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THE BEHAVIORAL FUNCTIONS OF THE CHOLINERGIC BASAL FOREBRAIN : LESSONS FROM 192 IgG-SAPORIN

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Abstract—Until recently, our understanding of the functional neuroanatomy of the cholinergic basal forebrain (CBF) has been hindered by the lack of a lesioning technique that is truly selective. The development of the immunotoxin 192 IgG-saporin (192-sap) has greatly improved our ability to create specific lesions of the CBF. Rats with such lesions have been studied in a wide variety of behavioral paradigms of learning, memory, and attention. Complete or near-complete destruction of the CBF results in deficits in a variety of behavior paradigms including passive avoidance, spatial tasks (water and radial mazes), delayed matching to position/sample, and attentional tasks. However, interpretation of many experiments is hampered by incomplete lesions and/or concomitant damage to cerebellar Purkinje neurons. Future studies will need to address these issues. Recent development of a similar immunotoxin that is effective in primates should permit more sophisticated behavioral analysis of CBF function. Additionally, immunotoxins selective for other types of neurons, such as the noradrenergic selective anti-DBH-saporin, will permit analysis of the behavioral functions of other diffusely projecting systems and how these other systems may interact with the CBF. © 1999 ISDN. Published by Elsevier Science Ltd. All rights reserved

The cholinergic basal forebrain (CBF), one of the diffusely projecting systems of the brain, consists of the cholinergic neurons in the medial septum (MS), vertical and horizontal limbs of the diagonal band of Broca (DBBv and DBBh), and nucleus basalis/substantia innominata (NBM/SI).[37] The CBF provides cholinergic input to the hippocampus (MS and DBBv), neocortex (NBM/SI), amygdala (NBM/SI), and olfactory bulbs (DBBh).[37] The physiological function of the CBF is thought to be to modulate the excitability of cortical and hippocampal neurons.[28] Anatomical and physiological data on the CBF encourage the notion that it modulates cognitive functions such as learning, memory, and attention. The evidence for this hypothesis has come from observations that muscarinic antagonists generally impair cognitive function in humans and animals, and cholinomimetics can reverse such impairment or enhance cognition when given alone.[9,21] Although the precise site within the CNS for cholinergic drug effects on behavior is uncertain, the CBF has become regarded as the probable anatomical substrate for the cognitive effects of cholinergic drugs.[20,26,32] The discovery that the CBF degenerates in Alzheimer's Disease (AD) and that the degree of this degeneration correlates with the degree of dementia[43,63] has strongly encouraged interest in the role of the CBF in cognition. A relative paucity of data addresses the behavioral role of the mesencephalic cholinergic neurons or the possibility of cholinergic drugs exerting behavioral effects by acting on that system.

Degeneration of the CBF in AD has motivated investigators to attempt to model, in animals, this pathological feature through a variety of lesioning techniques. The approaches employed have included fimbria-fornix transection,[33] intracerebral injection of the toxin AF64A,[58] and intracerebral injection of excitotoxins.[14,16,18,22,25,38,39,48,53] A major shortcoming of this work has been that each of these techniques lacks selectivity. Thus, it is difficult to ascribe any resulting behavioral deficits specifically to destruction of the CBF. In fact, studies that used intracerebral injection of excitotoxins

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Abbreviations : 192-sap, 192 IgG-saporin ; AChE, acetylcholine esterase ; AD, Alzheimer's Disease ; CBF, cholinergic basal forebrain ; ChAT, choline acetyl transferase ; DBB, diagonal band of Broca ; DBBh, horizontal limb of the diagonal band of Broca ; DBBv, vertical limb of the diagonal band of Broca ; DMTP, delayed matching to position ; DNMTP, delayed non-matching to position ; DNMTS, delayed non-matching to sample ; FG, Fluoro Gold ; HAChT, high affinity choline transport ; i.c.v., intraventricular ; MS, medial septum ; NADPH, nicotinamide-adenine dinucleotide phosphate diaporase ; NBM, nucleus basalis magnocellularis ; OX7-sap, OX7-saporin ; p75, low affinity neurotrophin receptor ; SI, substantia innominata.

cast some doubt on the behavioral role of the CBF because the excitotoxins that produced the greatest loss in cortical cholinergic markers did not produce the greatest behavioral impairments.[15,17]

