

Cortical cholinergic inputs mediate processing capacity: effects of 192 IgG-saporin-induced lesions on olfactory span performance

Janita Turchi and Martin Sarter

Department of Psychology and Neuroscience Program, The Ohio State University, 27 Townshend Hall, Columbus, OH 43210, USA

Keywords: acetylcholine, attention, basal forebrain, nonmatch-to-sample, rats

Abstract

An olfactory span task that required rats to discriminate an olfactory stimulus added to an increasing list of such stimuli (nonmatching-to-sample; NMTS) was employed to assess the role of the basal forebrain cholinergic system in the animals' olfactory working memory capacity. A separate group of animals was trained in a matching-to-sample (MTS) version of this task that did not tax span performance. NMTS animals required significantly more sessions to reach an olfactory span of 18 stimuli than MTS rats. Infusions of the cholino-immunotoxin 192 IgG-saporin into the basal forebrain resulted in decreases of cortical acetylcholinesterase (AChE)-positive fibre density ranging from 80% in frontodorsal and frontoparietal regions to 35% in the pyriform cortex and 24% in the olfactory bulb. Postsurgery span performance was significantly reduced in lesioned NMTS but not MTS animals. Span performance in lesioned NMTS animals recovered following 4 weeks of postoperative training; however, these animals' span remained vulnerable to the effects of increased intertrial intervals. The distribution of errors in lesioned animals indicated a recency effect. In NMTS animals, olfactory span performance during the initial two postoperative weeks correlated significantly with AChE-positive fibre density in neocortical but not olfactory areas. The privileged, automatic processing of olfactory stimuli in rats may have contributed to the transience of the lesion effect. The results support the crucial role of cortical cholinergic input in the mediation of aspects of processing capacity.

Introduction

The results from numerous experiments have consistently supported the hypothesis that basal forebrain corticopetal cholinergic projections mediate major aspects of attention and thereby influence learning and memory (Everitt & Robbins, 1997; Sarter & Bruno, 1997; Wenk, 1997; McGaughy *et al.*, 2000). The assumption that loss of cortical cholinergic inputs is responsible for the attentional effects of basal forebrain lesions has been supported by the observations that the effects of intrabasal infusions of the immunotoxin largely spare the basal forebrain cholinergic projections to the amygdala (Heckers & Mesulam, 1994; McGaughy *et al.*, 1996) and that the effects of intracortical infusions of 192 IgG-saporin reproduce the effects of intrabasal infusions of this toxin on attentional performance (Bucci *et al.*, 1998; McGaughy & Sarter, 1998). In intact animals, studies on attentional performance-associated cortical acetylcholine (ACh) release (Himmelheber *et al.*, 2000) and cholinergically mediated cortical neuronal activity (Gill *et al.*, 2000) indicated that the activity of cortical cholinergic input system correlates with demands on attentional processing.

Evidence addressing the role of cortical cholinergic inputs in divided attention performance, the mediation of processing capacity and/or executive mechanisms regulating the allocation of processing resources to competing tasks, as well as capacity-taxing demands on

mnemonic processing, has remained scarce, in part due to the complexities associated with testing such functions in laboratory animals. Using a cross-modal divided attention paradigm developed for use in rats (McGaughy *et al.*, 1994), 192 IgG-saporin-induced loss of cortical cholinergic inputs was demonstrated to impair the animals' performance exclusively during blocks of trials with 'modality uncertainty', when auditory and visual conditioned stimuli occurred randomly, thereby presumably eliciting competitive processing of the two sets of propositional response rules and thus taxing divided attention performance (Turchi & Sarter, 1997). The present experiment was designed to broaden the analysis of the role of the basal forebrain cholinergic system in the regulation of processing capacity, using a different conceptual approach to a test of processing capacity, namely short-term memory capacity assessed by a list learning paradigm.

Theories about limitations in primary memory have been strongly influenced by Miller's (1956) 7 ± 2 rule, although, more recently, human working memory capacity has been suggested to be even smaller than originally suggested (see the 'magical number 4' in Cowan, 2001). However, if list learning is assessed using recognition tasks, humans and animals exhibit impressively large spans (e.g. Roberts & Kraemer, 1981; Wright *et al.*, 1985; Steele & Rawlins, 1989). The extent to which animals recall early and late items more accurately than items in the middle of the list (primacy and recency effects) has remained a matter of debate (Gaffan, 1992; Deacon & Rawlins, 1995). The present experiment employed an olfactory span task to assess the effects of cortical cholinergic deafferentation on the

Correspondence: Dr Martin Sarter, as above.
E-mail: sarter.2@osu.edu

maximal number of items animals can discriminate from a new item within a particular session.

Similar to the present experiment (see Methods), Dudchenko *et al.* (2000) trained rats to dig a fruitloop (a type of sugary breakfast cereal) out of cups filled with a mix of sand and a ground spice. Animals were then trained to find the reward in the novel stimulus added to an increasing list of items. Dudchenko *et al.* observed that rats exhibit olfactory spans of up to 25 odours in this task.

Working memory capacity limitations have been conceptualized in terms of attentional capacity limitations, as the recall of information about a recent stimulus or from long-term memory represents a function of the attentional capacity available for this task (Cowan, 2001). Therefore, we predicted that the olfactory span of rats trained in this task would be limited by lesions of the basal forebrain cholinergic system, as such lesions have been hypothesized and demonstrated to limit attentional capacities (citations above). Thus, the results from the present experiment were expected to provide the basis for a broadening of the hypothesis which suggests that cortical cholinergic inputs mediate attentional resources for working memory capacity.

Methods

Subjects

The study was initiated with 24 male Fischer–Brown Norway rats (Harlan, Indianapolis, IN, USA) weighing approximately 200 g. All animals were housed individually in an environment controlled for temperature, light (12 animals [MTS group] were housed in a room with lights on at 02.00 h, the other 12 [NMTS group] were housed in a room with lights on at 06.00 h, each for 12 : 12 light–dark schedule) and humidity. Both groups of animals were trained between 7 and 11 h following lights on, i.e. MTS animals were trained between 09.00 h and 13.00 h and the NMTS animals were trained between 13.00 h and 17.00 h. Both groups received training 6 days/week. Animals were handled extensively for 2 weeks prior to behavioural training. Water was available *ad libitum* and the animals were moderately food restricted to 90% of their free feeding weights. Animal experimental procedures were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee. All research was conducted in facilities approved by the American Association of Accreditation of Laboratory Animal Care (AAALAC).

Apparatus

Rats were trained and tested on a transparent, rectangular, Plexiglas platform (90 cm × 110 cm), resting 90 cm above the floor on a desktop. Resealable polystyrene cups (6.3 cm in depth and 6.3 cm in diameter) were used to hold the stimulus scents (100 g of playground sand mixed with 1 g of spice). The desk upon which the Plexiglas platform rested was located in the centre of the testing room and the distal spatial cues were maintained constant throughout the experiment. As described below, the position of the spices on the platform was random for each individual trial. The following spices were employed in this study (obtained from regular grocery stores): anise, basil, caraway, cardamom, celery, chives, cilantro, cinnamon, clove, coriander, cumin, dill, garlic, ginger, marjoram, mustard, nutmeg, onion, oregano, paprika, rosemary, sage, tarragon, thyme, turmeric and vanilla.

