The study of behavioral dysfunctions

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Appendices

The appendices have been induced for various reasons. Appendix 6.1 gives an example of how we calculated the measure ‘choice correspondence of reinforced hole visits’ in the holeboard task. This measure can be considered as an index of the extent to which rats develop a fixed search pattern in a spatial holeboard discrimination task.

Appendix 6.2 describes the effects of age on the acquisition of a seven-choice task in a radial alley maze. Although the data have been published previously (Raaijmakers, et al., 1990), this publication is not readily available.

In Chapter 3.2 we hypothesized that lesioning of the nucleus basalis magnocellularis (nbm) would affect the number of proactive errors rats made when they were shifted to a new discrimination problem in the eight-arm radial alley maze, and the number of retroactive errors they made when they were shifted back to the originally acquired problem. Appendix 6.3 gives an operationalization of proactive and retroactive errors in the radial alley maze task, using the data from experiment 2 in Chapter 3.2, and tests the above mentioned hypothesis.

Appendix 6.4 describes an analysis we performed to test the hypothesis that the activity of the enzyme choline acetyltransferase (ChAT) in the cortex recovers over time after lesion of the nbm. The approach described is exploratory, because there were relatively few animals in some experimental groups.

Many colleagues and friends have contributed to the studies reported in this book, and their invaluable cooperation and input is acknowledged in Appendix 6.5.

Finally, Appendix 6.6 lists my scientific publications. This list shows that my scientific interests extend to non-spatial learning and memory, behavioral pharmacology, behavior genetics, and methodological contributions, i.e. to topics which are not explicitly dealt with in this book.

6.1
Calculation of the measure ‘choice correspondence of reinforced visits’ in the spatial holeboard discrimination task*

Introduction

The spatial holeboard discrimination task (Oades, & Isaacson, 1978) allows the simultaneous assessment of working memory (WM) and reference memory (RM) in rodents. In addition to these measures, an index for the development of a fixed food search pattern or food search strategy can be calculated. This index, the ‘choice correspondence of reinforced visits’, reflects the degree of correspondence between the sequences of reinforced, i.e. first visits, to the baited set of holes of two subsequent trials. It has been reported that normal rats develop a fixed food search pattern or food search strategy (Oades, & Isaacson, 1978, Oades, 1981a,b; Oades 1982; van der Staay, van Nies, & Raaijmakers, 1990; van der Staay, Krechting, Blokland, & Raaijmakers, 1990).

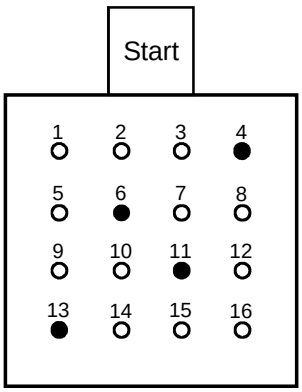

Figure 1. *Map of the holeboard. The holes were numbered 1 to 16, starting at the row closest to the startbox. Holes no. 4, 6, 11, and 13 were baited with one food pellet each.*

Worked example

The holeboard was constructed according to the descriptions given by Oades (1981a, 1982; see Chapter 3.1, Material and Methods, for details). All walls were made of transparent polyvinyl chloride (PVC). There were 16 holes in the gray PVC floor. The rats were trained to collect pellets from a fixed set of four holes. A rat was placed in a clear Plexiglas start-box connected to the holeboard in the middle of one side wall. A trial was initiated by raising the guillotine door of the start-box; it was

* This worked example has been published as part of the paper: van der Staay, F.J. (1999). Spatial working and reference memory of Brown Norway and WAG rats in the holeboard task. *Neurobiology of Learning and Memory*, **71**(1), 113-125.

terminated when the rat had found all the food pellets. Rats were trained daily with massed trials. A hole visit was scored when a rat pointed its nose toward to the edge of a hole, moved its nose over the edge, or poked its nose into the hole (Oades, 1981a). The configuration of baited holes is depicted in Figures 1 and 2.

The sequences of visits to the baited set of holes were compared from trial to trial, and the longest common sequence of two successive trials was determined. This measure reflects the variability of the spatial pattern of obtaining rewards, but it neglects all erroneous choices, i.e. visits to the unbaited holes and revisits to holes of the baited set. Examples of sequences in four successive trials are depicted in Fig. 2. The determination of the measure choice correspondence of reinforced visits, based on these examples, is further elaborated in Table 1.

