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Cognitive Effects of Neurotoxic Lesions of the Nucleus Basalis Magnocellularis in Rats: Differential Roles for Corticopetal Versus Amygdalopetal Projections

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The cholinergic hypothesis states that cholinergic neurons of the basal forebrain nucleus basalis magnocellularis (nbm) that project to cortical and amygdalar targets play an important role in memory. Biochemical studies have shown that these target areas are differentially sensitive to different excitotoxins (e.g., ibotenate vs. quisqualate). This observation might explain the finding from many behavioural studies of memory that different excitotoxins affect memory differentially even though they produce about the same level of depletion of cholinergic markers in the cortex and similar cortical electrophysiological effects. Thus, the magnitude of mnemonic impairment might be related to the extent of damage to cholinergic projections to the amygdala more than to the extent of damage to corticopetal cholinergic projections. This explanation might similarly apply to the observation that the immunotoxin 192 IgG-saporin produces mild effects on memory when injected into the nbm. This is because it damages cholinergic neurons projecting to the cortex but not those projecting to the amygdala. Studies comparing the effects on memory of ibotenic acid vs. quisqualic acid lesions of the nbm are reviewed as are studies of the mnemonic effects of 192 IgG-saporin. Results support the cholinergic hypothesis and suggest that amygdalopetal cholinergic neurons of the nbm play an important role in the control of memory.

Keywords: 192 IgG-saporin, amygdala, cholinergic hypothesis, cortex, ibotenic acid, memory, nucleus basalis magnocellularis, quisqualic acid, review

INTRODUCTION

About twenty years ago it became apparent from studies of post mortem brain tissue that cortico- and amygdalopetal acetylcholine-releasing neurons of the basal forebrain selectively degenerate in people with Alzheimer's disease (Coyle et al., 1983). Furthermore, the severity of dementia at the time of death was positively related to the extent of loss of cholinergic neurons (review; Bierer et al., 1995). Around the same time it was realized that significant cholinergic dysfunction occurs in the aged and that a similar relationship existed between the severity of this dysfunction and impairments in memory (Bartus et al., 1982). From these and releted observations (e.g., Drachman, 1978) the cholinergic hypothesis of geriatric memory dysfunction was born. More generally, the cholinergic hypothesis states that cholinergic

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neurons of the basal forebrain play an important role in the control of memory.

The cholinergic hypothesis was in for a rough ride. Many animal studies of the role of basal forebrain cholinergic neurons in memory utilized locally injected excitotoxins to make axon-sparing lesions. In the latter half of the 1980s decade, reports began to appear that different excitotoxins injected into the same structure in different groups of animals did not produce the same level of mnemonic impairment. Biochemical analyses showed that cortical cholinergic markers for damage to cholinergic neurons of the basal forebrain were equally affected by these excitotoxins. Similarly, some electrophysiological measures of neocortical activity (e.g., electroencephalographic activity, which is regulated by cholinergic inputs from the basal forebrain; review by Riekkinen et al., 1991a), also appear to be affected to a similar extent by different excitotoxins. The fact that a variety of excitotoxins produced similar reductions in cortical cholinergic markers and cortical physiology made it difficult to attribute their differential mnemonic effects solely to damage of the cortically projecting cholinergic system (Dekker et al., 1991). The cholinergic hypothesis therefore was brought into question. In this paper we will review these studies and show that excitotoxic damage to the cholinergic nucleus basalis magnocellularis (nbm) of the basal forebrain affects not only cholinergic projections to the cortex but also those to the amygdala and that the latter projections play an important role in memory.

Another criticism of the cholinergic hypothesis came out of observations from pharmacotherapeutics. An analogy can be drawn from the treatment of Parkinson's disease. This disorder results from the loss of dopaminergic neurons mostly from the substantia nigra of the ventral mesencephalon. Almost miraculous therapeutic effectiveness of dopamine replacement therapy was observed in sufferers of Parkinson's disease treated with the dopamine precursor L-dopa (Hornykiewicz, 1974). Yet, the analogous approach in the treatment of Alzheimer's disease met with very limited success (Ashford et al., 1989). If the cholinergic hypothesis was correct, cholinergic replacement therapy should have worked; the relative lack of success of this approach again brought the cholinergic hypothesis into question. However, not everyone agreed.

Sarter and his colleagues (Sarter et al., 1990; Sarter and Bruno, 1994) suggested that nbm neurons normally carry phasic bursts of activity (the cholinergic signal) in association with learning. They have argued that the failure of cholinergic replacement therapy in the treatment of Alzheimer's disease is related to the masking of the cholinergic signal by agents that act directly at cholinergic receptors or otherwise up-regulate tonic cholinergic activity. From his point of view, it is not the cholinergic hypothesis that is in error, but the mechanism of action of the pharmacotherapies that have been tried. In recent years the central driving force in the development of pharmacotherapies for the treatment of Alzheimer's disease has continued to be the cholinergic hypothesis and drugs now available, that have shown some therapeutic effectiveness in people in the early stages of the disease, enhance cholinergic neurotransmission (Kása et al., 1997). Thus, the cholinergic hypothesis continues to provide an important basis for linking the memory deficits in Alzheimer's disease with the loss of basal forebrain cholinergic cells.