Recently, the problem of selectively killing the CBF has been addressed by the development of the immunotoxin, 192 IgG-saporin (192-sap). 192-sap consists of the monoclonal antibody 192 IgG which is disulfide coupled to saporin, a ribosome-inactivating toxin derived from the plant *Saponaria officinalis* (soap wort). The antibody component of 192-sap is directed against rat p75, the low-affinity neurotrophin receptor. Targeting p75 serves as an ideal way of selectively killing the CBF because in the adult rat basal forebrain only the cholinergic neurons express p75.[10] When injected intracerebrally, 192-sap binds to the surface of p75 bearing neurons and is internalized by endocytosis. Once in the cytoplasm, the saporin moiety escapes endosomes and enzymatically inactivates the large ribosomal subunit halting protein synthesis and leading ultimately to cell death.[66] The present review will briefly evaluate the selectivity and effectiveness of 192-sap for producing lesions of the CBF and then describe what experiments using 192-sap have revealed about the behavioral function of the CBF.

SELECTIVITY AND EFFECTIVENESS OF 192-SAP

The selectivity and effectiveness of 192-sap for producing lesions of the CBF has now been shown by several laboratories. 192-sap selectively kills CBF neurons when injected into the ventricular system (i.c.v.), into the CBF directly, or into the target fields of the CBF.[64] I.c.v. injection of 192-sap lesions the CBF as well as the p75 bearing Purkinje cells of the cerebellum.[5,24,56,66,67] Such injections spare the cholinergic neurons of other brain regions,[67,24,65,66] the GABAergic neurons that are intermingled with the CBF,[30,31,46,54] the calbindin D-28K expressing neurons of the NBM,[24] and the NADPH-diaphorase expressing neurons of the NBM.[24] I.c.v. 192-sap injection also results in loss of cholinergic markers such as choline acetyl transferase (ChAT) activity,[29,31,34,40,51,56,57] acetylcholine esterase (AChE) positive fibers,[55] and high affinity choline transport (HAChT)[61] in the target fields of the CBF. When injected intraparenchymally into the CBF, 192-sap kills mainly in the proximity of the injection site but spares interspersed non-cholinergic neurons.[1,5,8,54,62] One study reported a delayed loss of cholinergic striatal interneurons after injection into the NBM,[24] but this finding has not been replicated. The sphere of CBF cell kill that results from intraparenchymal injection can be expanded by increasing the dose; however, non-selectivity becomes a problem at such dose levels (Wrenn and Wiley, unpublished observations). Intraparenchymal injections of 192-sap can also be performed in the target fields (hippocampus, cortex) of the CBF. Ohtake *et al.* has shown that injections into the hippocampus result in a mostly ipsilateral loss of MS/DBBv cholinergic neurons and that this effect is blocked by simultaneous injection of colchicine.[41] Similarly, intracortical infusions cause a loss of AChE fiber density in the cortex and a loss of NBM cholinergic neurons.[27,47]

The selectivity and lethality of 192-sap for CBF neurons was further demonstrated by Book *et al.* using a double labeling approach.[6] In this study the persistent retrograde tracer Fluoro-Gold (FG) was injected cortically one week before i.c.v. injection of 192-sap or saline. The FG pre-labeled both cholinergic and non-cholinergic basal forebrain neurons. After killing the cholinergic neurons, the NBM was stained immunohistochemically for ChAT. These authors found that only the neurons that were double-labeled for both FG and ChAT were lost in the 192-sap treated rats. Thus, 192-sap selectively killed the cholinergic neurons of the NBM while leaving the non-cholinergic neurons intact.

It is important to emphasize that much of the CBF projection to the amygdala is not lesioned by i.c.v. or intraparenchymal 192-sap. Many of the cholinergic neurons that project to the amygdala do not express p75; therefore, they are unaffected by 192-sap.[23,24,65] This sparing of the cholinergic innervation of the amygdala differs significantly from the pathology of Alzheimer's disease.[63]

In summary, 192-sap is the most effective and selective means available for producing lesions of the rat CBF. Its advantage over previous techniques is in its ability to kill the cholinergic neurons of the basal forebrain while leaving intermingled non-cholinergic neurons intact. Significant draw-

backs of i.c.v. 192-sap (the best approach for making high grade CBF lesions) include sparing of the cholinergic innervation to the amygdala and destruction of some cerebellar Purkinje neurons.