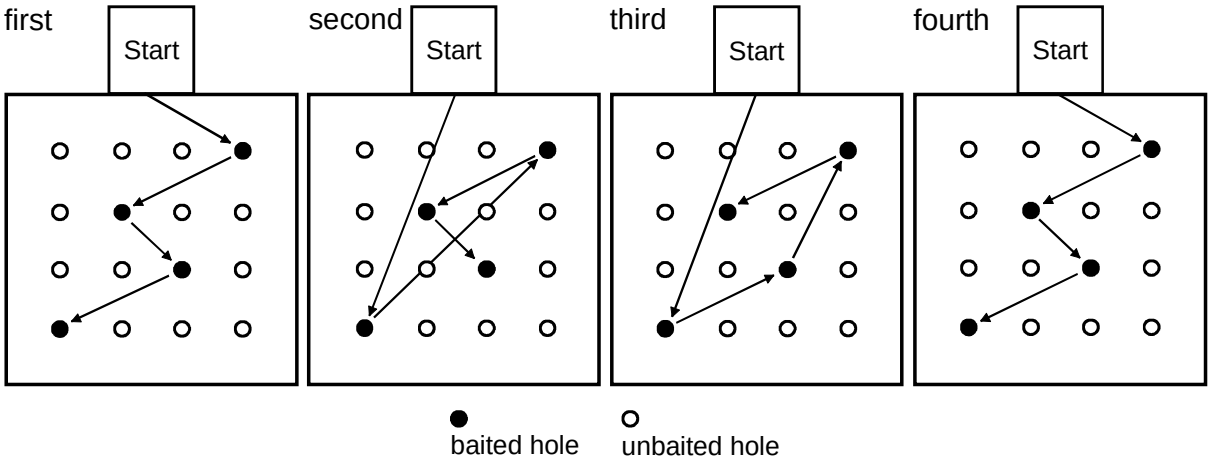

Figure 2. *Examples of the order in which the pellets were collected from the baited holes (see also Fig. 1) in four successive trials. The choice correspondence of reinforced visits was determined from the sequences in these four trials. The order of these visits from the startbox to the fourth reinforced visit is shown by arrows.*

Table 1. *Determination of the choice correspondence of reinforced visits. The sequences depicted in Fig. 2 were used in the following examples, in which the choice correspondence of reinforced visits was determined pairwise between trials 1 and 2, 2 and 3, and 3 and 4. The numbers of the holes of the common sequences are printed in bold. The choice correspondence of reinforced visits between trials 1 and 2, 2 and 3, and 3 and 4 was 3, 2, and 1, respectively.*

	Sequence of hole visits			
Trial	first	second	third	fourth
First trial: rat visited baited holes no.	**4**	**6**	**11**	13
Second trial: rat visited baited holes no.	13	**4**	**6**	**11**
Second trial: rat visited baited holes no.	13	**4**	**6**	11
Third trial: rat visited baited holes no.	13	11	**4**	**6**
Third trial: rat visited baited holes no.	**13**	11	4	6
Fourth trial: rat visited baited holes no.	4	6	11	**13**

The score 0 never occurred because rats always visited at least one hole in common in two successive trials. Note that the longest common sequence between two successive trials is expressed as the number of choices made during these common sequences and not as the number of common transitions between choices. In the latter case, the measure choice correspondence of reinforced visits would have ranged from 0 to 3, instead of from 1 to 4. These different operationalizations yield exactly the same statistical results.

6.2
Effects of age on the acquisition of a seven-choice task in a radial alley maze*

Introduction

Aging rats have consistently been found to suffer from impairments of spatial memory in cross-sectional studies. In fact, both working memory (WM) and reference memory (RM) appear to be impaired in complex spatial discrimination tasks (see Chapter 2.1, van der Staay, Krechting, Blokland & Raaijmakers, 1990; van der Staay, van Nies & Raaijmakers, 1990). In the present study, we assessed the sensitivity of a seven-choice task in a radial alley maze for age-associated changes in learning. This task mainly involves the RM. Young and old rats of the pigmented Brown-Norway (BN/BiRij) and of the albino Wistar (Wu:Cpb) strains, were tested.