The cholinergic hypothesis again was challenged in the 1990s with the development of an immunotoxic agent that is relatively specific for cholinergic neurons of the basal forebrain. 192 IgG-saporin was produced by coupling the ribosome-inactivating protein saporin to the monoclonal 192 immunoglobulin G (IgG) antibody against the low-affinity p75 nerve growth factor (NGF) receptor (Heckers et al., 1994). The cholinergic neurons of the basal forebrain express by far the highest level of p75 NGF receptors in the adult rat brain. Thus, 192 IgG-saporin could be used to selectively kill

those neurons. Reports appeared of extensive decreases in cortical markers for cholinergic neurons following 192 IgG-saporin injections into the basal forebrain but with relatively mild or no mnemonic impairments. Again, there appeared to be a dissociation between the loss of cholinergic neurons and memory impairment, bringing the cholinergic hypothesis into question. However, as shown clearly by Heckers et al. (1994), the basal forebrain cholinergic neurons that project to the amygdala do not bear p75 NGF receptors and therefore are not killed by 192 IgG-saporin. As the basoamygdalar cholinergic projections play an important role in memory, the relative weakness of 192 IgG-saporin as a memory-impairing agent could be understood, obviating the need to reject the cholinergic hypothesis. These studies also will be reviewed in the present paper.

EXCITOTOXIC LESIONS OF THE NBM AND MEMORY

Endogenous Excitotoxic and Neuroprotective Agents

Animal studies had shown that excitotoxic lesions of the nbm, like systemic treatments with the muscarinic cholinergic receptor blocker scopolamine, lead to impairments of memory (Salamone et al., 1984; Murray and Fibiger, 1985; Beninger et al., 1986). This suggested the intriguing hypothesis that an endogenous excitatory amino acid agonist could produce damage to the nbm and mnemonic dysfunction. One candidate was quinolinic acid (cf. Jhamandas et al., in press). This molecule is an endogenous metabolite of tryptophan and was reported to produce axon-sparing lesions of hippocampal and striatal neurons (Schwarcz et al., 1983). We tested the effects of this agent on nbm cholinergic cells. In biochemical analyses, quinolinic acid injected into the nbm produced decreases in acetylcholine outflow from cortical slices, high affinity choline uptake and acetylcholinesterase, an enzymatic marker for cholinergic neurons (El-Defrawy et al., 1985). Thus, the endogenous tryptophan metabolite quinolinic acid acted as an excitotoxin and was able to kill cortically projecting cholinergic neurons of the nbm.

Would quinolinic acid also produce mnemonic deficits when injected into the nbm? To answer this question, well-trained rats received nbm injections of quinolinic acid and were tested in a partially baited radial maze task that allows assessment of memory while controlling for possible non-mnemonic effects of the lesions. Results revealed that lesion animals were impaired in the component of the task requiring recent memory (Wirsching et al., 1989). Thus, the endogenous tryptophan metabolite quinolinic acid, when injected into the nbm, produces decreases in cortical cholinergic markers and impairments in memory like those seen in people suffering from Alzheimer's disease.

The metabolic pathway from tryptophan also produces kynurenic acid. This molecule acts as an endogenous glutamate receptor antagonist. Thus, co-injection of kynurenic acid with quinolinic acid into the nbm reversed the effects of quinolinic acid on cortical markers of cholinergic function including choline acetyltransferase (ChAT), acetylcholine outflow from cortical slices, high affinity choline uptake and acetylcholinesterase (Boegman et al., 1985). In behavioural studies of the mnemonic effects of quinolinic acid injections into the nbm and the possible protective effects of kynurenic acid, Wirsching et al. (1989) found that kynurenic acid protected rats against the memory impairments produced by quinolinic acid. In that paper, biochemical studies confirmed that kynurenic acid also prevented the decreases in cortical ChAT normally seen following intra-nbm quinolinate. Thus, there are endogenous substances that can act both as neurotoxins and as neuroprotective agents. Recently, we have shown that treatments that enhance endogenous levels of kynurenic acid can protect against the neurotoxic effects of

exogenously introduced neurotoxins (Harris et al., 1998; Miranda et al., 1999). If the loss of basal forebrain cholinergic neurons in Alzheimer's disease is related to the action of an endogenous excitotoxin, treatments that enhance levels of endogenous neuroprotective substances may prove useful in reducing neuron loss and the memory impairments that accompany it.

Differential Effects of Excitotoxins on Corticopetal and Amygdalopetal Cholinergic Efferents of the Nbm

Studies of the efferent connections of the basal forebrain cholinergic neurons that constitute the nbm reported the now widely known projections to the cortex but also other targets including, importantly, the amygdala (Fibiger 1982; Nagai et al., 1982; Woolf and Butcher, 1982; Carlsen et al., 1985). It was found that different excitotoxins differentially affected nbm efferents to the cortex and amygdala. Thus, Boegman et al. (1992a) examined the effects of a range of doses of the excitotoxins quinolinic acid, quisqualic acid, ibotenic acid, α-amino-3-hydroxy-4 isoxazole propionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA), injected into the nbm on ChAT, an enzymatic marker of cholinergic neuron viability, in the cortex and amygdala of rats. Results (Figure 1 A-E) revealed that all of these agents produced dose-dependent decreases in ChAT in both the cortex and amygdala. However, the potency on cholinergic neurons projecting to the two different target areas differed. Of the five excitotoxins reported by Boegman et al. (1992a), only quinolinic acid was more potent at some doses on amygdalopetal projections than on corticopetal ones (Figure 1A). For the other four excitotoxins, cortical ChAT was more affected than amygdalar ChAT. However, among these there were important differences. The slope of the function relating dose to percent decrease was about the same for cortical and amygdalar ChAT following ibotenic acid (Figure 1C) but the shapes of the two functions following quisqualic acid (Figure 1B) or AMPA (Figure 1D) were quite different. Decreases in cortical ChAT produced by quisqualic acid rose quickly to an asymptote at a relatively low dose whereas the function for amygdalar ChAT rose gradually. Thus, in the middle of the dose range tested, quisqualic acid would produce much larger decreases in cortical ChAT than in amygdalar ChAT; at higher doses, this difference would not be seen. This would be equally the case for AMPA. For ibotenic acid, on the other hand, over quite a wide dose range the effects on cortical and amygdalar ChAT would remain roughly proportional although cortical ChAT always would be more affected than amygdalar ChAT.

