimulus to the rat hindpaw with stimulation of the basal forebrain as few as 20 times enhanced the size of the cortical somatosensory-evoked potential by nearly 30% in the first minutes, and the effect was ~50% greater 1 h later. This long-lasting increase in cortical neuronal excitability could be prevented by prior systemic treatment with either MK-801 or with L-NAME, indicating that it shares some of the main characteristics of LTP (Verdier and Dykes 2001).

Finally, to achieve ACh concentrations in the dialysates large enough to be quantified with the HPLC assays presently available, collection periods of at least 5 min are needed. Information acquisition and behavioral responses usually occur within seconds. This time-scale difference makes it very difficult to demonstrate a precise correlation between activation of the cholinergic neurons and specific cognitive processes, and this is today the technical limit of microdialysis experiments. For example, we were not able (Giovannini et al. 1998) to correlate object recognition/discrimination task, which depends on the cortically projecting cholinergic pathway (Bartolini et al. 1996), to increased cortical cholinergic activity. Extracellular ACh levels did not increase significantly in the cortex of rats that discriminate objects while in a familiar environment. Therefore, either the basal activity of the cholinergic system is sufficient for the animal to perform object recognition, or only a brief burst of ACh release, undetectable in a 5-min collection period, takes place during the task (Giovannini et al. 1998). Similarly, an acquired operant task can be performed during the basal activity of the cholinergic system, while the acquisition of the behavior is accompanied by a strong increase of ACh release (Orsetti et al. 1996). Nevertheless, the sustained cholinergic activation, demonstrated by the high levels of extracellular ACh observed in the behavioral paradigms summarized in Table 1, indicates that many behaviors occur within or require the facilitation provided by the cholinergic system to the operation of pertinent neuronal pathways. The activation appears to be diffused throughout the forebrain cholinergic network, possibly with different regional intensity (Inglis and Fibiger 1995), and is a prerequisite of sustained attention (Sarter et al. 2001). In turn, sustained attention is the prerequisite of information acquisition, recall, and correct responses to environmental stimuli. Support for this hypothesis is provided by experiments with positron emission tomography (Furey et al. 2000) in volunteers subjected to a visual working memory task in which cholinergic enhancement improves memory performance, probably by augmenting the selectivity of perceptual processing during encoding.

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