Behavioural training, acquisition data, testing, maximal span cap and probe trials

During the handling phase, which preceded the initiation of behavioural training, rats were provided with several fruitloops in

their home cages in addition to their food pellets. Animals were first trained to dig for a piece of fruitloop (Kellogg's) buried in a sand-filled cup. Rats were placed on the platform for 20 min per day and exposed to pieces of fruitloop placed both directly upon the platform as well as atop a sand-filled cup. Over the next few sessions, the pieces of fruitloop were presented as partially, and then fully buried under approximately 1 cm of sand in the cup. Correct digging was defined as executed with the forepaw as opposed to nose. The animals required a mean of 10.37 ± 0.60 sessions to acquire this skill. Rats were then trained in either the MTS or the NMTS paradigm. For this step, each rat was presented with 10–15 trials per day involving only two stimuli on the platform per trial, and allowed to correct for erroneous digs (defined as any attempt, either via forepaw or nose, to obtain the reinforcement in a nonbaited cup). Training for the NMTS task involved presentation of the initial (sample) stimulus (e.g. sage) which was baited with fruitloop. The subsequent trials were comprised of this sample scent and a second, novel, scent cup (e.g. clove); for this task, only the novel (nonmatching) stimulus was rewarded. Conversely, only the initial sample stimulus (matching) was reinforced each trial for the MTS version of the task. Upon mastery of this rule (75% correct responses for 2 consecutive days), the rats commenced training for the span component of the task (see Fig. 1 for a schematic depiction of the NMTS version of the task).

In this phase, rats were presented with successively increasing numbers of odours (choices among three, then four cups, and so on). The position of all cups on the platform was rearranged in randomized fashion from trial to trial, thereby ruling out any spatial guidance and potential experimenter-odour cues (Mumby *et al.*, 1995; see the insert in Fig. 1 for an example of an NMTS session). Throughout the experiment, animals were always allowed to self-correct within each trial, as well as complete all trials per session to ensure equal exposure to the stimulus scents among all animals. The olfactory span for each rat in a given session was defined as the number of stimuli present on the platform, excluding the initial sample stimulus, for the last error-free trial completed by the animal. After finding that animals could perform a span of 18 (19 stimuli present on the table) accurately, six of the best animals in both the MTS and NMTS tasks were subjected to a test session culminating in 25 stimuli present on the table (span of 24). Even at this level of difficulty, animals still performed with high accuracy (olfactory spans of 23.33 ± 0.49 and 22.16 ± 0.79 for MTS and NMTS, respectively). Due to both the amount of time necessary to run 25 trials (25–30 min/animal) and the expectation that training at this level would result in equally homogenous preoperative span values, it was decided that the final task would consist of maximally 19 stimuli per training session. As anticipated, rats performing the NMTS version of the task required more sessions for both rule acquisition ($F_{1,22} = 99.000$; $P < 0.0001$) and attaining the maximal span than those performing the MTS version ($F_{1,22} = 313.675$; $P < 0.0001$; see Fig. 2).

On average, the interval between successive trials was about 35–45 s for trials involving 10 or more cups; this time was necessary to transfer the animal to the opaque plastic tub positioned on a neighbouring table, to add the new stimulus cup while rearranging the position of the cups on the platform, and to return the animal to the centre of the platform. The cups were distributed along the outer borders of the platform to allow the animals to move freely within the centre area of the platform.

Animals were considered suitable for surgery upon attaining a stable baseline defined by a span of no less than 16/training session for 7 consecutive days. Surgeries for both groups of animals were clustered about the same dates to ensure that animals would be

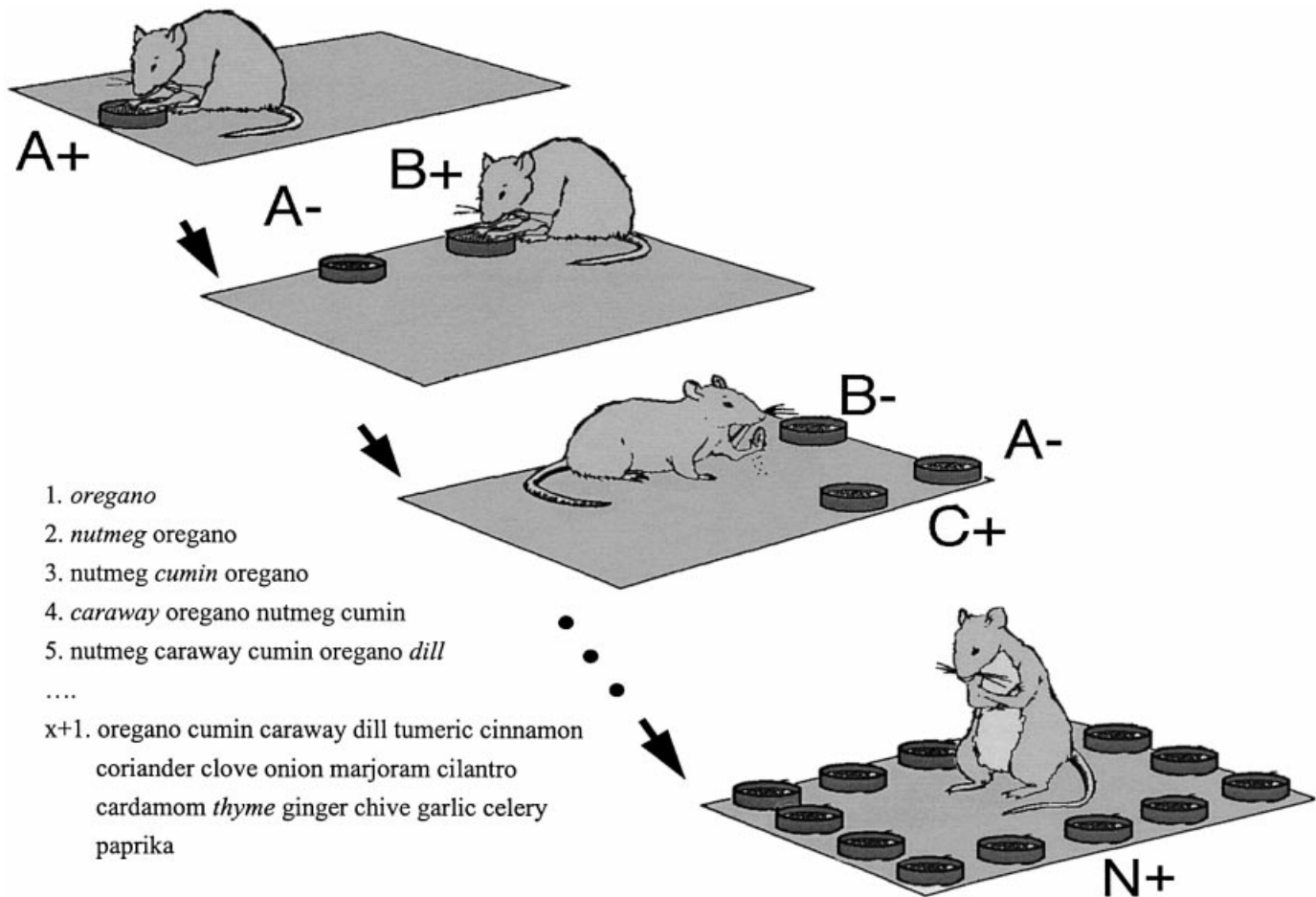


FIG. 1. Illustration of the nonmatching-to-sample (NMTS) olfactory span task (Dudchenko *et al.*, 2000; this figure, excluding the text insert, was provided by Dr Paul Dudchenko; Department of Psychology, University of Stirling, Scotland). Rats were trained to dig a fruitloop from a cup filled with sand (100 g) mixed with a spice (1 g) and to dig from a new spice in a subsequent trial. Thus, the animals were required to maintain a list of spices encountered in previous trials during a daily session (see text insert for an example of a daily session; spices in *italic* indicate the new and thus baited cup for each trial). The position of the cups was rearranged randomly for each new trial. The olfactory span, defined as the number of stimuli on the table, excluding the initial sample stimulus, until the animal made an error (i.e. a dig into a previously presented cup), was hypothesized to represent a measure of working memory capacity and executive function. In the MTS version of the task, animals were required to dig always from the first spice presented (that would be oregano in the example given in the insert) and thus could solve the task through pairwise comparisons ('nutmeg is not oregano, cumin is not oregano', etc.). Presumably, the MTS task does not tax working memory and related executive function and thus was predicted not to be affected by loss of cortical cholinergic inputs.

exposed to the same postoperative testing conditions. Animals were returned to behavioural training and testing 7 days after surgery.