During the late 1980s and the early 1990s, a number of reports appeared in the literature of differential mnemonic effects of different excitotoxins injected into the nbm. These reports were characterized by the observation that both excitotoxins, quisqualic and ibotenic acid in most cases, produced large decreases in cortical ChAT but affected memory differently. The mnemonic deficits produced by ibotenic acid were usually significantly larger than those produced by quisqualic acid. In some cases, no deficit in memory was seen after quisqualic acid. Moreover, the cortical ChAT depletion produced by quisqualic acid usually was reported to be greater than that produced by ibotenic acid. These data were seen to be contrary to the cholinergic hypothesis and many authors sought explanations in the differential degree of nonspecific damage produced by the different neurotoxic agents. However, the differential effects of these agents on nbm efferents to the cortex and amygdala provide a basis for understanding the results.

The results of eleven of these studies are summarized in Table I. All of these studies reported data from at least two different groups of rats, one group with ibotenic acid lesions of the nbm and the other with quisqualic acid lesions. In some cases the lesions were made with a single bolus injection into the nbm and in others two or

more injections were given at different rostral-caudal, dorsal-ventral, or medial-lateral sites within the nbm. When more than one injection was given, the dose (nmol) was determined by adding the amount injected into each site. All of the papers reported results of assays of cortical ChAT done following the completion of behavioural testing. From these, the percent decrease in cortical ChAT was calculated if it was not reported directly and in every case, frontal cortical ChAT values were used. Only one study (Beninger et al., 1994) reported amygdalar ChAT values. For the others, amygdalar ChAT percent decreases were estimated, based on the total dose of excitotoxin injected, by interpolating or extrapolating from the functions reported by Boegman et al. (1992a) and reproduced in Figure 1. A range of behavioural tasks for the assessment of memory was used in the studies. A number of studies used more than one test of memory. In most cases when more than one test of memory was used, the second test was inhibitory avoidance. In those cases, the inhibitory avoidance data were ignored (cf. Sarter et al., 1992). In no case are data from the same group of rats represented more than once. Most studies employed widely accepted behavioural methods for testing memory including variations of the water maze or radial maze tasks, delayed matching, conditional discrimination or T- or double Y-maze alternation tasks. The level of impairment was rated as "large" if performance was statistically significantly poorer than control and "none" when there was not a significant effect. In those cases where both excitotoxins produced a significant impairment compared to control but one was also significantly greater than the other, the smaller of the two was rated as "moderate".

A few general impressions result from inspection of Table I. Ibotenate tended to produce greater mnemonic impairments than quinolinic acid. Although there is quite a range of values, cortical ChAT percent decreases tended to be greater following quisqualic acid versus ibotenic acid and estimated amygdalar ChAT percent decreases tended to be greater following ibotenic acid versus quisqualic acid. ChAT percent decreases were averaged for each excitotoxin for the cortex and for the amygdala; means and standard errors of the means (SEMs) are shown in Table II. t-tests comparing ChAT percent decrease values for the two excitotoxins were carried out and results are reported in Table II. They confirmed that quisqualic acid produced greater cortical ChAT percent decreases and smaller estimated amygdalar ChAT percent decreases than ibotenate. There was no overlap in the level of impairment produced by the two excitotoxins. Ibotenate was reported to produce large impairments in every study; quisqualic acid produced either moderate or no impairment in every study. These data tell a consistent story. Excitotoxins that produce a greater decrease in amygdalar ChAT produce greater mnemonic impairments; it is the loss of nbm efferents to the amygdala, not those to the cortex, that appears to play a critical role in mnemonic impairments produced by excitotoxic lesions of the nbm.

The role of nbm cholinergic projections to the amygdala in memory was confirmed by the study of Beninger et al. (1994). This was the only study comparing the effects of quisqualic and ibotenic acid on memory that included biochemical assessment of cortical and amygdalar ChAT (see Table I). Results showed that the two excitotoxins produced similar decreases in cortical ChAT (51%) but significantly differential decreases in amygdalar ChAT (17% for quisqualate and 30% for ibotenate). In the double Y-maze test of recent memory, ibotenate produced a significantly greater impairment than quisqualate. The locus of ChAT depletions that correlated with impairments of memory was the amygdala. In a related study, Ingles et al. (1993) evaluated the effects of intra-amygdalar injections of the muscarinic cholinergic receptor antagonist scopolamine on memory in the same task. Their results revealed a dose-dependent impairment of memory, supporting the view that cholinergic neurotransmission in the amygdala plays an important role in memory.

