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Effects of selective thalamic and prelimbic cortex lesions on two types of visual discrimination and reversal learning

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Abstract

The effects of excitotoxic lesions of the mediodorsal nucleus of the thalamus, the anterior thalamic nuclei and of the prelimbic cortex were examined on two tests of discrimination and reversal learning. In experiment 1A (visual discrimination and reversal), rats were required to discriminate two stimuli, and respond to the stimulus associated with reward (the S+ stimulus). There was no effect of lesion on acquisition of this task. However, when stimulus–reward contingencies were reversed, animals with lesions of the mediodorsal nucleus of the thalamus made significantly more errors than control animals or animals of other lesion groups. In experiment 1B (conditional discrimination), animals were required to learn a rule of the type 'If stimulus A then go left, if stimulus B then go right'. No main effect of lesion on acquisition was observed in this experiment. To test the generality of the reversal effect obtained in experiment 1A, a second cohort of animals with the same lesions was tested on acquisition of the visuospatial conditional task immediately postsurgery, followed by the reversal of the conditional rule (experiment 2). As in experiment 1B, no main effect of lesion group was observed during acquisition of the task. However, lesions of the mediodorsal nucleus of the thalamus resulted in a mild impairment according to number of sessions required to attain criterion performance of the task when the response rule was reversed. The results of the present study provide evidence for a role for the mediodorsal nucleus of the thalamus in new learning, particularly when stimulus–reward contingencies are reversed. Furthermore, they show that the functions of this thalamic nucleus can be dissociated from those of the anterior thalamus and the prelimbic cortex.

Introduction

Human patients with damage to the thalamus can present with a variety of behavioural impairments, including memory deficits (Winocur *et al*., 1984; Victor *et al*., 1989; Clarke *et al*., 1994), attentional deficits (Rafal & Posner, 1987) and impairments in response inhibition (Leng & Parkin, 1988; Joyce & Robbins, 1991; Forstl & Sahakian, 1993). It has been suggested that these deficits may be due to damage in distinct regions of the thalamus. However, the lack of neuropathological cases where damage is confined to selective thalamic nuclei [mediodorsal (MD) or anterior (ANT) thalamus], makes it difficult to determine the separate contributions of these structures (for review, see Bentivoglio *et al*., 1997).

Work conducted using the laboratory rat has examined the effects of discrete and histologically verifiable lesions in specific regions of the thalamus. Such studies have focused almost exclusively on the MD thalamus, and have yielded equivocal results. For example, whereas some authors have reported impairments following MD thalamic lesions in tests of spontaneous and reinforced alternation (Weiss & Means, 1980; Vicedomini *et al*., 1982), others have found no impairments on such tasks (Brito *et al*., 1982; Green & Naranjo, 1986; Hunt & Aggleton, 1991, 1998; Neave *et al*., 1993). Furthermore, in cases where positive results were obtained, it is difficult to determine the critical task factor. To take the above examples, the observed deficits could conceivably be due to a requirement for response inhibition, as the MD thalamus projects strongly and reciprocally to the prefrontal cortex, a region which is often thought to be involved in aspects of response inhibition (Cohen *et al*., 1996; Robbins, 1996). In addition, these tasks require the use of spatial information, and regions of the thalamus, most notably the anterior thalamus, have been identified with spatial processing (Aggleton *et al*., 1995, 1996). Finally, such deficits might arise because of animals' inability to associate stimuli with reward, as large thalamic lesions have been reported to impair stimulus–reward learning in the monkey (Gaffan & Murray, 1990).

In the present study, we attempted to disentangle these possibilities by examining the effects of lesions on nonspatial discrimination reversal learning. This method addresses the foregoing issues in several ways. First, reversal learning can be analysed in stages to reveal distinct processes during learning (Jones & Mishkin, 1972; Dias *et al*., 1996; Bussey *et al*., 1997). The number of errors committed during the early stages of a reversal, when the subject's performance is increasing from below chance levels, can give an indication of the subject's ability to extinguish the previous stimulus–reward association. Subjects with impaired response inhibition would be expected to be impaired at this stage of reversal. In contrast, the number of errors committed in the later stages of reversal, when the subject's performance is increasing to levels above chance, gives an indication of the subject's ability to acquire the new stimulus–reward association. Thus, response inhibition can be teased apart from stimulus–reward learning.

Second, in the present study, two types of reversal learning were examined: reversal of a stimulus–reward association, and reversal of a stimulus–response association (visuospatial conditional learning). This allowed us to test the generality of any observed effects. Third,

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the use of nonspatial stimuli removes the confound of spatial information. This may be particularly important when comparing the effects of MD and ANT thalamus lesions. Fourth, both MD and ANT thalamus lesions were included to examine the effects on learning of lesions in these two thalamic regions. Finally, lesions of the prelimbic cortex (PL) were included in the study as Bussey *et al*. (1997) found that frontal cortex lesions, including the prelimbic region, disrupted the later stimulus–reward learning stage of reversal learning. Both the medial and lateral portions of the MD thalamus project to the prelimbic cortex (Condé *et al*., 1990). It was therefore of interest to determine whether lesions of this cortical region will have similar effects to MD lesions on stimulus–reward reversal learning, as this would suggest that these structures interact as part of a fronto-thalamic circuit underlying this type of learning.

Materials and methods

Subjects

The subjects were male Lister Hooded rats (Harlan Olac, UK), housed in pairs in a temperature-controlled room (21 °C) under diurnal conditions (14-h light: 10-h dark). All testing occurred at a regular time during the light period. The animals were food deprived and maintained at 85% of their free feeding weight throughout the experiment with water available *ad libitum*.

Surgical procedures

Animals received neurotoxic lesions of the prelimbic cortex (PL), the mediodorsal thalamic nuclei (MD), the anterior thalamic nuclei (ANT) or sham control surgery (Sham). All animals were anaesthetized by intraperitoneal injection of Sagatal (0.1 mL/100 g) and placed in a Kopf stereotaxic headholder (David Kopf Instruments, Tujunga, CA, USA). The scalp was retracted to expose the skull and a craniotomy was made above the mid-sagittal sinus to expose the target region of the brain. The incisor bar was set at +5.0.

For lesions of the prelimbic cortex, animals received bilateral injections of 0.33 μL, 0.09 M *N*-methyl-D-aspartic acid (NMDA) dissolved in phosphate buffer (pH 7.0–7.2) using a 1-μL Hamilton syringe. The stereotaxic coordinates for the prelimbic cortex were as follows: AP +4.0 mm from Bregma; L ±0.8 mm from the midline; DV –3.5 mm below the dura; and AP +2.7 mm; L ±0.8 mm; DV –4.0 mm. Each injection was made over 2 min and allowed to remain for a further 2 min before the needle was retracted. Animals of the MD lesion group received 0.36 μL 0.12 M NMDA at the following injection sites: AP –1.8 mm from Bregma; L ±0.7 mm from the midline; DV –6.2 mm below the dura. Each injection was made over 2 min and allowed to remain for a further 3 min for dispersion. For lesions of the anterior thalamus, animals received bilateral injections of 0.2 μL of 0.12 M NMDA at the following injection sites: AP –0.2 mm from Bregma, L ± 1.0, DV –6.2; and AP –0.2 mm, L ± 2.0, DV –5.3. All injections were made over 2 min and a further 3 min was allowed for dispersion.

There were also surgical controls (Sham) for each of the three lesion types. Animals acting as Sham (PL) and Sham (MD) lesioned groups received the same surgical treatments except no infusion of NMDA was made. A pilot group of anterior thalamic lesions revealed occasional bilateral damage to the dentate gyrus of the hippocampus. In order to control for this additional brain damage, surgical controls for the anterior thalamic group received bilateral injections of 0.01 μL of 0.12 M NMDA at the following injection sites: AP –0.2 mm, L ± 1.0 and DV –3.0 mm.