Probe trials were conducted to assess whether rats in either task type were using means other than olfactory discrimination among the stimulus scents to perform the task; these probe trials were scheduled for larger numbers of stimuli upon the platform to ensure significant demands on working memory load. For this purpose, both one session before the preoperative data collection period, as well as one session after the fourth week of postoperative training, the fruitloop was omitted from the baited cup in the trial with 15 stimuli present on the table. Furthermore, for one session in the fifth week of postoperative training, an entire set of clean cups was substituted between the trials containing 13 and 14 stimuli on the platform (these cups were filled with the same concentrations of spices as those of the preceding trial and had no markings of the animal present upon them). Each test was conducted for each animal in both task groups. An additional probe trial was conducted for two separate, nonconsecutive, sessions for each animal in each task group in the fifth week of postoperative data

collection to examine the effects of lesion upon performance of trials subsequent to a 4 min delay interposition; this delay was inserted between the trials involving 15 and 16 stimuli present on the platform and involved removal of the rat from the platform at the end of the fifteenth trial, as would have ordinarily occurred, to the opaque plastic tub on the neighbouring table, where the rat remained for the duration of the delay.

Surgical procedures

Ketamine (90 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.) were administered to the rats for anaesthesia. The animals were placed in a stereotaxic instrument (Kopf, Tujunga, CA, USA). The incision site was cleaned with Povidone solution and surgery was performed under aseptic conditions. Animals designated for lesions received infusions of the cholinergic immunotoxin 192 IgG-saporin (Chemicon International, Temecula, CA, USA; lot number 18070313, 0.21 µg/µL in Dulbecco's saline, 0.5 µL/hemisphere into the basal forebrain (from bregma: -0.8 mm, L ± 2.5 mm, V -7.2 mm; Paxinos &

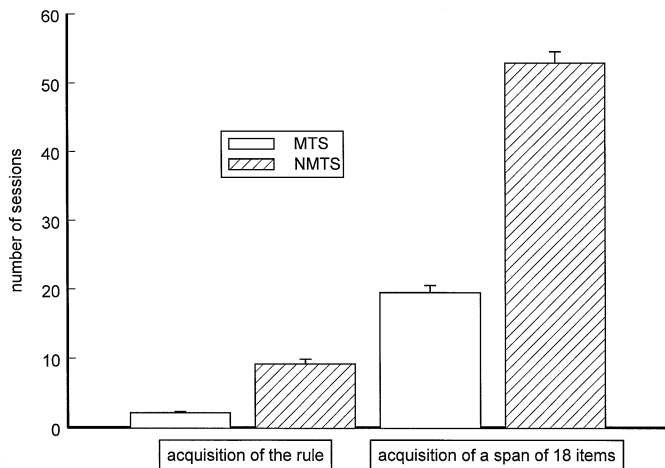


FIG. 2. Number of sessions ($M \pm SEM$) required by the animals to acquire the nonmatching-to-sample (NMTS) and matching-to-sample (MTS) task. The left pair of bars depicts the number of trials until the animals acquired the basic NMTS and MTS task rules, defined as the correct selection of the novel (NMTS) or previous (MTS) stimulus following the presentation of a second cup/stimulus. The right pair of bars depicts the number of sessions required to achieve the entire span of 18 items for the first time (see Methods).

Watson, 1986). Control animals received infusions of Dulbecco's saline (0.5 $\mu\text{L}/\text{hemisphere}$). Infusions for both groups of animals were made with a 1- μL Hamilton syringe, which was left in place for 2 min after each bolus injection. Postoperative care included wound treatment with lidocaine ointment, a prophylactic dose of amoxicillin (1 mL/kg, s.c.) and a 7-day recovery period with food and water available *ad libitum*. Upon conclusion of this recovery period, animals were returned to behavioural training in the final task.

Histological procedure

After completion of postsurgical behavioural training, rats were anaesthetized and transcardially perfused with 0.9% buffered saline (pH 7.2–7.4), followed by 10% formalin (v/v solution). Perfused brains remained in formalin for 4 h and were then placed in 30% sucrose phosphate buffer (w/v solution) for cryoprotection. Brain sections (40 μm) were processed to define acetyl cholinesterase (AChE)-positive fibres (Tago *et al.*, 1986). The validity of AChE-positive fibres as a method for revealing cortical cholinergic axons in rats has been frequently demonstrated. Exceptions include the medial prefrontal, cingulate and retrosplenial areas in which this method stains for AChE not associated with cholinergic neurotransmission (Lysakowski *et al.*, 1989; see also Mesulam & Geula, 1992 for the validity of this method in the human brain). Initially, sections were placed in 0.1% H_2O_2 for 30 min then rinsed in 0.1 M maleate buffer (pH 6.0) prior to placement in the first incubation medium. Sections were incubated for 60 min in a solution composed of 5 mg acetylthiocholine, 0.5 mL of 1.0 mL 5 mM potassium ferricyanide in 200 mL maleate buffer (pH 6.0). Rinses in 50 mM Tris buffer (pH 7.6) preceded placement of the sections in a second incubation medium, comprised of 0.05 g diaminobenzidine and 3.75 g nickel ammonium sulphate in 125 mL of 50 mM Tris buffer (solution pH = 6.4). Following 10 min of incubation, 12 drops of 0.1% H_2O_2 were added to this solution and sections remained for 12 min prior to final rinsing in 5 mM Tris buffer. Sections were subsequently mounted on gelatine-coated, air dried, dehydrated in ethanol and placed in xylene prior to coverslipping. Parallel sections from the

area of the infusion of 192 IgG-saporin into the basal forebrain were stained with cresyl violet for inspection of nonspecific damage.

Quantification of AChE-positive fibres

Employing procedures described in previous work (Holley *et al.*, 1994; McGaughy *et al.*, 1996), the relative cortical cholinergic fibre loss produced by the 192 IgG-saporin administration was quantified. Briefly, a focusing magnifier in a Vanox Olympus Research Microscope (Model AHBT; $\times 25$ magnification) provided the superimposed image of four orthogonal double cross-lines over each area of cortex to be quantified. AChE-positive fibres which crossed these four lines were then counted. Fibre counts were taken from the somatosensory cortex (area 3b; layers II–IV and V, respectively) and the motor cortex (area 4; layers II–IV and V). The background stain in the pyriform cortex and the olfactory bulb was too dark to allow a reliable count of fibres; therefore, the density of the stain in these areas was estimated measuring the exposure time (s) required to photograph this area (at a magnification of $\times 50$ and using the spot-metering mode of the microscope; 100 ASA; exposure adjustment, 1; reciprocity compensation factor, 0; see also Sarter & Dudchenko, 1991). Two measures from the pyriform cortex were taken from layers II and III/IV, respectively. As the olfactory bulb (OB) receives projections both from specific portions of the horizontal limb of the diagonal band of Broca as well as the sublenticular substantia innominata (Záborszky *et al.*, 1986; Gaykema *et al.*, 1990), density estimates were taken from the internal plexiform layer and internal granular layer of the OB. Two counts per hemisphere and per area were taken from two different sections per brain.