TABLE I Reported effects of the excitotoxins ibotenic acid and quisqualic acid injected into the nucleus basalis magnocellularis (nbm) on cortical choline acetyltransferase (ChAT) levels and on memory of rats in a variety of tasks. Included are the estimated effects of the excitotoxins on levels of ChAT in the amygdala (Amy) based on published dose-response curves (Boegman et al., 1992a)

Excitotoxin into nbm	*Dose (nmol)*	*% ChAT Decrease*		*Memory task*	*Level of impairment*	*Reference*
		Cortex	*Amy (E)*			
Ibotenate	60.0[a]	70	45	Water maze	Large	Dunnett et al. (1987)
	60.0[a]	17	45	Delayed matching	Large	Etherington et al. (1987)
	24.0	28	36	Conditional discrimination	Large	Robbins et al. (1989)
	12.5	45	26	Delayed matching	Large	Markowska et al. (1990)
	33.0[a]	46	38	Water maze	Large	Connor et al. (1991)
	42.0[a]	48	40	Water maze	Large	Riekkinen et al. (1991b)
	24.0	53	36	Water maze	Large	Riekkinen et al. (1991c)
	63.0[a]	35	46	T-maze alternation	Large	Wenk et al. (1992)
	30.0[a]	25	37	Radial maze	Large	Ammassari-Teule et al. (1993)
	48.0	36	42	Bättig maze	Large	Steckler et al. (1993)
	60.0	36	45	Bättig maze	Large	Steckler et al. (1993)
	50.0	51	30[b]	Double Y-maze	Large	Beninger et al. (1994)
Quisqualate	120.0[a]	76	37	Water maze	Moderate	Dunnett et al. (1987)
	120.0[a]	51	37	Delayed matching	None	Etherington et al. (1987)
	120.0[a]	43	37	Conditional discrimination	None	Robbins et al. (1989)
	60.0[a]	74	21	Delayed matching	Moderate	Markowska et al. (1990)
	60.0	59	21	Water maze	Moderate	Connor et al. (1991)
	42.0[a]	63	16	Water maze	None	Riekkinen et al. (1991b)
	96.0	66	30	Water maze	Moderate	Riekkinen et al. (1991c)
	60.0[a]	35	21	T-maze alternation	None	Wenk et al. (1992)
	120.0[a]	50	37	T-maze alternation	None	Wenk et al. (1992)
	150.0[a]	46	48	Radial maze	None	Ammassari-Teule et al. (1993)
	120.0[a]	42	37	Bättig maze	None	Steckler et al. (1993)
	60.0	51	17[b]	Double Y-maze	Moderate	Beninger et al. (1994)

a. These amounts are the sums of injections into at least two different ipsilateral sites.
b. These values were reported in the paper, not estimated (E) from Boegman et al. (1992a) as were the other values reported in this column.

Boegman et al. (1992b) reported that the quinolinic acid analogue phthalic acid has a particularly interesting biochemical profile with respect to its effects on cortical and amygdalar ChAT following injection into the nbm. As can be seen in Figure 1 F, phthalic acid produces a greater decrease in amygdalar ChAT than in cortical ChAT following injection into the nbm. The separation between effects in the two structures is even greater than that seen for quinolinic acid. Mallet et al. (1995) took advantage of this compound to further test the hypothesis that nbm

cholinergic efferents to the amygdala play an important role in memory. Using a dose of 300 nmol, we confirmed in biochemical assays that cortical ChAT was minimally affected (17% decrease) whereas amygdalar ChAT was decreased by 61%. For comparison, another group received quisqualic acid; cortical and amygdalar ChAT depletions were 50 and 17%, respectively. When the injected animals were tested in the double Y-maze, a significant impairment of recent memory was seen in the phthalate group; it was different from both the control and quisqualic acid lesion groups. These results confirm that depletions of nbm cholinergic efferents to the amygdala produce a greater impairment of memory than similar decreases of nbm cholinergic projections to the cortex.

Interestingly, some electrophysiological evidence also suggests that the mnemonic deficits seen after excitotoxic basal forebrain damage may, to some extent, be mediated by structures other than the neocortex. A generalized slowing of the neocortical electroencephalogram (EEG) and increased power in the low frequency (< 4 Hz) delta band are characteristic physiological markers of reduced cholinergic activity in the neocortex in rats (Dringenberg and Vanderwolf, 1998; Buzsaki and Gage, 1989; Riekkinen et al., 1991a). Further, Alzheimer's patients show an increase in delta activity which correlates with impaired cognition (Riekkinen et al., 1991a; Penttilä et al., 1985) and the reduction in cholinergic markers in postmortem tissue samples such as cell density in the basal forebrain and ChAT activity in cortex (Riekkinen et al. 1991a). Based on these findings, the EEG is used as an additional measure of the integrity of cholinergic cortical innervation and neuronal activity in the cortex of patients afflicted with Alzheimer' s disease.

How do different excitotoxic lesions of the basal forebrain affect the cortical EEG in rats? Unfortunately, a direct comparison of different neurotoxins is difficult at present since some toxins used for behavioral experiments have not been employed for EEG studies (e.g., AMPA, NMDA). Also, a variety of different EEG measures has been used to assess the effects of neurotoxic lesions in rats (e.g., percent of slow waves, absolute and relative delta power, ratios of power in low and high frequency bands, EEG coherence), complicating a direct comparison of different studies. However, it is noteworthy that at least some neurotoxins produce similar EEG changes, despite their differential effects on performance in memory tests. For example, both quisqualic acid and ibotenic acid have been shown to increase frontal cortex delta power by approximately 50% (Riekkinen et al., 1990a,b), while producing much smaller or no EEG changes in more posterior cortical regions. Thus, the effects of these two excitotoxins on activity in cortical networks (as assessed by EEG power) appear to be quite similar, whereas their effects on performance in memory tests are not. These data may suggest that the behavioral changes produced by quisqualate and ibotenate are, at least partially, related to disruptions of activity in regions other than the neocortex.