Histology

At the conclusion of the behavioural testing the animals were perfused transcardially with 0.9% saline followed by 10% formal saline. After dehydration by immersion in 30% sucrose, the brains were sectioned on a freezing microtome at 60-μm thickness. Every second section was mounted on a glass slide and stained with cresyl violet. The sections were used to verify lesion placement and to assess the extent of lesion-induced neuronal loss.

Apparatus

Preliminary training and behavioural testing were carried out in four automated touch-screen testing chambers (see Bussey *et al*., 1994). The apparatus consisted of a testing chamber and a video display unit (VDU) housed within a sound-attenuating box. The box was fitted with a fan for ventilation and masking of extraneous noise. The inner chamber (48 × 30 × 30 cm) consisted of a metal frame, clear Perspex walls and an aluminium floor. A 3-W houselight and tone generator were attached to the ceiling of the chamber. Located centrally at the rear of the chamber was a food magazine attached to a pellet dispenser (Campden Instruments, Loughborough, UK) which was situated outside the box. A panel light (3 W) illuminated the food magazine and animals gained access to the magazine via a hinged Perspex panel door monitored by a microswitch. A pressure-sensitive area of the floor (14 × 10 cm) was located directly in front of the food magazine. This was monitored by a microswitch to detect the rats' presence when in that area of the testing chamber.

The stimuli were presented on the VDU screen which was located at the other end of the chamber. Surrounding the VDU was a 'touch-screen' attachment (Touch-tech 501, Microvitec, Bradford, UK) comprising an array of horizontally and vertically arranged photocells located ≈1 cm from the VDU screen. As a consequence, the rats were not required to make direct contact with the screen but were required to approach the stimuli closely using a nose-poke response. A black perspex 'mask' was attached to the face of the VDU ≈2 cm away from the surface of the display. This mask served to block access to the VDU display except through response windows that measured 6 × 8 cm. Each window was separated by black perspex dividers to prevent accidental approaches to the adjacent response window. A shelf extending 7 cm from the surface of the mask supported by springs (to prevent the animal climbing onto it) was positioned just beneath the response windows ≈15 cm from the floor of the chamber. The combined effects of the response windows and the shelf was to force the animal to stop, rear up and stretch towards the stimuli with a head-on approach, thus facilitating the rat's attention to the stimuli. For the visual discrimination task, the stimuli consisted of a white rectangle and a white cross that were equated for luminance (see Fig. 1A). The stimuli for the conditional discrimination task were coloured shapes (see Fig. 1B).

General behavioural procedures

Rats were initially shaped to collect food pellets from the food magazine. During the first session, pellets were placed in the magazine panel with the magazine flap open. Following this, the rats were trained to collect pellets that were delivered under a variable interval 40 s schedule, together with the illumination of the magazine light and presentation of the tone. Once the animals were reliably retrieving a minimum of 60 pellets per session from the magazine, they were trained to respond to stimuli presented on the VDU. During this procedure, a large yellow square was randomly presented in one of the response windows. The square remained on the screen until the rat responded to it after which the rat was rewarded with a pellet, tone

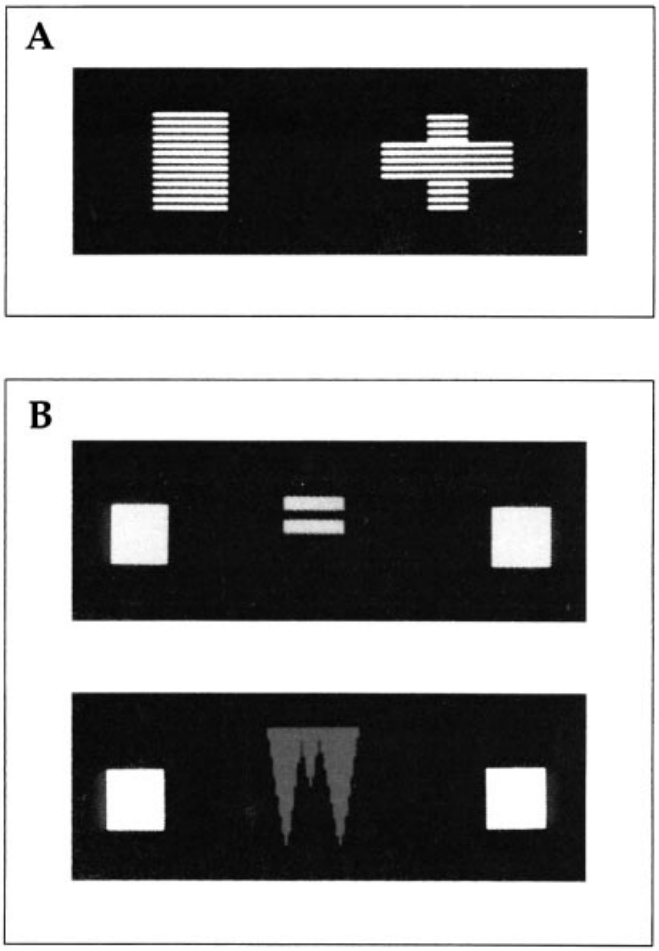


FIG. 1. Computer graphic stimuli. (A) Equiluminant rectangle and cross used in visual discrimination task. (B) Coloured shapes used in conditional discrimination task.

and magazine light. Once the rat was able to obtain 50 reinforcements within 20 min, it was moved onto the task.

In both behavioural tasks, each trial was initiated with the presentation of the stimuli, which were contingent upon the animal being located on the rear floor panel following a 5-s inter-trial interval (ITI). The rat was then required to approach the VDU screen and make a response by selecting a stimulus via a nose poke. Correct responses were followed by the disappearance of the stimuli and the presentation of a 45-mg Noyes reward pellet and a 1-s 4-kHz tone concomitant with the illumination of the food magazine. The next trial commenced once the rat had obtained its reward by pushing the magazine flap. This resulted in the food panel light being extinguished and the onset of the ITI. Incorrect responses resulted in the disappearance of the stimuli and the houselight being extinguished for a time-out period of 5 s. As part of a correction procedure, after an incorrect choice animals received the same stimulus configuration until the animals had responded correctly.

Performance measures

In addition to the number of sessions required to attain criterion performance, performance of the task was assessed using the following behavioural measures: (i) number of errors; (ii) average choice latency, which was the time from the onset of the choice stimuli to the time the rat made a nose-poke response to one of the choice stimuli; (iii) average magazine latency, which was the time from the correct nose poke to the time the rat entered the magazine to collect reward; and (iv) percentage response bias, which was the number of responses either to the right or left response window depending on a particular animal's bias, expressed as a percentage of total trials for that session. These measures were calculated on the basis of noncorrection trials only. In addition, a perseveration score was calculated, which was the number of responses to the same stimulus (in the same location) during correction trials. Thus, when there was a correction trial in place, the perseveration score was the number of correction trials minus 1 (i.e. subtracting the final response made by the animal when it produces the correct response). This perseveration score is the same as that used by Nonneman *et al.* (1974), in a study of the effects of prefrontal cortex lesions in rats. Note that in the present study, it cannot be determined whether the animal is repeating responses to the visual stimulus or the side on which it is presented. Thus, this measure is a general measure of perseveration.

Data were further analysed according to the method of Dias *et al.* (1996), based on a method used by Jones & Mishkin (1972). In this analysis, errors during reversal learning were broken down into two learning stages: errors committed before the attainment of chance-level performance (37% correct for 60 trials) and errors committed between 38% and 85% correct responses (see Bussey *et al.*, 1997). Each criterion required performance at the stated level for two consecutive sessions. Errors made during the first stage suggest that the animal is unable to inhibit responding to the previously rewarded stimulus and switch responding to the new stimulus. Thus, unlike the perseveration score (discussed earlier), this measure indicates that the animal makes repeated responses to the visual stimulus irrespective of the side on which it was presented. We therefore refer to these errors as 'stimulus-bound' perseverative responses (see Ridley, 1994).

Data analysis

Data for each variable were subjected to an analysis of variance (ANOVA) using the CLR ANOVA version 2.0 (Clear Lake Research, Houston, TX, USA) statistical package. Where *F*-ratio's were significant, the means were compared using Newman–Keuls' *post hoc* comparisons.