Statistical analyses

For the olfactory span task, the following measures were recorded per test session: the olfactory span of the animal (defined as the number of stimuli present on the platform, excluding the initial sample stimulus, for the last error-free trial completed by the animal), the total number of errors per trial (defined as any attempt, either via forepaw or nose, to obtain the reinforcement in a nonbaited cup), and the specific spice of each error. On rare occasions, an animal might fail to perform a trial due to excessive waste production/grooming, and/or climbing activity over the cups; on these rare occasions, this behaviour was recorded as an omission. However, given that animals had, during these instances, tended to inspect all of the stimuli present on the platform, thereby presumably updating their spans, animals were allowed to proceed, often without further error, to the next trial. The rarity of these events precluded meaningful analysis of omission effects. Intertrial interval length was also noted on various test sessions within the acquisition phase, as well as comments regarding the rat's behaviour during the session throughout the course of the experiment. The impact of surgery upon behavioural measures was examined by comparison of the average performance for 7 days prior to surgery with the performance of days 1–7, 8–14, 15–21, and 22–28 postsurgery, as maximal decreases in choline acetyltransferase occur approximately 2 weeks after the administration of 192 IgG-saporin (Waite *et al.*, 1994). Any impairments of performance observed in the first 2 weeks of postoperative training were resolved to preoperative levels of performance by the fourth week of postoperative training for all groups of animals (see Results section). This recovery of preoperative olfactory spans indicated the utility of an additional test, the 4-min intertrial delay insertion, to assess potential long-term effects of the lesion manipulation upon task performance.

The effects of lesion upon olfactory span was assessed using repeated measures ANOVA, with the within-subjects factor of period

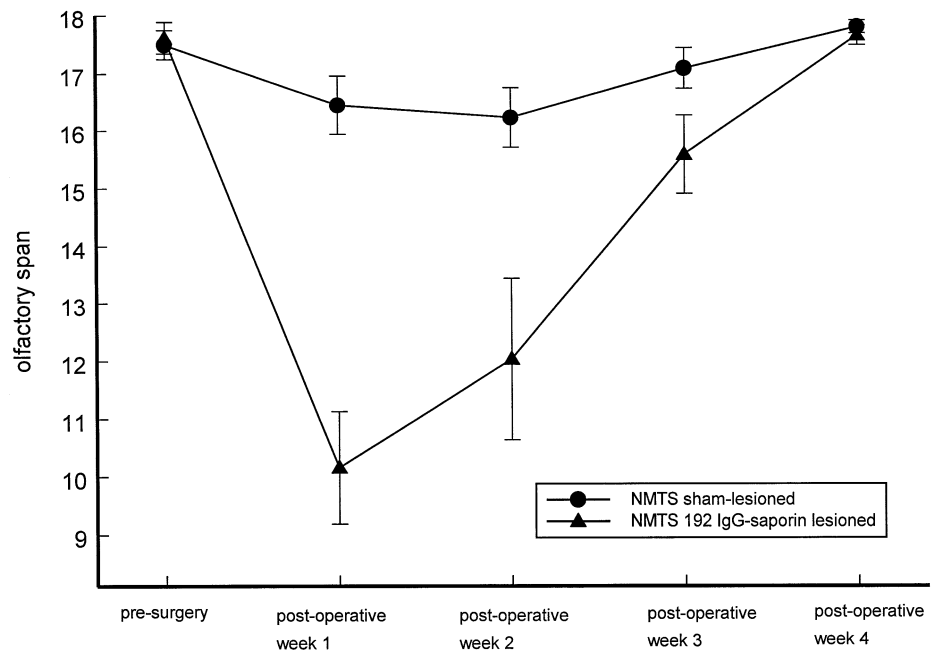


FIG. 3. Nonmatching-to-sample (NMTS) animals' pre- and postoperative performance in the olfactory span task. The 192 IgG-saporin-induced loss of basal forebrain cholinergic neurons significantly, albeit transiently, decreased the span of NMTS animals. Compared with sham-lesioned animals, the lesions did not affect matching-to-sample (MTS) performance (not shown).

(five levels) and the between-subjects factor of lesion type. One-way ANOVA's were conducted to reveal the locus of significant effects. The Friedman test was performed for nonparametric analysis of the distribution pattern of errors exhibited in the first 2 weeks of postoperative training; the number of erroneous digs each animal made within three stimulus clusters (stimuli 1–6, 7–12, and 13–18) within a given session were collapsed per animal for postoperative training days 1–14. Analysis examining the possibility of a differential pattern of erroneous digs was then conducted for each of the four groups separately; this test ranks the test variables (here the collapsed number of errors per stimulus cluster) and then reveals if at least one of the variables differs from the others. For all analyses, exact P -values are provided, as advocated by Greenwald *et al.* (1996). Relative amounts of cortical cholinergic fibres present after surgery, as revealed by the histological analysis (discussed earlier), were correlated with the behavioural data using Pearson's correlation coefficient. AChE-positive fibre counts were taken in frontoparietal, and pyriform cortices, as well as olfactory bulbs, and correlated with the average postsurgical olfactory spans for weeks 1 and 2. Additionally, ranked data indicating the relative magnitude of cholinergic fibre density per rat in each group (MTS or NMTS) were correlated to this measure of postoperative olfactory span using Spearman's rho (ρ). All statistical analyses were performed using SPSS-PC version 8.0 (SPSS, Chicago, IL, USA).

Results

Behavioural observations

Throughout the course of the experiment, and as corroborated by the control probe trials discussed below, the animals exhibited behaviour consistent with the use of olfactory discrimination to solve the olfactory span task, namely explicit sniffing of the stimuli present upon the platform. While MTS trained animals often located the correct (initial sample) stimulus without updating all of the stimulus scents per trial, NMTS animals, presumably reflecting the more effort-intensive processing required to perform

this version of the task, would consistently sniff each stimulus cup to update the entire list per trial and then choose the cup in which to dig. Nowhere was this distinction more evident than during the 4 min intertrial delay interposition between trials 15 and 16; the MTS animals spent little time examining the range of scent cups present on the platform after finding the correct cup, whereas the NMTS animals spent considerable time (even up to 4 min in one case) sniffing each stimulus cup multiple times before choosing to dig (see Discussion).

Olfactory span

As the maximal olfactory span for both tasks was capped at 18 (see Methods), there were no significant differences of preoperative performance level between the groups ($P > 0.1$ for all). However, lesions of the cholinergic neurons of the basal forebrain differentially affected the performance of MTS and NMTS animals. The lesion resulted in a significant decrease in the olfactory span of NMTS animals ($F_{1,10} = 21.225$; $P = 0.001$), with sham-lesioned animals' performance being greater than that of 192 IgG-saporin-lesioned animals for postoperative weeks 1 and 2 ($F_{1,11} = 32.609$; $P < 0.001$ and $F_{1,11} = 7.651$; $P = 0.020$, respectively; see Fig. 3).

Lesions of the basal forebrain, when compared to sham lesions, did not affect the span of MTS animals ($F_{1,10} = 0.297$; $P = 0.882$). The surgical manipulation *per se* produced an initial decrease in the span in both sham-lesioned and lesioned animals of this group ($F_{1,4} = 11.567$; $P < 0.001$) which, however, recovered by the second postoperative week (MTS sham-lesioned preoperative span, 17.47 ± 0.26 ; postoperative span week 1, 14.57 ± 1.6 ; postoperative span week 2, 17.50 ± 0.34 . MTS 192 IgG-saporin-lesioned preoperative span, 17.92 ± 0.07 ; postoperative span week 1, 13.85 ± 1.05 ; postoperative span week 2, 17.61 ± 0.32).