Consistent with this argument, Riekkinen and co-workers have shown that the progressive EEG slowing in Alzheimer's patients that accompanies the disease course correlates better with non-mnemonic measures such as praxis of the hand or speech comprehension than with performance on memory tests (Riekkinen et al., 1991a). Again, these data suggest that the cortical electrophysiological dysfunction present in Alzheimer's disease (and that is likely related to cholinergic cortical deficits), may not predict the degree of mnemonic deficits present in patients suffering from Alzheimer's disease.

To our knowledge, the effects of different neurotoxins on the electrophysiological activity of the amygdala have not been examined. Experiments of this kind would be useful to test whether a relation exists between the degree of amygdaloid cholinergic denervation, electrophysiological abnormalities in the amygdala, and behavioral deficits in tests of learning and memory.

TABLE II Means (±SEM) for cortical and amygdalar ChAT from TABLE I

Excitotoxin into nbm	*No. Studies*	*ChAT % Decrease*		*Level of Impairment*
		Cortex	*Amygdala*	
Ibotenate	12	40.8	38.8	Large
		(4.1)	(1.8)	
Quisqualate	12	54.7[a]	29.8[b]	None-moderate
		(3.8)	(3.0)	

a. Significantly different from ibotenate mean: $t(22) = 2.49$, $p < 0.03$.
b. Significantly different from ibotenate mean: $t(22) = 2.55$, $p < 0.02$. Because the variances for the estimated % ChAT decreases for the amygdala differed between groups, the amygdala data were re-analyzed using a Mann-Whitney U- Test and still were found to be significant, $p<0.05$.

TABLE III Reported effects of 192 IgG-saporin injected into the nucleus basalis magnocellularis (nbm), into the nbm, vertical limb of the diagonal band (vldb) and medial septum (ms), or intracerebroventricularly (icv) on frontal cortical and hippocampal (hipp) choline acetyltransferase (ChAT) levels and on memory in a variety of tasks in rats

Locus of Injection	Dose (ng)	% ChAT Decrease		Memory task	Level of impairment	Reference
		cortex				
nbm	10.0	28.0		Delayed alternation	None	Wenk et al. (1994)
	126.0	na		Water maze	Large	Berger-Sweeney et al. (1994)
	67.0	81.0		Water maze, delayed match	None	Torres et al. (1994)
	150.0*	62.5		Water maze, delayed match	None, mild	Baxter et al. (1995)
	504.0	31.7		Water and radial maze	None, mild	Dornan et al. (1996)
		cortex	hipp			
nbm +	504.0	59.0		Water and radial maze	None, mild	Dornan et al. (1996)
ms	504.0		61.4			
nbm +	202.4*	64.3		Water maze	None	Baxter et al. (1996)
ms/vldb	253.0*		78.2			
nbm +	300.0*	43.1		Delayed matching	Large	Robinson et al. (1996)
ms/db	450.0*		58.1			
icv	4,690.0	65.0	86.9	Water maze	Large	Nilsson et al. (1992)
	4,000.0	62.0	75.0	Delayed matching	Large	Steckler et al. (1995)
	340.0	- 3.0	-0.2	Water maze	None	Waite et al. (1995)
	1,340.0	52.4	65.6		None	
	2,000.0	68.6	88.3		Large	
	2,700.0	80.1	93.2		Large	
	4,000.0	78.4	92.9		Large	
	1,250.0*	21.0	23.0	Water maze	None	Leanza et al. (1995)
	2,500.0*	52.0	67.0		Large	
	5,000.0*	62.0	77.0		Large	
	1,300.0	59.0	69.0	Water maze	None	Waite and Thal (1996)
	2,700.0	89.0	94.0		Large	
	2,948.0*	na	na	Object discrimination	Large	Vnek et al. (1996)
	5,000.0*	65.0	90.0	Delayed matching	Large	Leanza et al. (1996)
	5,000.0*	na	na	Water maze, delayed match	Large	Leanza et al. (1998)

*These amounts are the sums of injections into at least two different ipsilateral sites for local injections and into both lateral ventricles for icv injections. Shaded rows indicate relatively low doses of icv 192 IgG-saporin that failed to produce impairments of memory.
Abbreviations: na = not available.