BEHAVIORAL EFFECTS OF I.C.V. 192-SAP

The cognitive effects of 192-sap induced CBF lesions have been examined in a variety of behavioral paradigms of learning and memory. The i.c.v. mode of injection has most consistently produced lesions that have lead to deficits in these paradigms, and the most informative of these studies have been those using a range of immunotoxin doses which has allowed correlations between lesion extent and behavioral deficits to be analyzed. Leanza *et al.* showed that only the highest doses used in their experiments resulted in impaired learning (acquisition) in the water maze and impaired passive avoidance retention.[31] These impaired animals had a greater than 90% reduction in CBF neurons that resulted in 70–90% reduction in ChAT activity in the target fields. Rats with less extensive lesions were unaffected. In similar work, Waite *et al.* found impaired acquisition and impaired working memory in the water maze and also impaired retention of single trial passive avoidance.[56,57] Again, impairments were seen only in rats with nearly complete lesions (89–94% reduction in hippocampal ChAT activity, 73–91% reduction in cortical ChAT activity).

Work in our own laboratory has shown that impaired passive avoidance performance arises at i.c.v. doses that produce $> 90\%$ MS/DBBv lesions combined with the $> 80\%$ NBM lesions. However, MS/DBBv lesions $> 90\%$ were insufficient to impair passive avoidance performance if the NBM lesion was $< 80\%$.[69] More recently, we have examined the dose-response effect of i.c.v. 192-sap on working memory in a six arm radial maze. We found a significant correlation between working memory impairment and lesion extent, and this impairment arose only in rats that harbored total CBF lesions greater than 75%. Similar to the passive avoidance results, rats with MS/DBBv lesions $> 85\%$ but with NBM/DBBh lesions $< 75\%$ were unimpaired in the working memory task. Thus, the currently available data show that behavioral deficits are most consistently observed in rats with total CBF lesions $\geqslant$ 75–85% but not with less complete lesions.

To date, at least seven other studies have reported deficient performance in learning and memory paradigms after i.c.v. injection of 192-sap. Each of these has used doses large enough to produce lesions of an extent similar to that in the studies described above. Studies by Leanza *et al.*, Berger-Sweeney *et al.* and Walsh *et al.* reported impaired water maze acquisition.[5,30,61] However, two of these[5,61] also reported impaired performance in a cued version of the task suggesting the presence of non-mnemonic performance deficits. Delay dependent deficits in a delayed matching to position (DMTP) task[29] and also in a delayed non-matching to position (DNMTP) task[34] have been reported. One report described a mixed delay-dependent/independent deficit in DNMTP[51] again suggesting non-mnemonic deficits. Acquisition, but not retention, of an object discrimination also has been reported to be impaired after i.c.v. 192-sap injection.[55]

In addition to the work in adult rats discussed above, 192-sap has been injected i.c.v. into neonates. In a series of experiments by Leanza *et al.*,[30] rats received a 192-sap i.c.v. injection at either P4 only, P4 and adulthood, or adulthood only. The rats that received the P4 only injection retained some surviving cholinergic neurons (especially in the DBB), and these rats were unimpaired in the water maze as adults. However, these surviving neurons were eliminated in the rats that received an additional i.c.v. injection in adulthood (P4 + adulthood), and these rats were impaired in the water maze. The rats that were injected only in adulthood were also impaired. The lack of behavioral impairment after neonatal i.c.v. injection also has been reported by Pappas *et al.* who injected on P7 and observed no deficit in water maze acquisition or T-maze alternation when the rats were tested as adults.[42] Finally, Ricerri *et al.*[45] used a P1 i.c.v. injection of 192-sap that produced only a 17% reduction in cortical ChAT activity and no reduction in hippocampal ChAT activity. These rats acquired and retained passive avoidance behavior as well as controls when tested on post-natal days 15–19. In contrast, P7 injected rats had substantially larger reductions in ChAT activity (78% hippocampus, 64% neocortex) and were impaired in passive avoidance acquisition but not retention when tested on post-natal days 15–19. In summary, neonatal i.c.v. injection of 192-sap, like i.c.v. injection into adult animals, results in impaired performance in behavioral paradigms of learning and memory only when the resultant CBF lesion is extensive.