Error analyses

The first 2 weeks of postoperative training were examined to assess whether a particular pattern was present in the error distribution. Erroneous digs in stimulus items 1–6, 7–12, and 13–18 were collapsed and the potential for a differential pattern in the number of

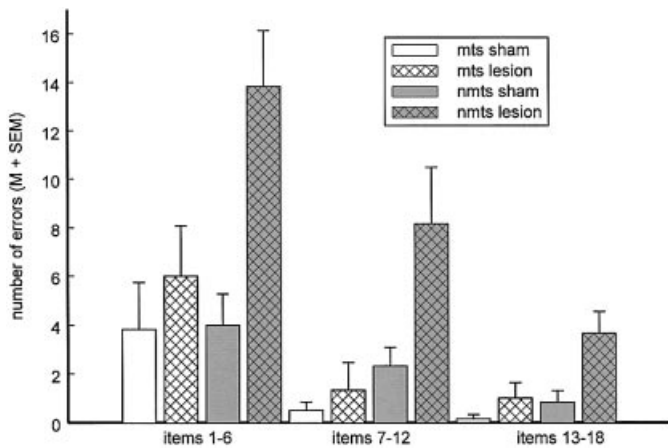


FIG. 4. Distribution pattern of the number of errors ($M \pm SEM$) exhibited by each group, representing data derived from the first 2 weeks of postoperative training. While neither sham-lesioned group (MTS or NMTS) exhibited a pattern to their erroneous digs, 192 IgG-saporin-lesioned animals in both the NMTS and MTS version of the task demonstrated a significant preponderance of erroneous digs for the first six stimuli of a given session.

errors among these three clusters of items was analysed for each group of animals using the Friedman test. Sham-lesioned animals of either task type, MTS or NMTS, did not exhibit significant differences between the number of errors of the three clusters of items ($\chi^2 = 4.353$, d.f. = 2, $P = 0.113$ and $\chi^2 = 3.391$, d.f. = 2, $P = 0.183$, respectively). A differential distribution of errors was found, however, for all 192 IgG-saporin-lesioned animals; each group revealed a greater proclivity for erroneous digs for the six initial stimulus scents of a given session (MTS: $\chi^2 = 6.381$, d.f. = 2, $P = 0.041$; NMTS: $\chi^2 = 11.000$, d.f. = 2, $P = 0.004$; see Fig. 4).

Effects of intertrial interval delay

As the effects of lesion upon NMTS olfactory span were ultimately resolved to preoperative levels by the fourth week of postoperative training for all animals, an additional test was administered to examine the possibility of longer-lasting ramifications of this surgical manipulation. The normal intertrial interval during testing sessions was approximately 40–45 s for the higher stimulus number sequences (13 and greater stimuli on the table; see Methods). In the fifth week of postoperative training, animals were presented with a 4-min delay insertion for one test session between the trials with 15 and 16 stimuli present on the platform. This test was repeated once for an additional session following a minimum of one session of preoperative span performance level. The span of lesioned MTS and sham-lesioned MTS and NMTS animals was unaffected by this delay and these animals performed the subsequent trials without error. In contrast, the delay resulted in an increased error rate in lesioned NMTS animals. Averaged over the two test sessions in which the effects of the 4-min delay were assessed and over the four remaining trials after insertion of this delay, these animals committed 2.0 ± 0.44 errors. The errors of these animals were not consistently restricted to the first trial following this delay but equally distributed over the four remaining trials following the 4 min delay.

Performance in control probe trials

Probe trials were conducted in the fifth week of postoperative data collection to assess whether rats in either task type were using means other than olfactory discrimination among the stimulus scents to

perform the task. When the reward was omitted for a given trial, all animals performed accurately irrespective of task type. Likewise, substitution of an entire set of clean cups between the trials containing 13 and 14 stimuli on the platform failed to elicit erroneous digs for any animal.

Histological findings and correlations between span and AChE-positive fibre density

Fibre counts from the motor cortex and somatosensory cortex (see Table 1) indicate the extent of the cortical cholinergic fibre loss. On average, the lesion reduced the fibre density in the motor cortex and somatosensory cortex by 80% and 76% (respectively; see Fig. 5). Relatively smaller decreases in AChE-positive fibre density were observed in the pyriform cortex (35%) and in the OB (24%). There was a strong trend for the lesion-induced decrease in fibre density to be greater in MTS animals when compared with NMTS animals for motor cortex (Mann–Whitney U -test; $U = 6.00$; $P = 0.054$) but there was no significant difference between these groups for somatosensory cortex ($U = 13.00$; $P = 0.423$). The AChE-positive fibre density estimates from the pyriform cortex and OB did not differ between the two groups of lesioned animals ($P > 0.86$ for both).

Analyses of the potential relationships between the postoperative olfactory span performance and fibre density data were based on the ranked mean olfactory spans from postoperative weeks 1 and 2 and the ranked means of the fibre counts from areas 3 and 4 (averaged over layers and hemispheres; see Methods; see Table 1). The data from areas 3 and 4 were collapsed as they correlated highly over all four groups of animals (Pearson's correlation coefficient; $r = 0.949$; $P < 0.001$).

For the NMTS animals, the relative number of extant cortical cholinergic fibres in areas 3 and 4 (collapsed) and 51a following the surgical manipulation correlated significantly with the level of postoperative olfactory span performance for both weeks 1 and 2 (Spearman's correlation coefficient, areas 3 and 4: week 1, $\rho = 0.893$, $P < 0.001$; week 2, $\rho = 0.671$, $P = 0.017$; area 51a; week 1, $\rho = 0.784$, $P = 0.003$, week 2, $\rho = 0.702$, $P = 0.011$). The data from the MTS animals did not exhibit any correlation between AChE-positive fibre density in these same cortical areas and postoperative performance levels for either week ($P > 0.33$ for all). Additionally, AChE-positive fibre density in the OB did not correlate with postoperative performance levels for either group of animals ($P > 0.20$ for all).

Discussion

Infusions of 192 IgG-saporin into the basal forebrain in animals performing a NMTS olfactory span task resulted in a robust but transient decrease in span performance. The performance of animals in a MTS version of this task was unaffected by the lesion. Following recovery of the postoperative NMTS performance, an increase in the intertrial interval impaired these animals' performance but remained without effect in sham-lesioned NMTS animals or in MTS animals. The span of NMTS animals correlated significantly with the density of AChE-positive fibres in neocortical but not in olfactory regions. Finally, lesioned animals exhibited a recency effect, as indicated by the finding that erroneous digs for stimuli presented early were more frequent than erroneous digs for stimuli presented in either in the middle or latter portion of the list. The discussion below focuses on the validity of the data generated by the task used in this experiment, the cognitive nature of the lesion-induced impairment in span, the reasons for the transient effect of the lesions, and the significance of