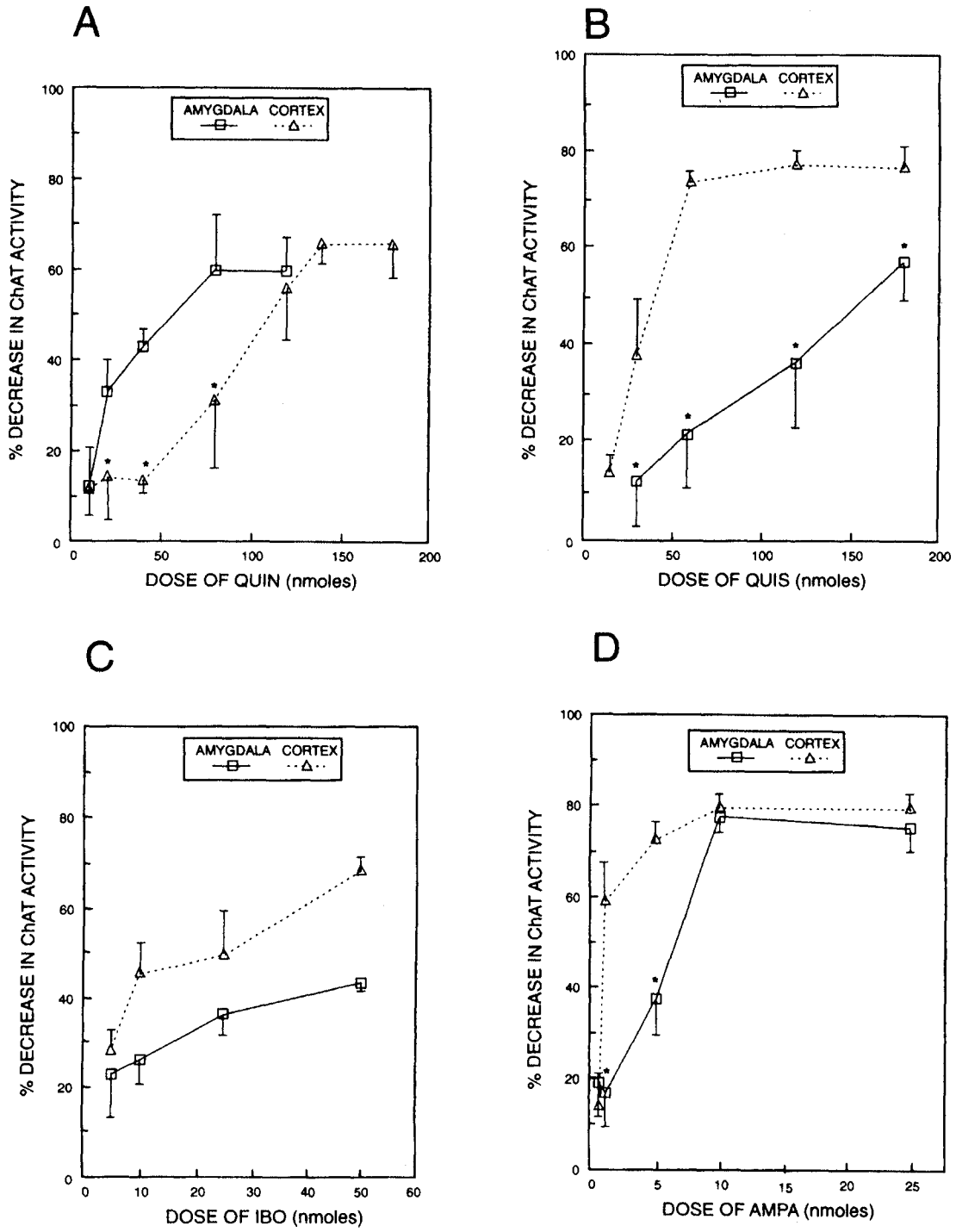
This section can be summarized by saying that there is good evidence that large and significant impairments of memory result from lesions of the nbm that lead to large decreases in amygdalopetal cholinergic efferents. The observations from the studies reviewed in this section (see Tables I and II) reconcile observed differences in the effects of different excitotoxins on memory with the cholinergic hypothesis by revealing the important role for cholinergic projections to the amygdala. It is noteworthy that the present focus on the amygdala should not lead to a rejection of a role for corticopetal cholinergic efferents of the nbm in memory. Indeed, a number of the studies using quisqualic acid to lesion nbm cholinergic neurons, although finding mnemonic deficits smaller than those produced by ibotenate, still did find significant deficits in memory in spite of modest effects on amygdalar ChAT. We have confirmed this in our own studies with quisqualic acid as reported in Table I (see also Biggan et al., 1991). The conclusion that performance of tasks requiring recent memory also depends on corticopetal nbm cholinergic efferents will help in integrating the findings from behavioural studies using the selective cholinergic neurotoxin, 192 IgG-saporin; these studies are the topic of the next section.

IMMUNOTOXIC LESIONS OF THE NBM AND MEMORY

Another wave of criticism of the cholinergic hypothesis came in the 1990s and stemmed from studies using the immunotoxin 192 IgG-saporin to destroy cholinergic neurons of the nbm. These studies tended to find relatively mild mnemonic impairments in spite of large depletions of cortical ChAT. However, as 192 IgG-saporin is selective for NGF receptor-bearing cholinergic neurons, and as NGF receptors are found on the corticopetal but not on the amygdalopetal cholinergic neurons of the nbm (Heckers et al., 1994), results can be seen to be consistent with the observations from different excitotoxins reviewed in the preceding section. Again, the cholinergic hypothesis should not be rejected on the basis of these observations of relatively weak mnemonic deficits in animals treated with intra-nbm injections of 192 IgG-saporin.

Studies of the role of basal forebrain cholinergic neurons in memory were dogged by the lack of a selective neurotoxin for cholinergic neurons. Whereas relatively selective agents were available for locally destroying dopaminergic (6-hydroxydopamine), serotonergic (5,7-dihydroxytryptamine) or noradrenergic neurons (DSP-4), no specific agents for targeting cholinergic neurons were available. This may have been responsible for the temporal lag in accumulating information about the behavioural functions of cholinergic neurons in comparison to the monoamines. In the absence of a selective neurotoxin, the best tools available to neuroscientists wishing to study the behavioural functions of the cholinergic neurons were the excitotoxins and many studies were reported using these agents, beginning around 1980. However, as discussed in the previous section, observations of similar actions of different excitotoxins on cortical ChAT but differential mnemonic effects brought the use of these agents into question. The uncertainty about the suitability of different excitotoxins to study the cholinergic system was increased by reports that these agents produced differential amounts of nonspecific damage. Thus, reports in the early 1990s of the development of 192 IgG-saporin (Wiley, 1992), a cholinergic neuron-selective neurotoxin were greeted with great enthusiasm by the neuroscience community.