Material and methods

Animals: in the first experiment, four 3-month-old and five 22-month-old BN/BiRij rats were used. In the second experiment, ten 5-month-old and eight 27-month-old Wistar rats (Wu:Cpb) were tested. The BN rats were supplied by TNO, Rijswijk, The Netherlands, whereas the Wistar rats were bred at the CPB, Zeist, The Netherlands. The rats were housed under a reversed day-night schedule in the animal facilities of the Psychological Laboratory, University of Nijmegen, The Netherlands.

Apparatus: the apparatus has been described in detail in Chapter 3. Briefly, the eight-arm radial maze consisted of a central platform from which eight arms radiated equidistantly. A cylindrical door that opened by moving down vertically allowed simultaneous access to the eight alleys. Symbols above the entrances of all alleys provided distinct intra-maze cues.

Procedure: the body weights of all animals were gradually reduced to 85% of their free-feeding values, and the animals were familiarized with the maze on 4 consecutive days in 10-minute adaptation sessions. When spatial discrimination training began (1 week after the start of the adaptation sessions) all rats had reached their 85% target weight. In both experiments, the rats were trained with massed trials (days 1 to 3, 2 trials per day; days 4 to 10, 3 trials per day; from day 11 on, 6 trials per day) until a criterion of seven error-free trials in a series of nine trials was reached. A trial was terminated as soon as the rat had found the food reward. A correction procedure was applied: during a trial, the rat could freely enter and re-enter all alleys, including the start alley. Two measures of the acquisition of the task were analyzed: 'trials to criterion' and 'errors to criterion'. Errors are visits and re-visits of the never-baited alleys, including the start alley, and were summed over all trials.

* The results of the experiments in this Appendix have previously been reported as part of the publication: Raaijmakers, W.G.M., van der Staay, F.J., Drinkenburg, W.H.I.M. & Blokland, A. (1990). Age differences and effects of lesions in the nucleus basalis magnocellularis on a seven-choice task in a radial alley maze. In: van Bezooijen, C.F.A., Ravid, R., & Verhofstad, A.A.J. (Eds.). *From gene to man.* Rijswijk: Stichting Gerontologie en Geriatrie (ISBN 90-9003996-1), pp. 159-163.

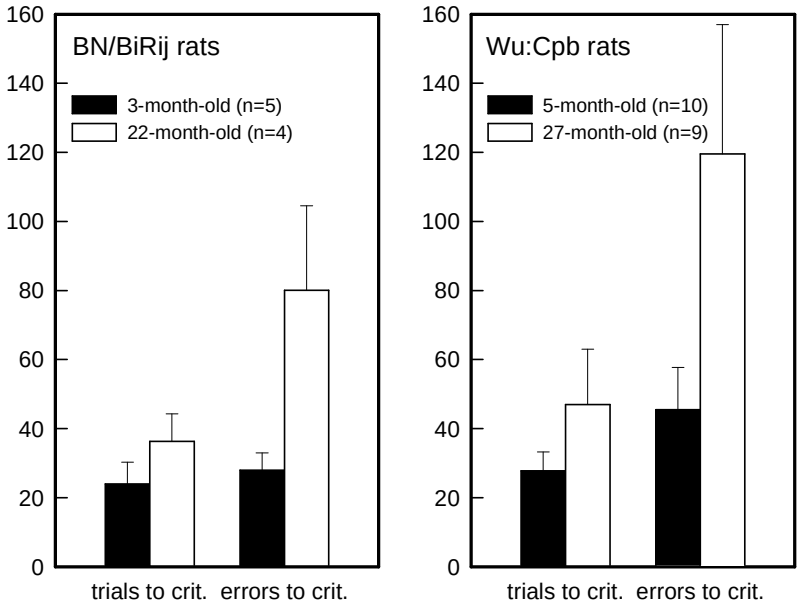

Figure 1. *Trials and errors to criterion of young and aged Brown Norway (BN/BiRij; left panel), and Wistar rats (Wu:Cpb; right panel) in a seven-choice task. The means and the standard errors of the means are depicted.*

Statistical analysis: age differences for trials and errors to criterion were analyzed separately for the two experiments by using Student's *t*-statistics.