TABLE 1. Postoperative olfactory span and cortical AChE-positive fibre density

Group/ Animal ID	Postoperative olfactory span (number of odours)			AChE-positive fibre density in cortex (%)				
	Week 1	Week 2	(Mean 1 + 2)	Motor (area 4)*	Somatosensory (area 3)*	(Mean of areas 3 + 4)	Pyriiform (area 51a)†	Olfactory bulb‡
MTS sham								
Pi4	12.9 (3.5)	17.0 (2)	14.9 (4)	20.5 (6)	17.3 (6)	18.9 (6)	0.14 (4)	0.17 (1.5)
Pi7	6.4 (4)	14.9 (5)	10.6 (5)	34.8 (5)	26.4 (5)	30.6 (5)	0.09 (6)	0.15 (4)
Pi9	12.9 (3.5)	16.4 (4)	14.6 (6)	43.4 (1)	38.9 (1)	41.1 (1)	0.17 (1)	0.15 (4)
Pu7	17.0 (1.5)	16.7 (3)	16.8 (2)	37.3 (3)	35.9 (3)	36.6 (3)	0.16 (2)	0.17 (1.5)
Pu9	17.0 (1.5)	17.0 (2)	17.0 (1)	40.0 (2)	38.3 (2)	39.1 (2)	0.15 (3)	0.15 (4)
Pi2	15.3 (2)	17.0 (2)	16.1 (3)	34.9 (4)	31.5 (4)	33.2 (4)	0.13 (5)	0.14 (6)
Mean ± SD	13.5 ± 3.9	16.5 ± 0.8	15.0 ± 2.3	35.2 ± 7.9	31.4 ± 8.3	33.2 ± 8.0	0.14 ± 0.03	0.16 ± 0.01
MTS-lesioned								
Pi6	9.9 (6)	17.0 (2)	13.4 (5)	19.0 (1)	9.1 (3)	14.0 (1)	0.08 (5)	0.23 (1)
Pi8	14.1 (2)	17.0 (2)	15.5 (2)	5.0 (3.5)	11.1 (1)	8.3 (2)	0.10 (3)	0.12 (4)
Pu2	13.3 (3)	17.0 (2)	15.1 (3)	0.6 (5)	10.0 (2)	5.3 (3)	0.11 (2)	0.10 (5)
Pu4	10.6 (5)	15.0 (6)	12.8 (6)	0.1 (6)	3.9 (4)	2.0 (6)	0.13 (1)	0.16 (2)
Pu8	12.3 (4)	17.0 (2)	14.6 (4)	5.0 (3.5)	0.3 (6)	2.6 (5)	0.09 (4)	0.13 (3)
Pi3	17.0 (1)	16.7 (5)	16.8 (1)	5.1 (2)	3.2 (5)	4.1 (4)	0.03 (6)	0.09 (6)
Mean ± SD	12.8 ± 2.6	16.6 ± 0.8	14.7 ± 1.4	5.8 ± 6.9	6.3 ± 4.4	6.0 ± 4.4	0.09 ± 0.03	0.13 ± 0.05
NMTS sham								
Pi10	13.7 (6)	15.7 (3)	14.7 (5)	34.4 (3)	32.8 (4)	33.6 (5)	0.18 (1)	0.13 (5)
Pu6	14.6 (5)	16.7 (1)	15.6 (2.5)	28.8 (6)	29.7 (6)	29.2 (6)	0.11 (5.5)	0.27 (1)
B1	15.6 (3)	13.4 (6)	14.5 (6)	35.0 (2)	32.5 (5)	33.7 (4)	0.12 (4)	0.20 (2.5)
Re3	15.1 (4)	16.4 (2)	15.7 (4)	31.3 (5)	36.3 (2)	33.8 (3)	0.11 (5.5)	0.20 (2.5)
Re4	16.9 (1.5)	14.9 (4)	15.9 (1)	33.4 (4)	35.4 (3)	34.3 (2)	0.16 (2)	0.19 (4)
Re5	16.9 (1.5)	14.3 (5)	15.6 (2.5)	37.7 (1)	37.2 (1)	37.4 (1)	0.13 (3)	0.11 (6)
Mean ± SD	15.5 ± 1.3	15.2 ± 1.3	15.3 ± 0.5	33.4 ± 3.1	34.0 ± 2.8	33.6 ± 2.6	0.14 ± 0.03	0.18 ± 0.06
NMTS-lesioned								
B1	11.6 (1)	9.0 (5)	10.3 (4.5)	7.3 (2)	7.3 (4)	7.3 (4)	0.09 (3)	0.08 (5.5)
Re2	5.4 (6)	5.3 (6)	5.3 (6)	6.3 (5)	1.4 (6)	3.8 (6)	0.08 (5)	0.28 (1)
B3	11.4 (2)	14.1 (1)	12.7 (1)	6.4 (4)	10.9 (2)	8.6 (2)	0.07 (6)	0.13 (2)
B14	8.9 (4)	13.9 (2)	11.4 (2)	6.6 (3)	10.1 (3)	8.3 (3)	0.09 (3)	0.10 (4)
B15	7.6 (5)	13.4 (3)	10.5 (3)	5.3 (6)	5.9 (5)	5.6 (5)	0.09 (3)	0.08 (5.5)
Pi5	10.1 (3)	10.6 (4)	10.3 (4.5)	14.1 (1)	21.0 (1)	17.5 (1)	0.11 (1)	0.12 (3)
Mean ± SD	9.2 ± 2.4	11.1 ± 3.5	10.1 ± 2.5	7.7 ± 3.2	9.4 ± 6.6	8.5 ± 4.7	0.09 ± 0.01	0.13 ± 0.08

*AChE-positive fibre counts averaged over the two counts taken from layers II–IV and V and over both hemispheres. †AChE stain density as measured by exposure time (s; see Methods). Data indicate averages over the two measures taken from layers II and III/IV over both hemispheres. ‡AChE stain density as measured by exposure time (s; see Methods). Data indicate averages over the two measures taken from the internal plexiform layer and the internal granular layer of the olfactory bulb (OB) of both hemispheres. Data in parenthesis indicate the ranks within the group (highest value, rank 1).

these findings with respect to current hypotheses about the functions of cortical cholinergic inputs.

As explained in the Methods section, the maximal number of olfactory stimuli presented to the animals had to be capped; therefore the present experiment was not designed to determine the maximal olfactory span of intact rats. Our data, similar to the findings by Dudchenko *et al.* (2000), suggest that this span may vary around 25 items. The fact that the effects of the lesion were not tested in interaction with the maximal olfactory span demands caution in interpreting the transient nature of the lesion effect, as the present results may not validly predict and possibly underestimate the consequences of such lesions in animals exhibiting maximal spans. However, as the lesion did not affect MTS animals, and as the performance of lesioned NMTS animals remained susceptible to increased intertrial intervals, the present data allow the conservative conclusion that the 192 IgG-saporin lesion at least transiently impaired olfactory span performance.

Collectively, the lack of effects of the probe trials, combined with the spatial re-arrangement of the stimuli prior to each trial and the selective effects of the lesion on NMTS performance, do not suggest that the animals were able to resolve the NMTS task by means other than identifying the lastly added item by comparing that item with the (growing) list of scents experienced thus far. As described in the

Results section, the systematic scanning of all olfactory stimuli in advanced trials by NMTS but not MTS animals suggests that these animals used each trial to update their lists. Importantly, this behaviour was also observed in trials in which the target item was coincidentally identified early. MTS animals could resolve the task solely by recalling the initial target stimulus and thus did not show this behaviour, reliably terminating exploration of other cups once they found the original stimulus of that session. Any speculation about alternative, noncognitive mechanisms mediating the performance of NMTS animals would need to explain the slower acquisition of the NMTS task, the selective lesion effect, and the selective and persistent vulnerability of lesioned NMTS animals to increases in intertrial length.

The distribution of errors performed by the lesioned rats indicated a recency effect (see Fig. 4). We might speculate this finding indicates that, as a result of the lesion, these animals more frequently identified an item that was added to the list early in the session erroneously as the new, lastly added item. Descriptively, the data shown in Fig. 4 suggest that the error distribution for all animals indicated a trend for a recency effect that was robustly augmented by the lesion in NMTS animals. Thus, the lesion may not have qualitatively altered the memory process but rather further limited the performance in trials with a relatively large number of items.