The results of some of these studies are shown in Table III. The table is organized into three sections: the first includes five studies where 192 IgG-saporin was injected directly into the nbm; the second section summarizes three studies involving injection of the immunotoxin into both the nbm and also other basal forebrain cholinergic nuclei including the medial septum (ms) and


A
AMYGDALA CORTEX
% DECREASE IN ChAT ACTIVITY
DOSE OF QUIN (nmoles)
B
AMYGDALA CORTEX
% DECREASE IN ChAT ACTIVITY
DOSE OF QUIS (nmoles)
C
AMYGDALA CORTEX
% DECREASE IN ChAT ACTIVITY
DOSE OF IBO (nmoles)
D
AMYGDALA CORTEX
% DECREASE IN ChAT ACTIVITY
DOSE OF AMPA (nmoles)

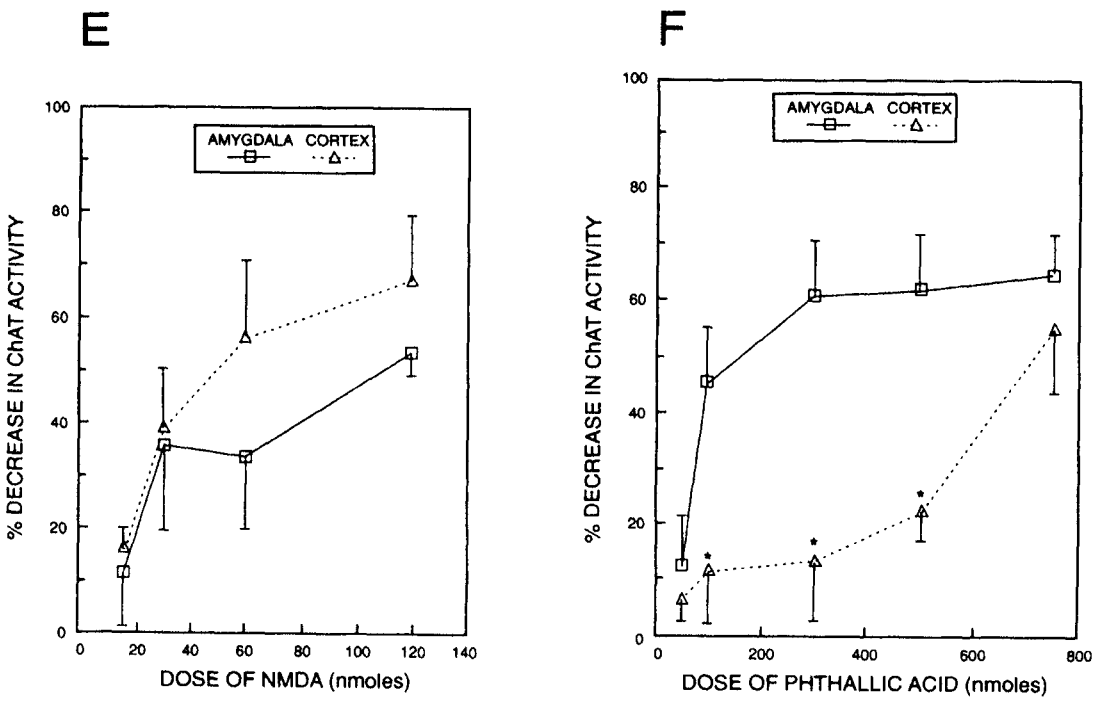


FIGURE 1 Dose-response functions for mean (±SEM) percent depletion of choline acetyltransferase (ChAT) in the cortex and amygdala following infusions (nmoles) of excitotoxins into the nucleus basalis magnocellularis. Excitotoxins tested were: A, quinolinic acid (QUIN); B, quisqualic acid (QUIS); C, ibotenic acid (IBO); D, α-amino-3-hydroxy-4 isoxazole propionic acid (AMPA); E, N-methyl-D-aspartic acid (NMDA); F, phthalaic acid. *amygdala and cortex differ at that dose by a t-test. Figs. A-E were adapted from Boegman et al. (1992a)

the diagonal band (db); the third section includes eight reports in which 192 IgG-saporin was injected into the lateral ventricle. For each study the dose of immunotoxin injected is indicated; in cases where more than one injection was made into a particular site, the amounts injected were summed. When 192 IgG-saporin was injected into the nbm, reported decreases in cortical ChAT are indicated where they were available. Studies involving injections into the nbm plus the ms or plus the ms and the db reported ChAT decreases in both the cortex and hippocampus, as indicated. Similarly, studies injecting the cerebral ventricles reported cortical and hippocampal ChAT decreases. Behavioural tests included most of the same tests that were used in the studies comparing ibotenate and quisqualate summarized in Table I. In some cases more than one test of memory was included as indicated except that tests of inhibitory avoidance learning were not included. Data from a single group of animals are only represented once in the table. The level of impairment was classified as "none" if no significant effect was seen and "large" if a significant effect was seen. The classification "mild" was used in cases where differences were seen on one task but not on another or where multiple trials were analysed and only some of them yielded significant effects.