Results

Experiment 1: the old BN/BiRij rats needed more trials ($t_7 = 2.5$, $p < 0.05$) and made more errors ($t_7 = 4.6$, $p < 0.05$) to reach the criterion than their young counterparts (see Fig. 1, left panel).

Experiment 2: a similar picture emerged for the Wistar rats. Again, the aged rats made more errors ($t_{16} = 4.2$, $p < 0.01$) and needed more trials ($t_{16} = 6.1$, $p < 0.01$) than the young rats before they reached the criterion (see Fig. 1, right panel).

Concluding remarks

The seven-choice task in the radial alley maze was found to be sensitive to age-associated deficits in learning. The performance of the young rats of the two rat strains, and the differences observed between the young and the old rats of a strain were similar. The aged Wistar rats might have had more difficulties acquiring this task (see Fig. 1) than aged BN rats, but the aged Wistar rats were 5 months older than the aged BN rats. Thus, the apparent poorer performance of the Wistar rats might have been because they were older. All rat were able to reach the criterion of seven error-free trials in a series of nine trials. The seven-choice task appears to be less demanding than the holeboard task (van der Staay, van Nies & Raaijmakers, 1990; see also Chapter 3.1) and the conefield task (van der Staay, Krechting, Blokland & Raaijmakers, 1990) where error-free trials are exceptions even after extended training.

6.3
Effects of nucleus basalis lesions on proactive and retroactive errors in a seven-choice task in a radial alley maze

Introduction

It is worthwhile evaluating the type of error a rat makes when it is switched from one problem to another in the seven-choice task in an eight-arm radial alley maze. Within the framework of the second experiment of Chapter 3.2, a proactive error occurs when an animal visits the goal alley of the previously acquired problem A while it is trained on problem B. A retroactive error occurs when a rat visits the goal-alley of problem B (that was acquired after problem A), while the animal is re-tested on problem A. Only the first visit in a trial is considered (see Table 1).

Table 1. *Definition of proactive and retroactive errors in the seven-choice task in a radial alley maze. The rats were first trained on problem A. The number of the start alley and of the goal alley, where the rat could find a food reward (a 45 mg food pellet), is given. Next, the rats were trained on problem B. Finally, the rats were re-tested on problem A. A proactive error was scored when a rat visited alley 6 first while being trained on problem B. A retroactive error was scored when a rat first visited alley 2 during re-testing on problem A.*

	problem A	**problem B**	**problem A**
The rat is started from alley:	1	7	1
The rat finds pellets in alley:	6	2	6
Type of error:		proactive error: the first visit is to alley no. 6	retroactive error: the first visit is to alley no. 2

In order to assess whether nucleus basalis magnocellularis (nbm) lesions affected the number of proactive or retroactive errors, we considered the first six trials of acquisition of problem B and the first six trials of the re-acquisition of problem A after acquisition of problem B, respectively.

The frequency of first visits to alley number 6 while a rat is being trained on problem B and the frequency of first visits to alley number 2 while a rat re-acquires problem A can be taken as measures of proactive and retroactive errors, respectively. However, erroneous and error-free trials appear within the first six trials. Therefore, we decided to use a ratio measure instead of the frequency measure. This method might be helpful to analyze proactive and retroactive errors in the seven-choice task.

Calculated example

The following operational definitions for proactive and retroactive errors were used:

Proactive errors are operationalized as: (number of trials -of first six- during acquisition of problem B, in which the goal alley of problem A was visited first) / (number of trials -of first six- during the acquisition of problem B, in which at least one erroneous visit was made).

Retroactive errors are operationalized as: (number of trials -of first six- during re-acquisition of problem A, in which the goal alley of problem B was visited first) / (number of trials -of first six- during the re-acquisition of problem A, in which at least one erroneous visit was made).