As mentioned above, i.c.v. injection of 192-sap kills some Purkinje cells of the cerebellum which correlates with the fact that approximately 50% of the Purkinje cells express p75.[10,44] The extent to which Purkinje cell loss may contribute to the behavioral deficits discussed above is unclear because a control group harboring Purkinje cell damage has rarely been employed. Another immunotoxin, OX7-saporin (OX7-sap), which kills Purkinje cells after i.c.v. injection[11] makes such control groups possible. The only study so far that has used OX7-sap as a control for comparison to 192-sap reported a passive avoidance deficit in 192-sap, but not OX7-sap, treated rats which indicates that Purkinje cell loss was not responsible for the behavioral deficit seen with 192-sap.[65] On the other hand, neonatal studies have shown that Purkinje cells are not lesioned by neonatally administered 192-sap. As discussed above, these rats often have incomplete CBF lesions and no behavioral impairment, but deficits do arise when an additional adult 192-sap injection is given. This additional injection not only completes the CBF lesion, but it also kills Purkinje cells. Thus, the difference in behavioral performance between the neonatally injected rats and the neonatal plus adult injected rats could just as easily be attributed to the Purkinje cell loss in the adults as it could be to the more extensive CBF lesion. In summary, a possible contribution of Purkinje cell loss in behavioral performance after i.c.v. 192-sap cannot be ruled out at present and demands further experimental examination.

All the data from behavioral studies of rats with i.c.v. injections of 192-sap are consistent with the hypothesis that p75-positive CBF neurons serve to modulate cognitive function. The most interesting feature of the relationship between the CBF and performance in behavioral paradigms of learning and memory is that only very high grade lesions of the entire population reliably give deficient performance. This observation points to the idea that the CBF possesses substantial reserve capacity and that lesions must cross a high threshold (in the range of 75–85%) to impact behavior. There is precedent for such a threshold relationship between lesion and functional loss from studies of other neural systems. In the monkey, for example, depletions of 90% or more of dopamine projecting systems are needed before motor impairments arise.[49] Such functional redundancy may be a feature of many neural systems. One can easily imagine natural selection favoring a system that can tolerate even a high degree of neuronal loss. On the other hand, the requirement for high grade CBF lesions to produce behavioral deficits may reflect shortcomings in the sensitivity and specificity of the behavioral tests. Perhaps, a more graded dose-response relationship between CBF cell loss and behavioral deficit would be apparent in behavioral paradigms that more directly test CBF function.

BEHAVIORAL EFFECTS OF INTRAPARENCHYMAL 192-SAP

As one would expect from the behavioral dose-response results obtained after i.c.v. injection of 192-sap, intraparenchymal injections, which produce small, circumscribed lesions of portions of the CBF, have not reliably produced learning and memory deficits. In one study by Wenk *et al.* 192-sap was injected into the NBM resulting in very small reductions in ChAT activity in the hippocampus (15%) and cortex (25–31%) and no effect on T-maze or passive avoidance performance.[62] Torres injected 192-sap into the MS, DBB, or the NBM and found no effect in the water maze.[54] Baxter *et al.* also found no effect on water maze acquisition and a delay independent working memory deficit in the water maze after intraparenchymal injection into the MS/DBBv or NBM/SI.[1] This same group also tested water maze performance in rats after injection into both the MS/DBBv and NBM/SI[2] as well as in old rats after MS/DBBv injection.[3] None of these groups were impaired. A similar lack of effect in the water maze was observed by Dornan *et al.* after MS and NBM injections.[12] One novel study even used a water version of the radial maze to test rats after MS/DBBv injection, but still no effect was found.[36] Finally, DNMTP tasks have also been used to assess the effects of intraparenchymal 192-sap. Steckler injected the immunotoxin bilaterally into the hippocampus and found no effect although the reduction in hippocampal ChAT activity in those rats was only 57%.[51]

The characteristic shared by all these negative findings after intraparenchymal injection of 192-sap is that the lesions were limited to a circumscribed part of the CBF and fell short of the diffuse, extensive lesions that are produced by i.c.v. injection. After intraparenchymal injection, even when

one part of the CBF is lesioned completely, other parts are left intact. As shown by the i.c.v. studies, incomplete lesions are inadequate to produce impairment in behavioral paradigms of learning and memory. As mentioned above, Baxter addressed this point directly by injecting rats in both the MS/DBBv and NBM/SI.[2] However, ChAT depletion in these rats was 78% and 64% in hippocampus and cortex, respectively. These reductions in ChAT activity fall short of those obtained after i.c.v. injection in rats that were impaired in the water maze as described by Nilsson[40] Leanza,[31] and Waite.[56] Thus, the negative findings in learning and memory paradigms after intraparenchymal injection likely reflect incomplete CBF lesions.