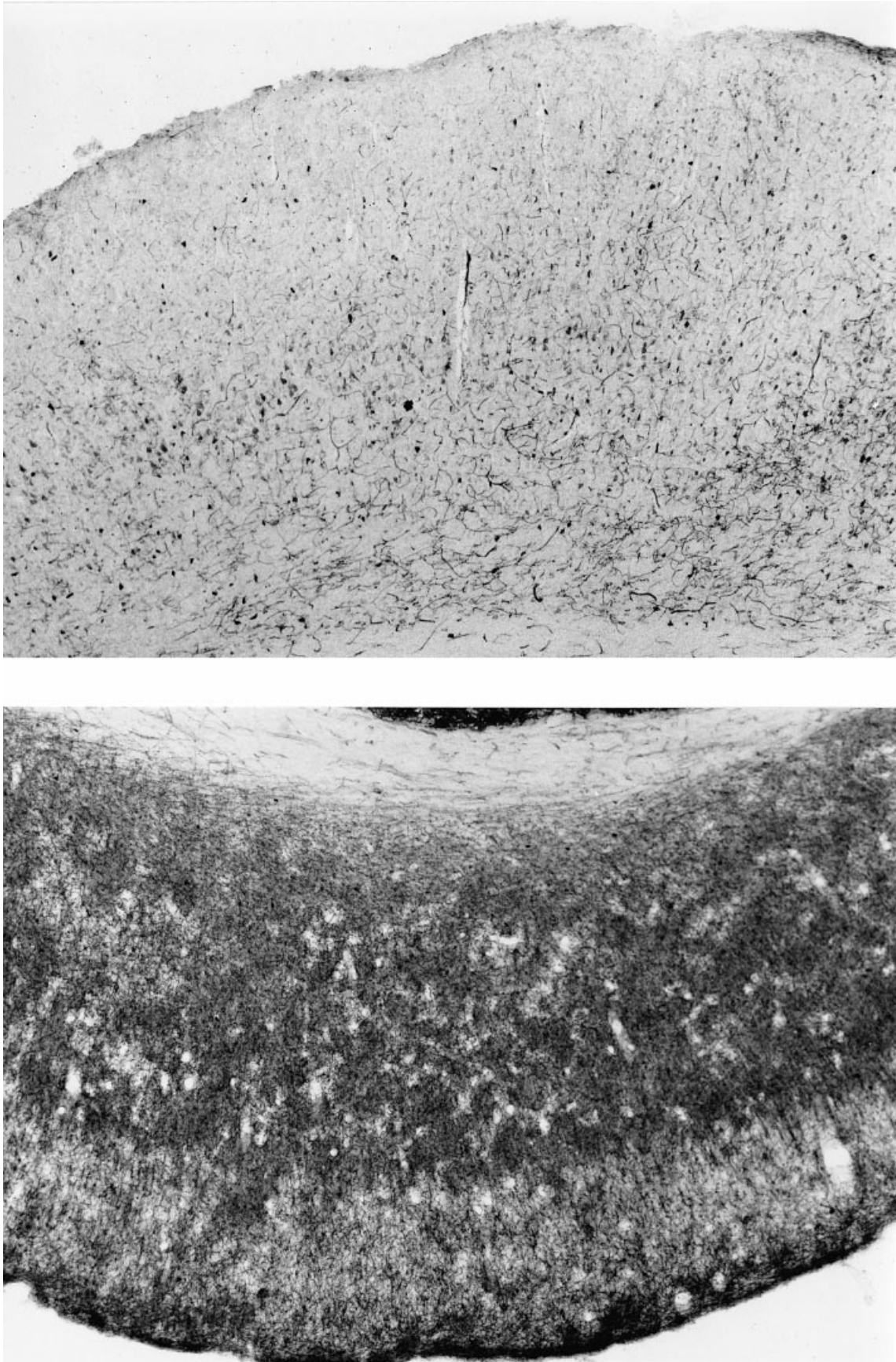


FIG. 5. Microphotographs of the somatosensory cortex (area 3; magnification $\times 11.5$) from a sham-lesioned (left) and a lesioned (right) rat (AChE-positive fibre stain; coronal sections). The left section is from matching-to-sample (MTS) sham-lesioned rat P19 (see Table 1). The loss of AChE-positive fibres in the right microphotograph (rat P15; Table 1) is evident, particularly in higher layers. Residual fibres are mostly present in layers V/VI. The AChE-positive fibre density in this area, motor cortex and pyriform cortex, but not in the olfactory bulb, correlated significantly with the olfactory span performance for postoperative weeks 1 and 2 in nonmatching-to-sample (NMTS), but not in MTS animals (see Results).

Recency effects have been consistently observed in humans and animals (e.g. Roberts & Smythie, 1979; Capatini *et al.*, 1992). The lesion-induced recency effect in NMTS animals suggests that the lesion resulted in a decay of the memory for early items (see also the effects of large excitotoxic lesions in Kesner *et al.*, 1987), presumably by limiting the attentional capacity available for maintaining long lists of items in store.

The transient effect of the lesion on NMTS span performance may be related to the particular nature of olfactory memory in rats, as well as the resilience of olfactory discrimination functions in rodents following insult to the olfactory system (see Yee & Costanzo, 1995). Importantly, while direct muscarinic antagonism in the OB has been shown to impair short-term olfactory memory in rats (Ravel *et al.*, 1994), similar cholinergic and even larger lesions fail to disrupt basic olfactory recognition performance and short-term memory (Wirth *et al.*, 2000). The absence of correlations between decreases in AChE-positive fibre loss in the OB and pyriform cortex and span performance in the present study supports the view that the lesion effects were not due to disruption in fundamental olfactory processes. Likewise, probe trials and analyses of the differences in the discriminability of target stimuli (not reported as such differences were not found) did not indicate any lesion-induced impairment in basic olfactory discriminative abilities. As the present results are hypothesized to reflect the lesion-induced limitations in attentional capacities, the explanation of the transient nature of the lesion effect relates to the attentional demands for olfactory discrimination performance in rats. In rats, olfactory memory may be more 'promiscuous in its accessibility by novel routes of expression' (Eichenbaum, 1998; p. 665) than other types of (declarative) information. Thus, the special, privileged status of olfactory information processing that is reflected by the direct olfactory-hippocampal pathway and extraordinary learning capacities (see also May Lu *et al.*, 1993) may be associated with a relative lack of vulnerability of olfactory memory to attentional determinants of memory. Reid & Morris (1993) criticized the idea that olfactory memory provides a 'privileged access to higher cognitive processes' by stressing the inflexibility of olfactory memory as revealed by poor reversal learning of olfactory stimulus-reward associations. Such findings reflect the highly 'automatic' nature of olfactory memory and its insensitivity to 'higher' cognitive processes, including limited attentional resources. In this context, an olfactory span task to assess the effects of cholinergic lesions on memory capacity may be less productive than, for example, tasks employing visual stimuli (McGaughy *et al.*, 1996). It is important to note, however, that even the fully recovered postoperative NMTS span performance remained vulnerable to the increased intertrial interval, indicating that such performance is not entirely 'protected' from the influence of 'higher' cognitive variables and that interactions between the length of the item list and increased retention intervals may reveal olfactory capacity limitations in lesioned rats (see also Roman *et al.*, 1993). In fact, it appears likely from this discussion, that a similar experiment which maximizes olfactory span performance would reveal a persistent span reduction.

The present data, at a minimum, demonstrate both a transiently decreased olfactory span and an increased error rate, following longer retention intervals for lesioned NMTS animals, as well as a pronounced recency effect in 192 IgG-saporin-lesioned animals of either task type. The span performance of MTS animals tested using the same stimuli and testing conditions, but which were not required to maintain a growing list of stimuli, remained unaffected by the lesion. Thus, the effects of the lesion are interpreted cautiously as due to an augmented decay in the memory for early items and, in the

context of evidence for impaired attentional capacities from other experiments (references above), are speculated to have resulted from the lesion-induced impairment in the attentional resources required for normal memory capacity (Cowan, 2001).

Acknowledgement

This research was supported by National Institutes of Health Grants NS32938 and AG10173.

Abbreviations

ACh, acetylcholine; AChE, acetylcholinesterase; IgG, immunoglobulin G; MTS, matching-to-sample stimuli; NMTS, nonmatching-to-sample stimuli; OB, olfactory bulb.