Table 2. *Number of trials in which a proactive or a retroactive error was made, number of trials in which at least one error was made, and ratio measure for both types of errors per rat and treatment condition (total).*

Treatment	**Rat no.**	**Acquisition problem B: proactive error ratio**			**Re-acquisition problem A: retroactive error ratio**		
		errors	trials	measure	errors	trials	measure
Intact							
	1	3	4	0.75	6	6	1.00
	2	4	6	0.67	1	1	1.00
	3	1	5	0.20	1	1	1.00
	4	3	4	0.75	2	4	0.50
	5	1	4	0.25	2	2	1.00
	6	2	5	0.40	0	3	0.00
	total	14	28	0.50	12	17	0.71
Sham-lesioned							
	1	1	4	0.25	1	4	0.25
	2	1	6	0.17	0	5	0.00
	3	0	2	0.00	3	3	1.00
	4	2	4	0.50	0	3	0.00
	5	0	5	0.00	1	1	1.00
	6	4	6	0.67	2	4	0.40
	total	8	27	0.30	7	20	0.35
nbm-lesioned							
	1	4	4	1.00	0	0	0.00
	2	1	2	0.50	0	3	0.00
	3	0	5	0.00	1	4	0.25
	4	0	2	0.00	2	5	0.40
	5	0	6	0.00	0	1	0.00
	6	0	5	0.00	1	1	1.00
	total	5	25	0.20	4	14	0.29

If, for example, a rat made at least one incorrect visit in four of the first six trials, and if a proactive error was made in three of the four erroneous trials, i.e. the first visit in a trial was to alley number 6, then the measure for proactive errors was 3/4 = 0.75. For each rat we determined the absolute number of erroneous trials in the first six trials and the absolute number of trials with a proactive or a retroactive error and calculated the ratio measure. We transformed these ratio measures into rank scores.

Using Kruskal Wallis one-way analysis of variance by ranks we then assessed whether the nbm lesion affected the number of proactive or retroactive errors. For treatment effects on proactive errors, H (the statistic obtained in the Kruskal-Wallis analysis) was: $H_2 = 3.41$, $0.20 > p > 0.10$ (with correction on ties; X^2-approximation with df = 2). The analysis did not confirm our expectation that the nbm lesion affects the incidence of proactive errors. For treatment effects on retroactive errors, the Kruskal-Wallis analysis revealed: $H_2 = 3.40$, $0.20 > p > 0.10$ (with correction on ties; X^2-approximation with df = 2). Again, the analysis did not confirm our expectation that the nbm lesion affects the incidence of retroactive errors.

Concluding remarks

Although we found no support for the notion that the nbm lesion affected proactive or retroactive errors, it is worth investigating whether the proposed analysis is sensitive enough to detect treatment-induced effects. If alley visits were completely random, then every alley had a chance of 1/6 = 0.17 of being visited as first location (remember that error-free trials were not considered. If the correct goal alley is included then the chance is 1/7 = 0.14 for each alley). As can be seen from the rows *total* of Table 2, the ratios were higher than 0.16, indicating that proactive and retroactive errors occurred at an above chance level, especially in the intact group. This group might suffer from proactive and retroactive errors, i.e. they suffered from interference from the previously acquired problem(s). However, as both the numerator and the denominator of the ratio measures can vary because both the number of erroneous trials and the number of trials with the specific proactive or retroactive errors can range from 0 to 6, it is not possible to make a simple comparisons with the (theoretical) chance level.

6.4
Is there recovery of cortical cholinergic activity in young and aged rats after bilateral lesioning of the nucleus basalis magnocellularis?

Introduction

There is controversy whether or not cortical cholinergic activity recovers after lesioning of the nucleus basalis magnocellularis (nbm) of rats (e.g. Wenk & Olton, 1984; Thal et al., 1988; Shaughnessy, et al., 1996). We found in Chapter 3.3 that after a 10-week survival period, choline acetyltransferase (ChAT) activity was only moderately reduced in the frontal and parietal cortices of nbm-lesioned young and aged Wistar rats. We had already performed a pilot-study (here called experiment I) in which we collected samples of the frontal and parietal cortices and of dorsal hippocampus from ten 4-month-old (3 sham-lesioned and 7 nbm-lesioned) and five 28-month-old (3 sham-lesioned and 2 nbm-lesioned) male WU:Cpb rats 1 week after bilateral lesioning of the nbm in order to measure the activity of choline-acetyltransferase (ChAT). Using these data, we performed an exploratory analysis to address the question whether spontaneous recovery of cortical ChAT activity occurred in the animals used in the experiment described in Chapter 3.3 (here called experiment II). The number of brain samples used in this analysis is given in Table 1.

