The role of the medial supramammillary nucleus in the control of hippocampal theta activity and behaviour in rats

Wei-Xing Pan and Neil McNaughton
Department of Psychology and Centre for Neuroscience, University of Otago, POB56, Dunedin, New Zealand

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Abstract
The medial supramammillary nucleus (mSUM) controls the frequency of hippocampal theta activity, completely in anaethsetized rats and partially in free-moving rats. mSUM could therefore influence hippocampal contributions to cognition and emotion. Using chemical lesions of mSUM in rats, we tested whether mSUM is involved in controlling several hippocampal-dependent functions: (i) defensive behaviour (open field, fear conditioning); (ii) behavioural inhibition (fixed interval schedule, differential reinforcement of low rates schedule); and (iii) spatial learning (water maze). Theta frequency was measured in all these tasks. mSUM lesions produced a pattern of changes in motivated/emotional behaviours (hyperactivity in defensive and operant tasks) similar to the pattern produced by hippocampal lesions, but had no significant effect on spatial learning. mSUM lesion decreased theta frequency modestly (by $\approx 0.4$ Hz) in behaving rats if the amount of movement was unchanged. There was not always a parallel between changes in theta frequency and behaviour; behaviours changed despite unchanged theta in defensive tasks and learning changed little despite a lower frequency of theta in the water maze task. This suggests that mSUM function impacts on emotional behaviour more than cognition, and can modulate theta and behaviour independently.

Introduction
The medial supramammillary nucleus (mSUM) is a small nucleus that overlies the mammillary bodies medially, mSUM connects with the septo-hippocampal system (Vertes, 1988; Vertes, 1992) and is involved in the control of the frequency of hippocampal theta rhythm (Kirk & McNaughton, 1991; Kirk & McNaughton, 1993; Kocsis & Vertes, 1994; Bland et al., 1995; McNaughton et al., 1995; Kirk, 1998). This suggests that mSUM may be involved in the functions of the hippocampus, which are thought to impact on both cognition and emotion.

We have previously reported that infusion of chlordiazepoxide into mSUM produced only a modest decrease in theta frequency in freely moving rats and only a modest disruption of spatial learning (Pan & McNaughton, 1997). Similarly, electrolytic lesions of the mammillary body and mSUM in rats do not impair spatial learning in the radial arm maze (Jarrard, 1983; Sziklas & Petrides, 1993; Sziklas et al., 1995), which is sensitive to hippocampal lesions. These data suggest that mSUM may not be critical for cognitive aspects of hippocampal function. It might, however, be involved with more emotional aspects.

An emotional role for mSUM is suggested by the fact that mSUM is one of only two structures in the brain that show a decrease in fear-induced c-fos expression on treatment with low-dose diazepam (Beck & Fibiger, 1995). Fos immunoreactivity in mSUM was also induced by exposure of rats to the ethologically based animal models of anxiety, the elevated plus-maze (Silveira et al., 1993) and novel open field (Wirtshafter et al., 1998). Further, fox protein is expressed in mSUM after cold or warm ambient exposure (Kiyohara et al., 1995; Miyata et al., 1995) or swim stress (Cullinan et al., 1996). Therefore, mSUM may play a role in responses to physiological and/or emotional stress.

The present project used fibre-sparing lesions of mSUM, to test whether mSUM is involved equally across a range of classes of hippocampal-dependent tasks: (i) defensive behaviour; (ii) behavioural inhibition; and (iii) spatial learning.

Defensive behaviour has been categorized into three levels (Blanchard & Blanchard, 1988). The first, least intense, level is that of exploratory behaviour or anxiety when the danger is uncertain. To assess this we used the open field. The second level is that of freezing or fear when danger has been identified but is not immediate. To assess this we used a conventional fear-conditioning task and assessed freezing both to the conditional stimulus and to the context provided by the conditioning chamber. The third, most intense, level involves fight/flight or rage/panic when danger is immediate. We did not assess this.