Experiment 1A: the effect of excitotoxic lesions of the mediodorsal thalamus, the anterior thalamic nuclei and the prelimbic cortex on visual discrimination and reversal learning

Behavioural procedures

Following surgery (Sham $n = 7$; PL $n = 10$; MD $n = 9$; ANT $n = 10$), animals were given a 14-day postoperative recovery period followed by pretraining (discussed earlier). On completion of the pretraining phase, the animals were moved onto the visual discrimination behavioural task.

Acquisition

In the visual discrimination task, the same pair of stimuli were presented on every trial (see Fig. 1A). One stimulus was designated the S+ and the other the S–, counterbalanced across animals. The stimulus configuration on each trial (i.e. which stimulus was on the left and which was on the right) was determined pseudorandomly. A nose poke to the S+ was rewarded with a tone, magazine light and a reward pellet. A nose poke to the S– was followed by extinction of

the houselight and a 5-s time-out period. Each session consisted of 60 trials. All animals were required to learn the correct, reinforced stimulus to a criterion of 85% on two consecutive sessions, as described previously (Bussey *et al*., 1997). A correction procedure was used such that following an incorrect choice, animals received the same stimulus configuration (i.e. S+ and S– remained in the same left/right locations) until the rat responded correctly.

Reversals

Following acquisition of this discrimination, the reward contingency was reversed so that the previously nonrewarded stimulus was now the correct, reinforced stimulus (i.e. S+ became S– and vice versa). Once the rat had attained the 85% criterion on this reversal, the reward contingencies were reversed again. A total of three reversals were given. Each session consisted of 60 trials.

Results

Histological analysis

The cytoarchitectonic borders and nomenclature are taken from the atlas by Zilles (1985). The largest and smallest of the lesions for each group are depicted in Fig. 2 and photomicrographs are presented in Fig. 3. Examination of the cresyl violet-stained sections revealed that one animal from the MD lesion group, two animals from the PL lesion group and one animal from the anterior thalamic lesion group presented with either an incomplete lesion or too extensive a lesion and were thus discarded from the behavioural analyses. In all other cases the area of the lesion was centred on the appropriate target region for that lesion group. Thus, the final numbers in each group for subsequent behavioural analysis were Sham = 7; PL = 8; MD = 8; ANT = 9.

Animals with lesions of the PL cortex showed extensive bilateral cell damage that began at the frontal pole (Fig. 2A, section 1) and continued caudally to the level of the genu of the corpus callosum (Fig. 2A, section 4). In all these cases, bilateral cell loss was evident in the prelimbic area with only the most caudal regions being spared. MD lesioned animals displayed extensive cell loss throughout the mediodorsal thalamic nuclei, which included the central, medial and lateral nuclei. In most cases sparing occurred only at the most lateral and ventral limits of the nuclei (Fig. 2B, section 4). The region comprising the nuclei was shrunken with almost complete neuronal loss. The lesions in the ANT group consistently involved all three anterior thalamic nuclei (anteroventral, anterodorsal and anteromedial), with some sparing of the anterodorsal nucleus (Fig. 2C, sections 3 and 4). In all cases, there was extensive shrinkage of tissue associated with the lesion (see photomicrographs, Fig. 3C). In most cases, the damage slightly extended into the midline nuclei. Five of the anterior thalamic lesioned animals showed either very slight unilateral or bilateral damage to the dentate gyrus of the hippocampus. In the Sham-ANT group of animals, there was similar slight bilateral damage to the dentate gyrus, which resembled the extraneous damage observed in the anterior lesioned groups. Sham-PL and Sham-MD animals did not show any neuronal damage.

Behavioural results

Acquisition of the visual discrimination

During the initial phase of the task, animals were required to discriminate two stimuli, one designated S+ and the other S–. Preliminary analysis of the Sham data revealed that there was no significant difference between the various Sham groups (Sham-PL, Sham-MD, Sham-ANT) in terms of sessions ($F_{2,4} = 1.98$, $P > 0.05$), or errors ($F < 1$) in attaining criterion performance, despite the dentate damage incorporated within the Sham-ANT group of animals. Thus, all these animals were treated as a single Sham group during subsequent analyses.

ANOVA revealed that there was no significant effect of lesion group on acquisition of the visual discrimination task either in terms of the