Results

Experiment I: effects of the nbm lesion on the ChAT activity in the frontal and parietal cortices and in the dorsal hippocampus after a 1-week survival period were analyzed by an Age (4-month-old versus 28-month-old) by Lesion (sham-lesioned versus nbm-lesioned) analysis of variance (ANOVA).

Frontal cortex: age-related differences in ChAT activity were not found. One week after lesioning of the nbm, the ChAT activity was reduced by about 47% (Lesion: $F_{1,11} = 43.19$, $p < 0.01$) compared with that of the sham-lesioned control rats. The nbm lesion did not affect the ChAT activity of young and aged rats differently.

Parietal cortex: the ChAT activity in the parietal cortex was not different in the young and the old animals. The nbm lesion, however, caused a reduction in ChAT activity in the parietal cortex of about 35% (Lesion: $F_{1,11} = 34.83$, $p < 0.01$). The reduction was similar for the two age groups (no Age by Lesion interactions were found).

Dorsal hippocampus: neither age differences nor lesion effects on hippocampal ChAT activity were observed.

Experiment II: the corresponding analyses of ChAT activity after a 10-week survival period are given in detail in the *Results* section of Chapter 3.3. In short, ChAT activity in the frontal and parietal cortices of

both young and aged rats was reduced by 25% and 16%, respectively. There was no differential effect of age on the decrease in the activity of this enzyme after lesioning of the nbm. The ChAT activity in dorsal hippocampus was not affected by the lesion.

A first step in the analysis of 'recovery' was to compare the ChAT activity in the frontal and parietal cortices of the sham-lesioned animals of both experiments by using an Age (4-month-old versus 28-month-old) by Survival period (1 week versus 10 weeks after lesioning) ANOVA. This analysis revealed that the ChAT activity in the two cortical brain samples was the same in the sham-lesioned rats that were killed 1 week or 10 weeks after lesioning. Likewise, there were no age differences nor was there an interaction between Age and Survival period. Therefore, we pooled the data for the sham-lesioned rats of both ages from the two experiments (n = 25), and used these data to calculate the mean ChAT activity of the two brain regions. These means provide the best estimates of the baseline ChAT level of the frontal and parietal cortex, respectively.

Table 1. *Number of brain samples (frontal cortex, parietal cortex, dorsal hippocampus) from sham-lesioned and nbm-lesioned young and old male WU:Cpb rats, dissected either 1 or 10 weeks after bilateral lesioning of the nucleus basalis magnocellularis (nbm).*

Survival (weeks)	**Brain sample**	**4-month-old rats**		**28-month-old rats**	
		sham-operated	nbm-lesioned	sham lesioned	nbm-lesioned
1 (experiment I)	frontal cortex	3	7	3	2
	parietal cortex	3	7	3	2
	dorsal hippocampus	3	7	3	2
10 (experiment II)	frontal cortex	9	9	10	9
	parietal cortex	9	9	10	9
	dorsal hippocampus	6	6	4	4

Because we did not find age differences in the ChAT activity of the nbm-lesioned rats in experiment I (1 week survival), we calculated the average ChAT activity in all nbm-lesioned animals (n = 9) and subtracted this average from the baseline estimate [see Table 2; (a - b)]. These difference scores provide an estimate of the acute effect of the lesion in the frontal and parietal cortices.

We now questioned whether the decrease in ChAT activity in the two cortical samples of the nbm-lesioned rats collected after 10 weeks (experiment II) was smaller than the decrease observed after 1 week. Because ANOVAs did not reveal any indication of age effects, we pooled the data of the nbm-lesioned rats (n = 18) of both age groups. We determined the difference scores between the baseline ChAT activity (as determined from the pooled sham-lesioned rats of both experiments; see Table 2) and the ChAT activity of the nbm-lesioned rats in experiment II. From this difference score, i.e. the estimate for acute lesion effects, we subtracted the difference between the baseline estimate and the ChAT activity after 10 weeks per nbm-lesioned animal to obtain a 'recovery score'. A positive score indicated that the ChAT activity decreased less after nbm lesions in rats surviving 10 weeks than it did in rats that survived 1 week; i.e. that recovery had occurred.