If mSUM lesions are similar to hippocampal lesions we would expect increased ambulation in the open field and decreased freezing in contextual fear conditioning (Cannon et al., 1992; Rossi-Arnaud & Ammassari-Teule, 1992; Stefanelli et al., 1993; Phillips & LeDoux, 1994). Such results could be the result of hyperactivity, but they are also what Gray (1982) would predict on the view that the hippocampus carries out the function of behavioural inhibition. To explicitly test behavioural inhibition we used a fixed interval schedule (FI) and a differential reinforcement of low rates of response schedule (DRL). The former allows over-responding with no loss of reinforcement and so represents a relatively mild requirement for behavioural inhibition. The latter involves loss of reinforcement for over-responding and so represents a stronger requirement for behavioural inhibition, as well as allowing assessment of explicit timing behaviour.
The predominant view in the literature is that the main function of the hippocampus is to control memory, especially spatial memory (O’Keefe & Nadel, 1978; Vertes, 1986; Nadel, 1991; O’Keefe, 1993). If mSUM really plays an important role in the functions of the hippocampus, it should be involved in controlling spatial memory. We previously reported (Pan & McNaughton, 1997) that infusions of chlordiazepoxide (CDP) into the mSUM region produced very modest, but statistically significant, impairments in learning in a single-day water maze and a similarly modest decrease in theta frequency (by 0.35Hz). However, the learning impairment did not occur until about the 10th trial in this case. Because we used 20 trials of continuous training in this single-day paradigm, the rats were exposed to the water for a relatively long time (> 10 min) and their body temperature would have decreased with testing time. Given that body temperature will affect theta frequency (Whishaw & Vanderwolf, 1971), there is an argument that the decrease in body temperature in the later stages of training made a major contribution to the observed learning impairment. Indeed, we observed that the theta frequency decreased logarithmically with training time. Critically, rats treated only with long cooling water exposure before training showed a serious deficit of learning and decrease in theta frequency in this task (Pan & McNaughton, 1997). We therefore tested spatial learning in the water maze. Four days of testing with four training trials per day were used instead of 20 trials within 1 day to minimize the decreases in body temperature and dissociate them from the extent of training.

In all the tasks, we also assessed the relationship between theta frequency and behaviour as well as the extent to which lesion of mSUM altered theta frequency. The hippocampal EEG was recorded during the tasks except for the water maze where it was recorded in a separate period of swimming before training in contrast to our previous recording during training, in case the attachment of the cable was affecting learning. All of the experiments were carried out in the same rats so that differences between effects across tasks could not be attributed to variation in lesion site. The tasks were ordered to produce minimal interference between tasks.

Materials and methods

Animals

Forty-two male Sprague-Dawley rats (290–400 g) were obtained from the University of Otago, Department of Laboratory Animal Sciences. They were maintained in groups of four at a temperature of 22 °C under natural light provided by an external window, supplemented by fluorescent lights (on at 06.00, off at 18.00 h). Water was available ad libitum. Food was available ad libitum except as indicated for specific experiments below.

Surgery

Lesions were obtained by neurotoxin microinjection and this and electrode implantation were carried out stereotaxically (Kopf, USA) under anaesthesia with sodium pentobarbital (60 mg/kg, i.p.) under sterile conditions. Silica capillary tubing (VS-140±40; Scientific Glass Engineering, UK; 140 µm external diameter and 40 µm internal diameter) was used as an injection needle to minimize nontoxic damage to mSUM and immediately dorsal structures. Microinfusions of 0.3 µL of 0.015 M RS-α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA; Tocris Cookson Inc., USA) made up in 0.01 M phosphate buffer (pH 7.4) were made at the rate of 0.1 µL/min via a 10-µL Hamilton syringe connected to an electrical microdrive. Control animals received similar injections of the phosphate buffer. The co-ordinates were AP 4.5, ML 0.8 (at an angle of 6° to the vertical), DV 8.5 from the skull, with the skull flat between lambda and bregma.

The recording electrodes consisted of two stainless steel wires, each Teflon-coated, with a 70-µm outside diameter, twisted together with the tips separated by 2.5 mm, and were implanted with the upper tip in the dentate gyrus of the dorsal hippocampus (Bregma AP 3.5, ML 2.0, DV 3.0 mm for the upper tip from the skull). A stainless steel skull screw served as ground. The electrodes were affixed to the skull using six stainless steel screws and dental acrylic. Immediately after surgery, the neurotoxin-injected rats exhibited minor seizures. These were treated with diazepam (2 mg/kg i.p.) when necessary. After surgery the rats were housed singly. All animals were allowed one week to recover from surgery prior to any testing. Nine rats did not recover from anaesthesia. Before behavioural testing, there were 17 lesion and 16 control rats.