Not all 192-sap studies using the intraparenchymal approach have yielded negative results in learning and memory paradigms. Torres found impaired passive avoidance retention after injection into the NBM and impaired DNMTP performance after injection into the MS.[54] A delay independent effect on a water maze working memory task was reported by Baxter,[1] and Dornan reported a deficit in the acquisition of a working memory task in the radial maze after injections into the MS alone, NBM alone, or both locations together.[12] In fact, conventional radial maze experiments typically have yielded behavioral deficits after intraparenchymal injection of 192-sap. Walsh *et al.* injected various doses into the MS and found dose-dependent deficits that were related to the cognitive demands of a delayed non-matching to position radial maze task.[60] In a similar study, Shen injected 192-sap into the MS and found impaired working memory in the radial maze but no effect on reference memory.[50] Walsh has also studied the effects of intrahippocampal and intracingulate 192-sap injections on DNMTP radial maze performance.[59] Interestingly, rats that received cingulate injections had a delay dependent deficit while rats that received hippocampal injections had a deficit that was not related to delay.

While lesion extent can explain the differing behavioral results between a number of the intraparenchymal studies and the i.c.v. studies, this explanation is inadequate to explain why intraparenchymally injected rats, with lesions of similar extent, have impaired spatial memory in the radial maze but not the water maze. It may be that detecting a deficit is related to the difficulty of what the rat is asked to do by the experimenter. Recalling a single platform location in the water maze is undoubtedly less demanding than recalling a list of locations in the radial maze. Thus, it may be necessary to use relatively demanding tasks, which are more sensitive, to detect memory impairment in rats with narrowly circumscribed CBF lesions. Whether or not a deficit is observed within a given experiment depends upon an interplay between extent of lesion and the demands of the task. Perhaps even animals with subthreshold lesions induced by low i.c.v. doses of 192-sap would be impaired in a difficult DNMTP radial maze task that employs long (1–4 h) delays such as that used by Walsh and colleagues.

Intraparenchymal injections of 192-sap also have been used to study the effects of CBF lesions on attentional processing. Injection into the NBM/SI impairs the incremental attention that normally occurs when the predictive relationship between two cues is shifted.[8] In these same rats decremental attention, as assessed by latent inhibition, was unaffected. 192-sap injection into the MS/DBBv disrupts decremental attention as assessed by both latent inhibition and a serial conditioning task.[4] In contrast, another study found no effect on latent inhibition by MS/DBB injection.[13] The conflicting results obtained in latent inhibition after MS injection may reflect methodological differences. The study that obtained positive results used visual stimuli as the conditioned stimuli and food cup behavior as the conditioned response while the study that obtained negative results used a conditioned taste aversion paradigm.

McGaughy *et al.* have reported that injections into the NBM/SI disrupt the ability to detect signals with no change in the ability to reject non-signals.[35] Injections into the NBM also impair the ability to discriminate between stimuli,[52] and unilateral NBM lesions alter the response to spatially lateralized visual targets.[7] These attentional effects of intraparenchymal injection of 192-sap raise the possibility that the performance deficits of 192-sap treated rats in behavioral paradigms of learning and memory may be downstream manifestations of disrupted attentional processing. The conundrum is that if normal attentional processing is a necessary pre-condition for learning and memory, why then have intraparenchymal injections consistently yielded effects in attentional paradigms but not learning and memory paradigms? As discussed in this study, the answer may lie in the level of difficulty of the behavioral paradigms of learning and memory. A lesion that causes a subtle change in attentional processing may only cause a mnemonic deficit when cognitive load is quite high.

CONCLUSIONS

The immunotoxin 192-sap has given investigators the ability to selectively lesion the CBF. The behavioral study of rats with high grade immunotoxic CBF lesions has shown that the CBF, probably by virtue of its input to higher brain centers (hippocampus, neocortex), plays some role in cognitive functions such as learning, memory, and attention. The development of other immunotoxins, such as ME20.4-saporin which lesions the primate CBF,[19] will permit more sophisticated behavioral analysis of CBF function. Additionally, immunotoxins selective for other types of neurons, such as the noradrenergic selective anti-DBH-saporin,[68] will allow future work to focus on the functional neuroanatomy of other diffusely projecting brain systems and how these other systems may interact with the CBF. However, much additional work with 192-sap is needed to reveal the precise behavioral function(s) of the CBF, and experiments with OX7-sap may clarify the contribution of cerebellar Purkinje neuron damage to behavioral deficits seen with i.c.v. 192-sap.

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