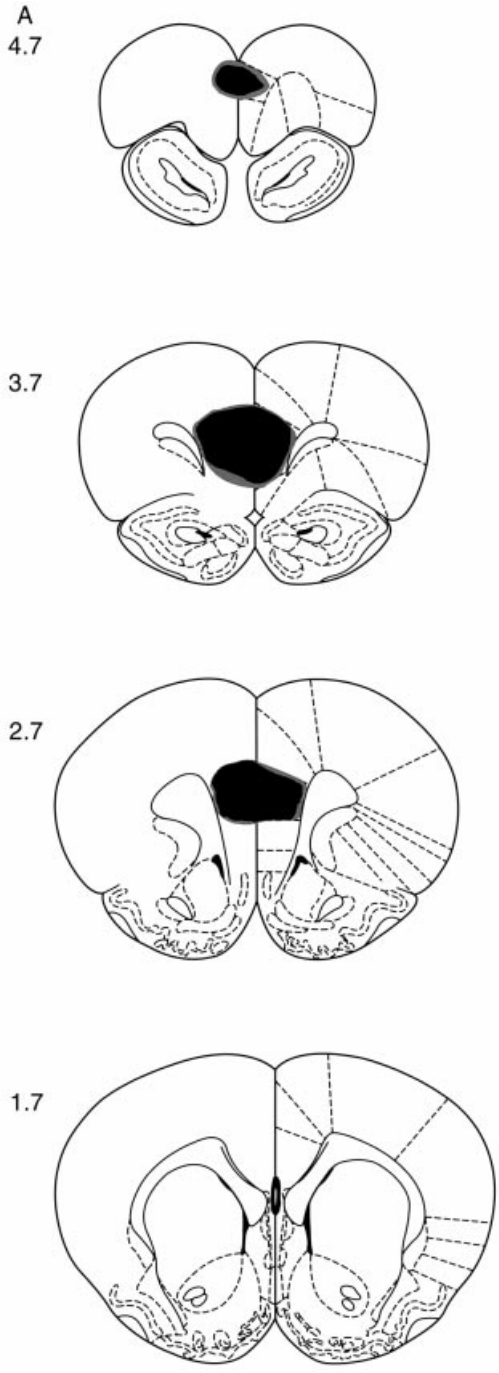
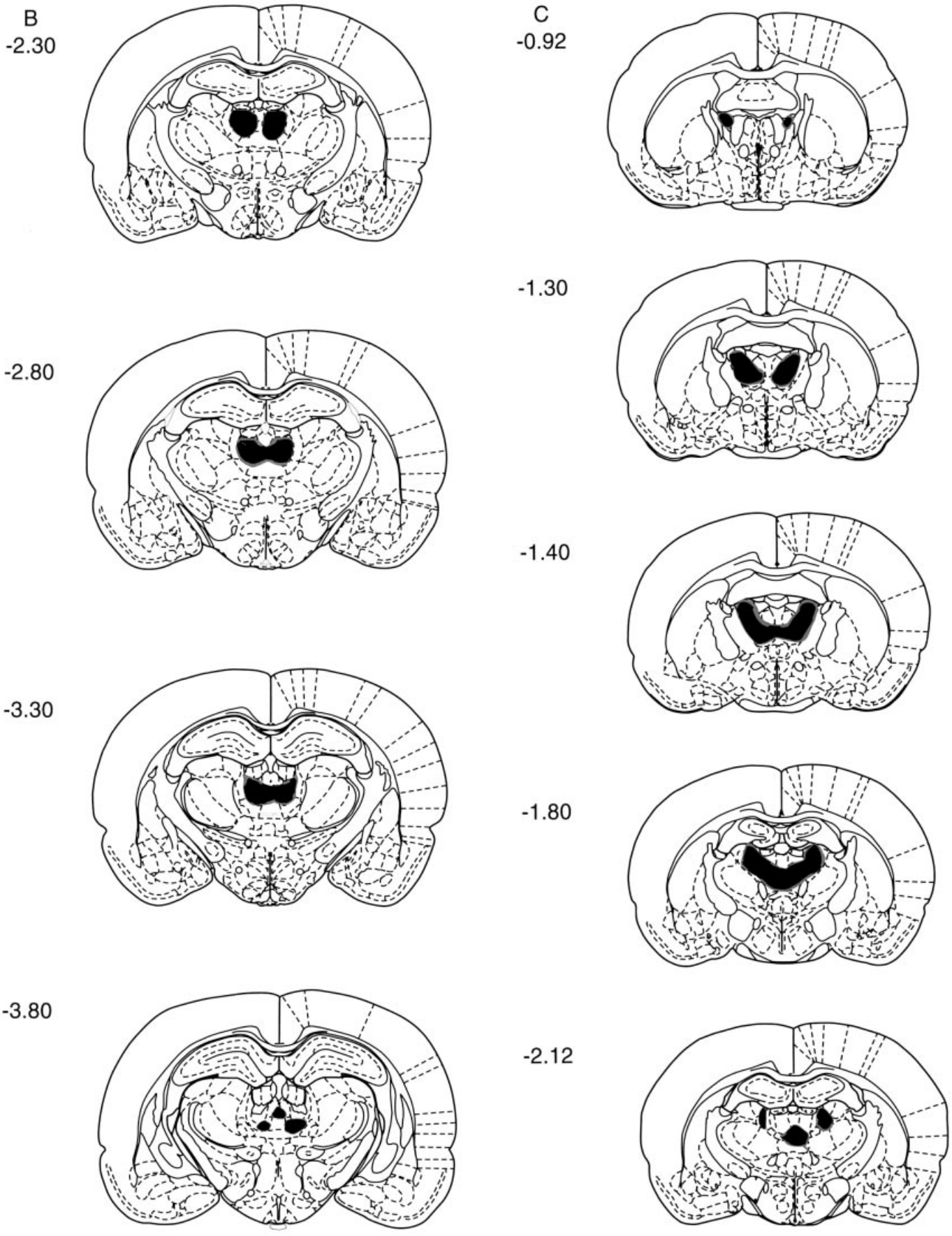
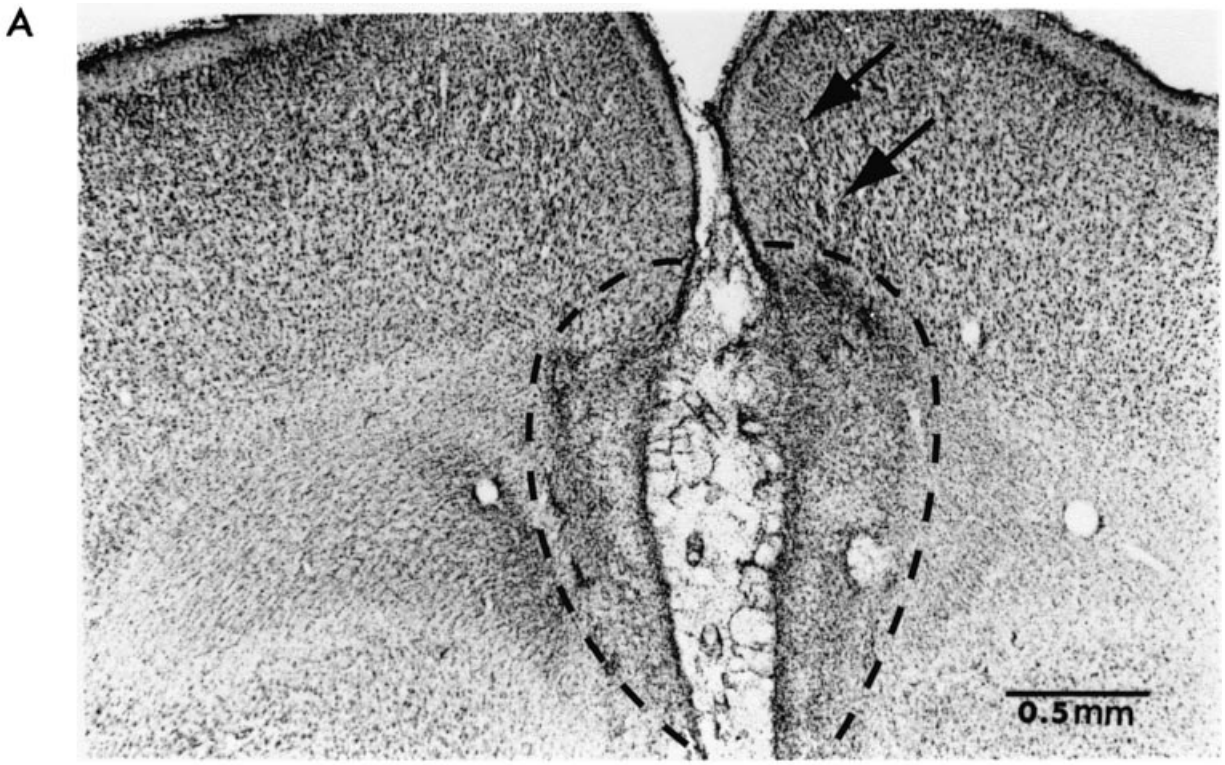


FIG. 2. (See parts B and C on facing page.) Diagrammatic reconstructions illustrating the extent of the largest (grey) and smallest (black) lesions of the prelimbic cortex (A), mediodorsal thalamic nuclei (B) and anterior thalamic nuclei (C). Numbers indicate sections relative to Bregma according to the atlas of Paxinos & Watson (1997).


B
-2.30
-2.80
-3.30
-3.80
C
-0.92
-1.30
-1.40
-1.80
-2.12


A
0.5mm
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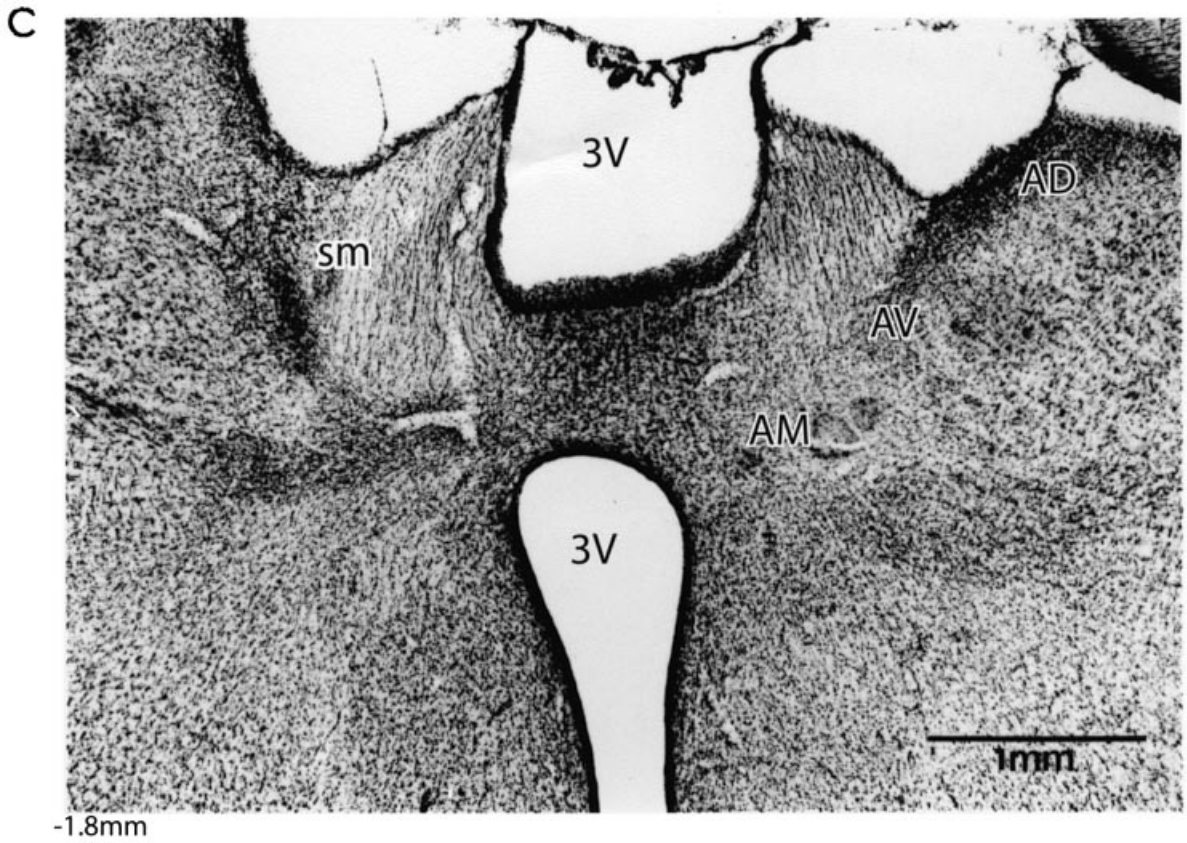