Studies involving the injection of 192 IgG-saporin locally into the nbm are summarized in the first section of Table III. With one exception, these studies found no or mild effects

of these treatments on memory, in some cases in spite of large decreases in cortical ChAT. Similarly, combined lesions of the nbm plus ms or nbm plus ms and db, summarized in the second section of Table III, yielded a large impairment in one case but no or mild effects in the other two. Shown in the third section of Table III are the effects of icv injections of a large range of doses of 192 IgG-saporin. The shaded areas indicate lower doses that produced no effect, in spite of substantial decreases in cortical and hippocampal ChAT in some cases. Higher doses produced significant impairments of memory; these often were associated with very large decreases in both cortical and hippocampal ChAT.

Results of studies evaluating the effects on memory of 192 IgG-saporin can be summarized as follows. Lesions localized to the nbm or made to the nbm plus additional basal forebrain cholinergic nuclei (ms, db) generally produced no or mild effects on memory. When large doses of immunotoxin were injected into the ventricles that resulted in large decreases in both cortical and hippocampal ChAT, memory impairments were seen.

The effects of local injections appear to be consistent with the results from studies in which the nbm was injected with the excitotoxin quisqualic acid. Like quisqualic acid, 192 IgG-saporin produced large decreases in cortical ChAT but modest mnemonic impairments. Over part of the dose range of quisqualic acid that was used to determine the effects of this agent on cortical and amygdalar ChAT (Figure 1B), it produced large decreases in the cortex compared to small decreases in the amygdala. In this way quisqualate can be seen to be similar to 192 IgG-saporin which would destroy cortically projecting cholinergic neurons of the nbm but not those projecting to the amygdala. Thus, the relatively mild effects of quisqualic acid and 192 IgG-saporin on memory are consistent with their relatively mild or minimal effect on amygdalar ChAT.

As was mentioned at the end of the section on excitotoxins, the argument for a role for the amygdalopetal nbm cholinergic neurons in memory does not preclude a role for the corticopetal ones in memory. Thus, there is plenty of evidence supporting a role for cholinergic projections to the cortex in aspects of cortical activation and learning and memory (e.g., Richardson, 1991). Perhaps the significant impairments of memory produced by large icv doses of 192 IgG-saporin reveal this mnemonic effect of nbm cholinergic efferents to the cortex. Alternatively, large icv doses of 192 IgG-saporin could produce their mnemonic effects through an action in the cerebellum where Purkinje cells are destroyed. This possibility is discussed in a recent review by Baxter and Chiba (1999) and further studies are needed to sort out the contribution of cerebellar damage to the reported effects of icv injections of 192 IgG-saporin.

CONCLUSIONS

In this review we have argued that the hypothesis that the cholinergic neurons of the basal forebrain play an important role in memory need not be rejected on the basis of observations reported over the past 20 years that, at first blush, appear to contradict the hypothesis. Thus, different excitotoxins that produce similar decreases in cortical markers for cholinergic neuron viability differentially affect memory and the cholinergic neuron-specific immunotoxin 192 IgG-saporin has relatively mild effects on memory. It is ironic that the observations from both of these cases can be reconciled equally by a consideration of the impact of the treatments on cholinergic projections from the nbm to the amygdala. The degree of EEG slowing after quisqualate and ibotenate lesions of the basal forebrain may be roughly equivalent for both toxins, confirming the hypothesis that the differential potency of these two compounds in mnemonic tests may be mediated, in part, by forebrain structures other than the neocortex. Thus, treatments that decrease cortical ChAT but leave intact amy-

gdalar ChAT have a relatively small impact on memory; those that decrease amygdalar ChAT have a large impact on memory.

Others have focused on the contribution of the amygdala to memory. For example, Kesner (1988; Kesner et al., 1990) reviews studies comparing the mnemonic effects of nbm lesions with those of lesions to nbm efferent target areas including the frontal and parietal cortex and the basolateral amygdala. It was amygdala lesions, not cortical lesions that reproduced the mnemonic deficits seen following nbm lesions. These studies utilized electrolytic lesions making conclusions about specific neurotransmitters impossible. In a complex series of studies combining electrolytic lesions and pharmacological treatments, Riekkinen et al. (1993) similarly showed that cholinergic projections from the nbm to the amygdala seemed to be critical for inhibitory avoidance learning. The profile of results from these studies is strikingly similar to that reviewed in this paper for excitotoxin- and immunotoxin-produced damage.

Perhaps because of the apparently negative findings with respect to memory from studies of excitotoxic and immunotoxic lesions of the nbm as reviewed here, focus has shifted in recent years from memory to attention in studies of nbm function (see reviews by Wenk, 1997; Everitt and Robbins, 1997). Some authors have argued that attention and memory are difficult to separate and form parts of a common process from activation to acquisition (Richardson, 1991). The eventual full integration of the results of studies of attention and memory and their relationship to cholinergic neurons of the basal forebrain will have to await further study.

In the present paper some of the recent challenges to the cholinergic hypothesis of memory have been reviewed. Results can be seen to continue to support a role for basal forebrain cholinergic neurons in memory. Continued study of the cognitive function of these neurons will provide new insights into the neurochemical workings of the brain and provide a rational basis for the development of effective pharmacotherapies for the treatment of disorders of cognition related to the loss of cholinergic neurons of the nbm.

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