References

- Bucci, D.J., Holland, P.C. & Gallagher, M. (1998) Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *J. Neurosci.*, **18**, 8038–8046.
- Capatini, E., Della Sala, S., Logie, R.H. & Spinnler, H. (1992) Recency, primacy, and memory: reappraising and standardising the serial position curve. *Cortex*, **28**, 315–342.
- Cowan, N. (2001) The magical number 4 in short-term memory: a reconsideration of mental storage capacity. *Behav. Brain Sci.*, **24**, in press.
- Deacon, R.M.J. & Rawlins, J.N.P. (1995) Serial position effects and duration of memory for nonspatial stimuli in rats. *J. Exp Psychol. Anim. Behav. Proc.*, **21**, 285–292.
- Dudchenko, P.A., Wood, E.R. & Eichenbaum, H. (2000) Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant impairments on spatial span, recognition, and alteration. *J. Neurosci.*, **20**, 2964–2977.
- Eichenbaum, H. (1998) Using olfaction to study memory. *Ann. NY Acad. Sci.*, **855**, 657–669.
- Everitt, B.J. & Robbins, T.W. (1997) Central cholinergic systems and cognition. *Annu. Rev. Psychology*, **48**, 649–684.
- Gaffan, E.A. (1992) Primacy, recency, and the variability of data in studies of animals' working memory. *Anim. Behav.*, **20**, 240–252.
- Gaykema, R.P.A., Luiten, P.G.M., Nyakas, C. & Traber, J. (1990) Cortical projection patterns of the medial septum-diagonal band complex. *J. Comp. Neurol.*, **293**, 103–124.
- Gill, T.M., Sarter, M. & Givens, B. (2000) Sustained visual attentional performance-associated prefrontal neuronal activity: Evidence for cholinergic modulation. *J. Neurosci.*, **20**, 4745–4757.
- Greenwald, A.G., Gonzales, R., Harris, R.J. & Guthrie, D. (1996) Effects sizes and *P*-values: what should be reported and what should be replicated? *Psychophysiology*, **33**, 175–183.
- Heckers, S. & Mesulam, M.-M. (1994) Two types of cholinergic projections to the rat amygdala. *Neuroscience*, **60**, 383–397.
- Himmelheber, A.M., Bruno, J.P. & Sarter, M. (2000) Increases in cortical acetylcholine release during sustained attention performance in rats. *Cogn. Brain Res.*, **9**, 313–325.
- Holley, L.A., Wiley, R.G., Lappi, D.A. & Sarter, M. (1994) Cortical cholinergic deafferentation following the intracortical infusions of 192 IgG-saporin: a quantitative histochemical study. *Brain Res.*, **663**, 277–286.
- Kesner, R.P., Adelstein, T. & Crutcher, K.A. (1987) Rats with nucleus basalis magnocellularis lesions mimic mnemonic symptomatology observed in patients with dementia of the Alzheimer's type. *Behav. Neurosci.*, **101**, 451–456.
- Lysakowski, A., Wainer, B.H., Bruce, G. & Hersh, L.B. (1989) An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. *Neuroscience*, **23**, 291–336.
- May Lu, X., Slotnick, B.M., & Silberberg, A.M. (1993) Odor matching and odor memory in the rat. *Physiol. Behav.*, **53**, 795–804.
- McGaughy, J., Everitt, B.J., Robbins, T.W. & Sarter, M. (2000) The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins. *Behav. Brain Res.*, **115**, 251–263.
- McGaughy, J., Kaiser, T. & Sarter, M. (1996) Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the

- behavioral impairment in relation to cortical, AChE-positive fiber density. *Behav. Neurosci.*, **110**, 247–265.
- McGaughy, J. & Sarter, M. (1998) Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG 7142. *Behav. Neurosci.*, **112**, 1519–1525.
- McGaughy, J., Turchi, J. & Sarter, M. (1994) Crossmodal divided attention in rats: effects of chlordiazepoxide and scopolamine. *Psychopharmacology*, **115**, 213–220.
- Mesulam, M.-M. & Geula, C. (1992) Overlap between acetylcholinesterase-rich and choline acetyltransferase-positive (cholinergic) axons in human cerebral cortex. *Brain Res.*, **577**, 112–120.
- Miller, G.A. (1956) The magical number seven, plus or minus two: some limits on our capacity for processing information. *Psychol. Rev.*, **63**, 81–97.
- Mumby, D.G., Kornecook, T.J., Wood, E.R. & Pinel, J.P. (1995) The role of experimenter-odor cues in the performance of object-memory tasks by rats. *Anim. Learn. Behav.*, **23**, 447–453.
- Paxinos, G. & Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Sydney.
- Ravel, N., Elaagouby, A. & Gervais, R. (1994) Scopolamine injection into the olfactory bulb impairs short-term olfactory memory in rats. *Behav. Neurosci.*, **108**, 317–324.
- Reid, I.C. & Morris, R.G.M. (1993) The enigma of olfactory learning. *Trends Neurosci.*, **16**, 17–20.
- Roberts, W.A. & Kraemer, P.J. (1981) Recognition memory for lists of visual stimuli in monkeys and humans. *Anim. Learn. Mem.*, **9**, 587–594.
- Roberts, W.A. & Smythie, W.E. (1979) Memory for lists of spatial events in the rat. *Learn. Motiv.*, **10**, 313–336.
- Roman, F.S., Simonetto, I. & Soumireu-Mourat, B. (1993) Learning and memory of odor–reward association: selective impairment following horizontal diagonal band lesions. *Behav. Neurosci.*, **107**, 72–81.
- Sarter, M. & Bruno, J.P. (1997) Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. *Brain Res. Rev.*, **23**, 28–46.
- Sarter, M. & Dudchenko, P. (1991) Dissociative effects of ibotenic acid and quisqualic acid-induced basal forebrain lesions on cortical acetylcholinesterase-positive fiber density and cytochrome oxidase activity. *Neuroscience*, **41**, 729–738.
- Steele, K. & Rawlins, J.N.P. (1989) Rats remember long lists of nonspatial items. *Psychobiology*, **17**, 450–452.
- Tago, H., Kimura, H. & Maeda, T. (1986) Visualization of detailed acetylcholinesterase fiber and neuron staining in rats brain by a sensitive histochemical procedure. *J. Histochem. Cytochem.*, **34**, 1431–1438.
- Turchi, J. & Sarter, M. (1997) Cortical acetylcholine and processing capacity: effects of cortical cholinergic deafferentation on crossmodal divided attention in rats. *Cog. Brain Res.*, **6**, 147–158.
- Waite, J.J., Wardlow, M.L., Chen, A.C., Lappi, D.A., Wiley, R.G. & Thal, L.J. (1994) Time course in cholinergic and monoaminergic changes in rat brain after immunolesioning with 192 IgG-saporin. *Neurosci. Lett.*, **169**, 154–158.
- Wenk, G.L. (1997) The nucleus basalis magnocellularis cholinergic system: one hundred years of progress. *Neurobiol. Learn. Mem.*, **67**, 85–95.
- Wirth, S., Lehmann, O., Bertrand, F., Lazarus, C., Jeltsch, H. & Cassel, J.-C. (2000) Preserved olfactory short-term memory after combined cholinergic and serotonergic lesions using IgG-saporin and 5,7-dihydroxytryptamine in rats. *Neuroreport*, **11**, 347–350.
- Wright, A.A., Santiago, H.C., Sands, S.F., Kendrick, D.F. & Cook, R.G. (1985) Memory processing of serial lists by pigeons, monkeys, and people. *Science*, **229**, 287–289.
- Yee, K.K. & Costanzo, R.M. (1995) Restoration of olfactory mediated behavior after olfactory bulb deafferentation. *Physiol. Behav.*, **58**, 959–968.
- Záborszky, L., Carlsen, J., Brashear, H.R. & Heimer, L. (1986) Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis on the projection neurons in the nucleus of the horizontal limb of the diagonal band. *J. Comp. Neurol.*, **243**, 488–509.