FIG. 3. (See parts A and B on facing page and C above.) Photomicrographs of coronal sections (Nissl stain) showing cytotoxic lesions in representative sections from animals for the PL (A), MD (B) and ANT (C) groups. The dotted line in panel A shows bilateral degeneration of cells confined to the prelimbic cortex. The frontal cortex and area Cg1, which overlie the prelimbic region, are completely spared (see arrows). (B) Bilateral cell loss in the mediodorsal thalamic nuclei (MDl, MDc and MDm). Note enlargement of the third ventricle (3V). The midline nuclei appear to be spared. (C) Extensive damage to the anterior group of thalamic nuclei (AM, AV, AD) is evident. Note significant shrinkage of the tissue resulting in enlarged ventricles and expansion of the stria medullaris (sm). Numbers indicate approximate sections relative to Bregma according to the atlas of Paxinos & Watson (1997).

number of sessions ($F < 1$; see Fig. 4A) or the number of errors ($F < 1$) taken to reach the 85% criterion (see Fig. 4B). There was no main effect of lesion group with respect to choice latency ($F < 1$) (means in seconds: Sham, 9.22; PL, 10.5; ANT, 10.2; MD, 8.10) and no significant group differences in terms of magazine latency ($F_{3,28} = 2.18$, $P > 0.05$) (means in seconds: Sham, 1.83; PL, 4.39; ANT, 2.38; MD, 1.78).

There were also no significant differences between groups in terms of the perseveration score ($F_{3,28} = 2.52$, $P > 0.05$) (means: Sham, 14.7; PL, 14.4; ANT, 23.4; MD, 17.2) and there was no significant main effect of lesion group on percentage response bias ($F < 1$) (means: Sham, 13%; PL, 10.7%; ANT, 12.0%; MD, 10.6%).

Reversals

Sessions and errors to criteria

Following successful acquisition of the visual discrimination, the reward contingencies were reversed so that S+ became S– and vice versa. Again, acquisition of each reversal was set at 85% correct, responding over two consecutive sessions. A total of three reversals was given.

Figure 5A shows errors committed by animals during the three reversals of the discrimination. A two-way ANOVA revealed a significant main effect of lesion group in terms of the number of errors made by animals during the three reversals ($F_{3,28} = 2.92$, $P < 0.05$). Newman–Keuls *post hoc* analysis revealed that this effect was due to animals of the MD lesion group making significantly more errors ($P < 0.05$) during each of the reversals relative to animals of the other three groups.

The number of errors committed during the two learning stages, summed across all three reversals, is shown in Fig. 5B. A significant main effect of learning stage was obtained ($F_{1,28} = 55.9$, $P < 0.001$), with significantly more errors made during the second (38–85%) stage of learning for all groups compared with the first stage. There was also a significant lesion group–stage interaction (see Fig. 5B), as animals of the MD group made more errors during the second stage of learning ($F_{3,28} = 3.34$, $P < 0.05$). That is, these animals made more responses at and above chance than animals of the other groups. There was no significant effect of lesion group on stimulus-bound perseverative responses during stage 1 of learning.

A two-way ANOVA revealed that although there was no significant main effect of lesion group on the number of sessions taken to acquire each reversal ($F_{3,28} = 2.51$, $P = 0.07$), there was a strong trend. This effect was due to animals with MD lesions taking, on average, more sessions to complete each reversal than animals of the other three groups, although these differences were not significant (mean number of sessions: Sham, 28.1; PL, 19.3; ANT, 24.2; MD, 38.9).

Latency measures

There was no significant main effect of group in terms of choice latency during the reversals ($F < 1$) (means in seconds: Sham, 9.8;

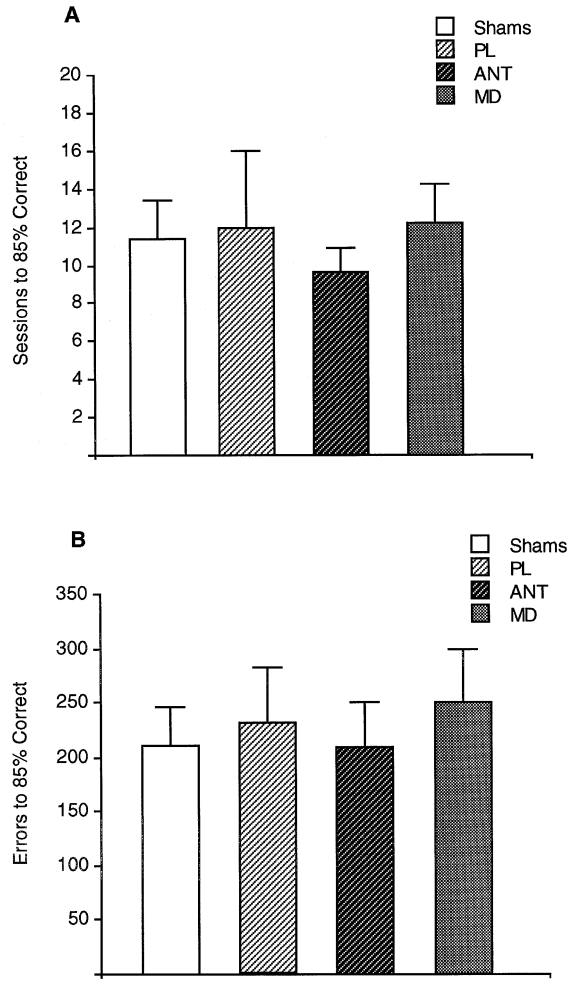

FIG. 4. Performance of rats on the acquisition of the visual discrimination task. (A) Number of sessions, (B) number of errors taken to acquire 85% criterion.