The resulting 'recovery scores' were positive (mean ± SEM for frontal cortex: 4.92 ± 1.08, for parietal cortex: 6.26 ± 0.97) and deviated from zero as confirmed statistically by Student's *t*-tests (frontal cortex: $t_{17} = 4.57$, $p < 0.01$, parietal cortex: $t_{17} = 6.48$, $p < 0.01$). Thus, the 'recovery scores' indicate that the effect of the nbm lesion was greater at 1 week than at 10 weeks.

Table 2. *Estimates of the baseline ChAT activity, based on all sham-lesioned rats from experiment I (n = 6) and experiment II (n = 19), and the average ChAT activity in the frontal and parietal cortices for all nbm-lesioned rats in exp. II (n = 18) were used to calculate estimates of the acute nbm-lesion effects.*

	ChAT activity (nM acetylcholine * hour^{-1} * mg protein^{-1})	
	Frontal cortex	**Parietal cortex**
a) Estimate of baseline ChAT activity sham-lesioned rats (exps. I & II)	29.32	26.29
b) Average ChAT activity nbm-lesioned rats (exp. I)	16.61	15.85
Estimate of nbm lesion effect on ChAT activity after 1 week (a - b)	12.71	10.44
Recovery score (a - b) - (a - ChAT activity of nbm-lesioned rats after 10 weeks)	4.92	6.26

Discussion

These results support the impression that ChAT activity in the cortical samples was partly restored 10 weeks after lesioning of the nbm. One should, however, keep in mind that some sample sizes were very small, and there may have been insufficient sensitivity to detect age by survival time interactions. For this reason we did not consider these data suitable to assess whether nbm lesions differentially affect ChAT activity, and whether recovery processes are affected by age. However, the determination of ChAT activity, as performed in these experiments, has been found to be highly reliable, even with small samples, and to be very sensitive to treatment-induced changes (e.g. van der Staay, 1989, p. 106-107).

Further evidence supporting the notion of neurochemical recovery of ChAT activity has been reported by Shaughnessy, et al. (1996). In their study, rats received bilateral infusions of colchicine into the nucleus basalis. Colchicine is a neurotoxic alkaloid that acts as an anti-mitotic, binds to tubulin and disrupts axoplasmic transport. ChAT activity in the frontal and parietal cortices, striatum and hippocampus was assessed in animals surviving 5 or 12 weeks post-lesion. ChAT activity was reduced by about 30% 5 weeks after lesioning in both cortical samples, but was not affected in the striatum and hippocampus. Twelve weeks after lesioning, ChAT activity in the frontal cortex was still reduced by about 20%, whereas ChAT activity in the parietal cortex had returned to control values.

However, it is not clear whether long-term recovery is due to sprouting of residual cholinergic innervation from the lesioned nbm. Cossette and co-workers (1993) reported that the cortical ChAT activity in rats returned to near normal levels within 3 months after unilateral lesioning of the nbm. Based on immunostaining of vasoactive intestinal polypeptide (VIP), which appears to be co-localized in intracortical acetylcholine (ACh) neurons in rodents, and immunostaining of ChAT, Cossette and colleagues suggest that the recovery was mainly due to an increase of intrinsic ACh innervation.

Recently, Shaughnessy and colleagues (1998) suggested that another population of cholinergic fibers, for example those originating in the horizontal limb of the diagonal band, might be the source of recovery. This suggestion is based on the observation that the recovery, indicated by an increase in cortical fiber density, occurred earlier in the deeper than the more superficial layers of the cortex. To solve the questions concerning recovery after lesioning of the nbm, further research is needed.

6.5
Acknowledgments

Scientific research is exciting, and involves the input of many people. This last aspect was emphasized when I came to writing this acknowledgment. There would be no such a thing as a 'Habilitationsschrift' without the invaluable cooperation and contribution of many colleagues and friends. I express my special thanks to my colleagues Drs. Arjan Blokland, Pim Drinkenburg, Ervin Horváth, Wijnand Raaijmakers, and Bernard Schmidt for their cooperation and for their fruitful feedback and discussions.

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