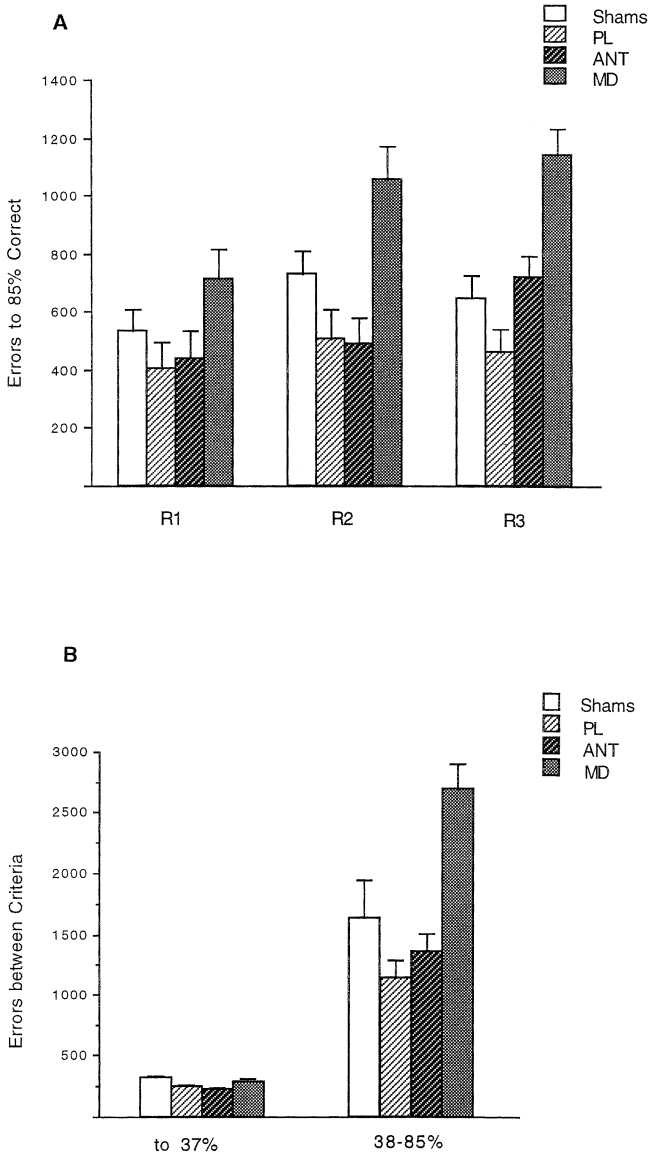

FIG. 5. Performance of rats on reversal learning of the visual discrimination task. (A) Number of errors made by each animal during each reversal, (B) number of errors committed during two learning stages summed across all three reversals.

PL, 10.0; ANT, 10.0; MD, 7.13). However, a significant lesion group–reversal interaction was observed ($F_{2,56} = 3.30$, $P < 0.01$). *Post hoc* analysis revealed this to be a result of the PL animals that were slower to respond to the choice stimuli during the first reversal only (means in seconds: Sham, 8.0; PL, 12.63; ANT, 8.47; MD, 6.34).

No significant main effect of group was observed for magazine latency ($F_{3,28} = 1.78$, $P > 0.05$) (means in seconds: Sham, 1.55; PL, 1.76; ANT, 1.74; MD, 1.47). However, there was a significant main effect of reversal ($F_{2,56} = 3.76$, $P < 0.01$), indicating that magazine latency decreased for all animals following the first reversal (means in seconds: reversal 1, 1.73; reversal 2, 1.58; reversal 3, 1.59)

Perseveration score

A significant main effect of lesion was obtained for the perseveration score ($F_{3,28} = 2.96$, $P = 0.05$). *Post hoc* comparisons revealed that this was due to animals of the PL, ANT and MD lesion groups making more perseverative responses than the Sham control group (means: Sham, 9.61; PL, 15.26; ANT, 17.95; MD, 14.74). A significant main effect of reversal was also obtained ($F_{256} = 39.54$, $P < 0.001$), which indicated that perseverative responding decreased for all animals as training proceeded across reversals (means: reversal 1, 19.9; reversal 2, 14.02; reversal 3, 9.25)

Percentage bias

There was no significant main effect of group on this measure ($F < 1$) (means: Sham, 15.45%; PL, 12.87%; ANT, 13.95%; MD, 13.83%) or an effect of reversal ($F_{2,56} = 0.88$, $P > 0.05$) or a group–reversal interaction ($F < 1$).

Experiment 1B: effects of excitotoxic lesions of the mediodorsal thalamus, the anterior thalamic nuclei and the prelimbic cortex on visual conditional discrimination learning

Behavioural procedure

Following the acquisition and reversal of the visual discrimination task, rats were required to learn a rule of the type, 'if stimulus A then go left, if stimulus B then go right'. For this task, a three-window

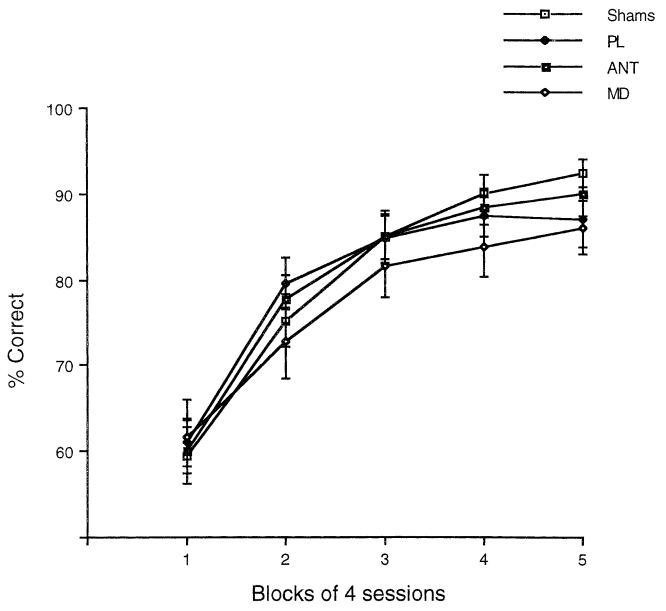

FIG. 6. Mean percentage correct accuracy across 20 sessions in blocks of four, for all animals during acquisition of the conditional discrimination task (experiment 1B).

mask was used. One window was located centrally and the other two were located symmetrically, either to the left or right of the central window.

Each trial began with the presentation of one of two discriminative stimuli that were presented pseudorandomly in the central window (see Fig. 1B). The rat was required to make a nose poke to this stimulus, which resulted in the presentation of two white squares (the choice stimuli) in the left and right windows. A nose poke to the appropriate stimulus (left or right square depending on the discriminative stimulus) resulted in a tone, magazine light and a reward pellet. Incorrect responses were followed by a 5-s time-out period. Both discriminative stimuli were presented an equal number of times during a session. As described previously (Bussey *et al.*, 1997), if the rat did not respond to the choice stimulus within a 2-s limited hold period, the choice stimuli disappeared and the house-light was extinguished for a 5-s time-out period. This ensured that a rat's response to a choice stimulus was always a head turn to the right or left while rearing, followed by a nose poke to the stimulus. Each session consisted of 60 trials. The animal had reached criterion of acquisition of this task once it had attained 85% correct over two consecutive sessions. All animals received a total of 20 sessions of acquisition on this task.

Results

Behavioural results

Acquisition

Analysis of variance revealed no significant difference between groups in choice accuracy during acquisition of this task ($F < 1$). All animals did, however, show an improvement in accuracy across the 20 sessions ($F_{19,532} = 68.39$, $P < 0.001$). As shown in Fig. 6, the mean percentage correct accuracy on the task for all animals in session 1 was 62%. By session 20, animals were obtaining a mean accuracy of 90%.

Sessions and errors to criterion

Analysis of variance revealed no significant effect of lesion group on acquisition of the conditional visual discrimination task, either in terms of the number of sessions to attain criterion performance ($F < 1$) (mean number of sessions: Sham, 9.6; PL, 9.3; ANT, 9.6; MD, 8.4) or in errors to acquire the discrimination to the criterion of 85% correct ($F < 1$) (mean errors: Sham, 180.14; PL, 175.5; ANT, 155.5; MD, 141.0).

Latency measures

There was no main effect of lesion group with respect to choice latency ($F_{3,28} = 2.16$, $P > 0.05$) (means in seconds: Sham, 1.31; PL, 1.81; ANT, 1.70; MD, 1.17) and no significant group differences in terms of magazine latency ($F_{3,28} = 1.23$, $P > 0.05$) (means in seconds: Sham, 1.46; PL, 1.50; ANT, 1.60; MD, 1.33).

Perseveration score

There were no significant differences between groups in terms of perseverative responding ($F_{3,28} = 1.08$, $P > 0.05$) (means: Sham, 4.49; PL, 5.36; ANT, 6.25; MD, 4.15).

Percentage bias

There was no significant difference between groups in terms of the percentage bias to respond to one particular response side ($F < 1$) (means: Sham, 6.2%; PL, 6.9%; ANT, 6.4%; MD, 6.3%).

Experiment 2: effects of lesions of the mediodorsal thalamus, the anterior thalamic nuclei and the prelimbic cortex on visual conditional discrimination learning and reversal

In experiment 1A, all animals were able to acquire a visual discrimination with no differences between lesion groups. However, animals with excitotoxic lesions of the MD thalamus were found to be significantly impaired in learning reversals of this visual discrimination. Experiment 2 was designed to test the generality of this effect. Specifically, we investigated whether the reversal learning impairment following MD lesions was specific to stimulus–reward learning or whether such lesions would impair reversal of a conditional, stimulus–response task.

In experiment 1B, no effect of lesion group was obtained on visual conditional discrimination learning, the results suggesting that all lesion groups, including animals with lesions of the MD thalamus, could acquire this task normally. However, as this task was the last assessed in experiment 1, the lack of effect could have been related to recovery of function. Furthermore, the possibility of transfer effects from the first to the second tasks must also be considered. Thus, in experiment 2, the effects of these lesions were again examined on the acquisition of the conditional discrimination in a new cohort of task-naive rats immediately postoperatively and this was followed by reversal of the conditional rule.

Methods

Subjects

The subjects were male Lister Hooded rats (Harlan), housed in pairs and food deprived, as in experiment 1. The animals were assigned to one of the four groups and the appropriate surgeries performed (see general Methods section for details). The group numbers were as follows: Sham, 9; PL, 10; MD, 10; ANT, 10. All animals used in this

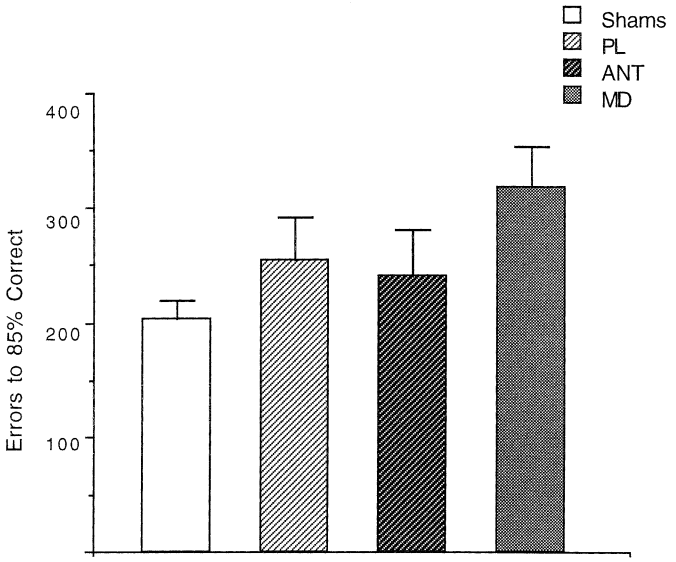

FIG. 7. Number of errors made to 85% correct during reversal learning of the conditional discrimination task (experiment 2).

study were treated in accordance with the UK 1986 Animals (Scientific Procedures) Act.

Behavioural procedures

Following surgery, animals were given a 14-day postoperative recovery period and rats were required to learn a rule of the type, 'if stimulus A then go left, if stimulus B then go right', identical to that required in experiment 1B. The behavioural procedures were the same as those described for experiment 1B with the exception that the experiment was extended such that once animals had attained criterion performance on this task the conditional rule was reversed such that the animals, using the above example, were required now to learn 'if stimulus A then go right, if stimulus B then go left'.

Results

Histological analysis

Examination of the cresyl violet-stained sections revealed that two animals of the anterior lesion group and one of the MD group presented with either an incomplete lesion or too extensive a lesion, and were thus discarded from the behavioural analysis. In all other cases the area of the lesion was centred on the appropriate target region for that lesion group and were similar to those obtained in experiment 1 (see Fig. 2). Thus, the final group numbers for each lesion group were as follows: Sham, 9; PL, 10; MD, 9; ANT, 8.

Behavioural results

Acquisition

Preliminary analysis of the Sham data revealed that there was no significant difference between the Sham animals (Sham-PL, Sham-MD, Sham-ANT) in choice accuracy across the 20 acquisition sessions ($F < 1$) and therefore these animals were treated as a single Sham group during subsequent analyses. Similar to the acquisition results of experiment 1B, an analysis of variance revealed that there was no significant group difference in choice accuracy during acquisition of this task ($F < 1$). All animals showed an improvement in accuracy across the 20 sessions ($F_{19,589} = 83.92$, $P < 0.001$). The mean percentage correct accuracy on the task for all animals in session 1 was 51%. By session 20, animals were obtaining a mean accuracy of 89%.

Sessions and errors to criterion

Analysis of variance revealed that there was no significant effect of lesion group on acquisition of the conditional visual discrimination task either in terms of the number of sessions to attain criterion performance ($F < 1$) (mean number of sessions: Sham, 7.3; PL, 7.8; ANT, 7.8; MD, 8.0) or in errors to acquire the conditional discrimination to the criterion of 85% correct ($F < 1$) (mean errors: Sham, 138.4; PL, 147.0; ANT, 147.5; MD, 149.6).

Latency measures

There was no main effect of lesion group with respect to choice latency ($F < 1$) (means in seconds: Sham, 1.55; PL, 1.86; ANT, 1.61; MD, 1.86) and no significant group differences in terms of magazine latency ($F_{3,32} = 2.03$, $P > 0.05$) (means in seconds: Sham, 1.65; PL, 2.41; ANT, 1.68; MD, 1.88).

Perseveration score

There was no significant difference between groups in terms of perseverative responding to a particular conditional stimulus ($F_{3,32} = 1.17$, $P > 0.05$) (means: Sham, 7.1; PL, 7.4; ANT, 7.5; MD, 9.6).

Percentage bias

There was no significant differences between groups in terms of the percentage bias to respond to one particular response side ($F_{3,32} = 1.18$, $P > 0.05$) (means: Sham, 6.8; PL, 6.5; ANT, 6.8; MD, 8.5).

Reversal

Sessions and errors to criterion

Having successfully acquired the initial conditional visual discrimination, the conditional rule was now reversed for all animals. Again, criterion was set at 85% correct for two consecutive sessions. ANOVA revealed that there was a significant main effect of lesion group on the number of sessions taken to acquire the reversal of the conditional rule ($F_{3,32} = 2.97$, $P < 0.05$). *Post hoc* analysis revealed that this effect was due to the animals of the MD lesion group taking more sessions to reach the 85% criterion in comparison with the sham animals ($P < 0.05$). The MD group did not, however, differ significantly from the other lesion groups (mean number of sessions: Sham, 10.0; PL, 12.0; ANT, 13.0; MD, 15.0). Furthermore, as shown in Fig. 7, there was a strong trend for an increase in the number of errors made by the MD lesion group during the reversal, although this effect failed to reach significance ($F_{3,32} = 2.04$, $P > 0.05$).

These data were also analysed according to two learning stages, as described earlier (see Performance measures). There was no main effect of lesion on the number or errors committed to reach criterion performance ($F_{3,32} = 1.33$, $P > 0.05$). A significant main effect of learning stage was obtained ($F_{1,32} = 72.79$, $P < 0.0001$), with significantly fewer errors made during the second (38–85%) stage of learning for all groups compared with the first stage. However, there was no significant lesion group–stage interaction.

Latency measures

No significant main effect of lesion group was observed in terms of choice latency during the reversal ($F < 1$) (means in seconds: Sham, 1.71; PL, 1.84; ANT, 1.45; MD, 1.65). There was no significant main effect of lesion on magazine latency ($F_{3,32} = 2.38$, $P > 0.05$) (means in seconds: Sham, 1.29; PL, 1.62; ANT, 1.49; MD, 1.58).

Perseveration score

There was no significant main effect of lesion with respect to perseverative responding when the conditional rule was reversed ($F_{3,32} = 1.12$, $P > 0.05$) (means: Sham, 11.0; PL, 10.7; ANT, 11.9; MD, 14.0).

Percentage bias

No significant main effect of group was observed in terms of percentage bias to respond to one particular response side ($F < 1$) (means: Sham, 8.7; PL, 10.4; ANT, 8.1; MD, 9.8).

Discussion

The present study investigated the effects of excitotoxic lesions of the mediodorsal nucleus of thalamus, the anterior thalamic nuclei and the prelimbic cortex, on visual discrimination and reversal learning in the rat. While there was no effect of these lesions on the acquisition of a single-pair visual discrimination task, lesions of the MD thalamus significantly increased the number of errors committed during the reversals of this discrimination (experiment 1A). In experiment 1B, none of the lesions impaired the acquisition of a conditional discrimination, a finding that was replicated in a second cohort of animals (experiment 2). In contrast to experiment 1B, in experiment 2 the effects of these lesions on conditional discrimination learning were examined immediately postoperatively, thus reducing possible transfer effects from the previous discrimination task. While acquisition of the task was unaffected, when the conditional response rule was reversed, animals with lesions of the MD thalamus were mildly impaired according to sessions required to attain criterion performance, and there was a strong trend for an increase in the number of errors committed. The lesions did not affect magazine latency, correct latency or percentage of bias in these experiments. As the MD lesion led to a deficit in reversal learning only, and not when switching from between very different tasks such as stimulus–reward and conditional learning, it seems unlikely that the deficit was due to general anxiogenic or frustrative effects related to task switching (Beracochea & Krazem, 1991; Krazem *et al*., 1995). There was no effect of lesions of the anterior thalamic nuclei or of the prelimbic cortex on any of the tasks examined.

Detailed analysis of data from experiment 1A revealed that the reversal impairment following MD damage was not due to an inability to suppress responding to the previously rewarded stimulus. Instead, these animals made many nonperseverative errors in the later stages of reversal, indicating difficulty in acquiring the new stimulus–reward association. This finding does not support the idea that the MD thalamus is necessary for response inhibition, and instead supports the view that the MD thalamus is important for new stimulus–reward associative learning (Gaffan & Murray, 1990). Analysis of reversal learning provides a unique tool for the study of stimulus–reward learning, as discrimination learning is thought to involve at least two processes: discriminating the stimuli perceptually and associating the stimuli with reward or nonreward (Mackintosh, 1974). Given that rats learned to discriminate perceptually the stimuli during initial acquisition, analysis of reversal learning allows the study of stimulus–reward learning under conditions in which the requirement for perceptual learning is minimized (Jones & Mishkin, 1972). This provides a plausible explanation as to why, contrary to Gaffan & Murray's (1990) findings, MD lesioned animals in the present study were not impaired in the acquisition phase of the task. Given that the visual system of the monkey is superior to that of the rat, for the monkey perceptual discrimination of the stimuli may be a relatively minor component of acquisition; monkeys may be able tell the stimuli apart almost immediately. Therefore, to compare stimulus–reward learning in the rat with that in the monkey, it may be necessary to allow the rat to learn first to discriminate perceptually the stimuli. The paradigm used in the present study allowed this, and as a result the effects of thalamic lesions on stimulus–reward learning in the rat and monkey were found to be comparable.

The type of impairment observed in the MD lesion group in experiment 1A is strikingly similar to that observed in a previous study (Bussey *et al*., 1997), which examined the effect of excitotoxic medial frontal cortex lesions on reversal learning, using the same apparatus and similar stimulus material to that used in the present study. Thus, it seems likely that both the MD thalamic lesion in the present study and the medial frontal cortex lesions in the study of Bussey *et al*. (1997) disrupted thalamocortical circuits important for this type of learning. In addition, similar to the medial frontal lesion, the MD lesioned animals were not spatially biased, did not show impairments in response latencies and, as for the medial frontal lesions, the MD lesioned group made more errors on each reversal than animals of the other groups. This further supports the comparability of the effects of medial frontal cortex and the MD thalamus

The profound impairment in reversal learning observed by Bussey *et al*. (1997) was observed following lesions of the medial frontal cortex that included prelimbic and overlying cingulate cortex rostral to the genu of the corpus callosum. In contrast, in the present study, rats with selective excitotoxic lesions of the PL cortex were unimpaired on this task. Thus, the present findings for the PL region, taken together with those obtained following medial frontal lesions (Bussey *et al*., 1997), implicate pre- or perigenual anterior cingulate cortex within the medial prefrontal cortex, rather than the PL cortex in this type of learning. This hypothesis remains to be tested directly.

The pattern of reversal learning deficit observed in the present study is unlike other reversal deficits reported in previous studies (e.g. Hunt & Aggleton, 1998), in which lesions of the MD thalamus were shown to produce a pronounced increase specifically in perseverative responding during both acquisition of a T-Maze matching-to-place task and reversal to the nonmatching version of the task. These deficits, however, were obtained using tasks that are spatial in nature and which often have required a shift from an innately preferred response bias (e.g. nonmatching-to-position). In the first experiment of the present study, a pronounced deficit in reversal of stimulus–reward association was obtained using a task that was not spatial in nature and did not require shifting from an innately preferred response bias. It is conceivable that these task differences contributed to the perseverative errors in the experiment of Hunt & Aggleton (1998), but the precise reason for this discrepancy remains unclear.

In contrast to the behavioural effects observed following MD lesions, there was no effect of ANT thalamic lesions on any of these tasks. In previous studies where substantial deficits have been obtained in animals with lesions of the ANT thalamic nuclei, the spatial component of the tasks may have been an important factor. The lack of impairment on nonspatial tasks is consistent with the view that the anterior thalamus is important for spatial, but not nonspatial, memory (for review, see Aggleton & Brown, 1999).

In addition to the main effect of MD lesions on stimulus–reward learning, there was also a trend towards an increase in perseverative score (a measure of the tendency to repeat a response within a trial, as distinct from perseveration to the previous S+ stimulus) in all three lesion groups in experiment 1A. There are at least two reasons why this trend is unlikely to account for the main effect of MD lesions on discrimination learning. First, only the MD group was impaired in stimulus–reward learning, whereas all three lesion groups showed the

trend towards an increase in perseverative score. Second, there was no relationship between perseverative score and impairment in stimulus–reward learning; the MD lesion group had the lowest perseverative score, but were severely impaired on stimulus–reward learning, while the ANT and PL groups had the highest perseverative score, but committed the lowest number of errors during stimulus–reward learning (indeed, the number of errors committed in the stimulus–reward learning phase by these groups was numerically less than that of controls).

In summary, the results of the present study provide several important pieces of information regarding the functions of the MD thalamus and ANT thalamic nuclei, and their relationship with the prefrontal cortex. First, the results suggest a functional dissociation between the MD and ANT thalamus, the former being more important for stimulus–reward learning. Second, similar patterns of impairments in reversal learning following MD thalamus and medial frontal cortex lesions suggest the existence of a functionally distinct thalamocortical pathway important for stimulus–reward learning. Although in general, MD but not ANT thalamic nuclei project to the prefrontal cortex, there are some selective projections from the anteromedial nucleus of the ANT thalamic nuclei to the prefrontal cortex, including the PL region. The findings of a behavioural dissociation between lesions of the MD and ANT thalamic nuclei indicate that the ANT thalamic projection to the prefrontal cortex serves a different function from those of the MD projection to the prefrontal cortex. Third, previously observed deficits in reversal learning following lesions to the medial frontal cortex (Bussey *et al*., 1997) were unlikely, due to damage to the prelimbic cortex, and instead pre- or perigenual anterior cingulate cortex is implicated in this type of learning. While direct inputs to the prefrontal cortex from the MD thalamus may be involved at some level in stimulus–reward associative learning, inputs to the prefrontal cortex from the anterior thalamus may be important for the encoding of spatial and episodic memory (Aggleton & Brown, 1999).

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Abbreviations

ANT, anterior thalamic nuclei; ITI, inter-trial interval; MD, mediodorsal thalamic nuclei; NMDA, *N*-methyl-D-aspartic acid; PL, prelimbic cortex; VDU, video display unit.

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