Rats were operated and then tested behaviourally in batches, with group membership counterbalanced within batches, and three such batches were pooled to produce the total numbers given above.

EEG recording

The electrodes were connected via Amp phenol gold connectors to a dual field effect transistor, preamplified (Grass Instruments, Quincy, MA, USA; P511K, 1–30Hz bandpass filter) and extracellular field activity was digitized at 100 Hz for subsequent analysis, as was a second ‘event’ channel (see procedure for individual experiments). The peaks of theta rhythm were detected and frequency calculated by a simple computer algorithm. The algorithm detected peaks and troughs in sequence outside a window that excluded small amplitude oscillations such as gamma waves. This provided very accurate estimates of frequency but did not guarantee that the activity being assessed was theta. All peak detections, and the presence of theta, were therefore individually checked for accuracy by the experimenter and peak detections corrected if necessary.

Scheduling of tasks

The same rats sequentially experienced Open Field, Water Maze, FI, DRL and Fear Conditioning testing. Note that the fear conditioning was run as the final test to prevent any effect of shock administration on the other tests, whereas the open field test, although a test of defence, is not greatly different from the handling which is normal before learning tasks and so was placed before these tasks, which might have affected it. There was a 3-day interval between successive tasks and the total time between the ambulation testing and fear conditioning was 58 days.

Histology and assignment to groups

Animals were deeply anaesthetized (sodium pentobarbital, 60 mg per rat, i.p.) and perfused transcardially with saline followed by 10% formalin. The brains were removed and placed in sucrose–formalin for 3 days, and were frozen and sectioned (40 µm) coronally. The sections were stained with thionin. The lesion positions were checked under the microscope and were reconstructed according to the atlas of Paxinos & Watson (1998).

Based on histological results (see section 3), later analyses were carried out with the rats assigned to the following groups:

MSUM lesion

Lesion of mSUM with an extent from 30% to 90% of the nucleus (7 rats).
Lesion of lateral hypothalamus (LH) unilaterally. The lesions were not in or above mSUM and encompassed an area as big as that of the mSUM lesions (5 rats);

Above-mSUM lesion

Lesion of an area immediately above mSUM, but not encroaching on LH. This area has no specific designation in Paxinos and Watson (1998). The lesions encompassed an area as big as that of the mSUM lesions (5 rats).

 Controls

Vehicle-injected rats with no sign of any lesion (n = 16)

It should be noted that the numbers of rats in each group given above are those entering the study. The same rats were tested consecutively in the Open field, Water Maze, FI and DRL and then, finally, the Fear Conditioning task. Four animals lost their electrodes or became sick over this long period and so the number of animals tested was less than the above numbers in some later tests. Specifically, one control rat lost its electrodes during FI training, two control rats lost their electrodes during DRL training, one lesion rat became sick during DRL training and two control rats became sick after DRL training. All these rats were excluded from final analysis of those and later tests. There were, thus, 11 control rats and 16 lesion rats at the start of the Fear Conditioning task.

Operant testing

A 24.5 × 22.5 × 23 cm operant chamber (Camden Instruments) was used to train and test all rats. The experiments were controlled and data were collected by a BBC microcomputer using the SPIDER real time control system (Paul Fray, Cambridge, UK).

In the present two tasks, EEG was recorded (and the rat connected to the cable) during training only on the last day, so as to minimize the interruption of behaviour by the cable. The responses made by the rat and reinforcements received were recorded on the second EEG channel concurrently. The theta frequency in the 1 s before each bar press was measured.

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FI schedule

After 3 days of increasing periods of food deprivation and 5 days of 23-h food deprivation, the rats entered pretraining. First, the rats were trained with a noncontingent random-time 30-s schedule for four days. A 45-mg reward pellet (Camden Instruments) was delivered noncontingently at the end of the interval (0–60 s) that was produced by a random number generator. The lever was retracted from the box throughout the 30-min training session. For the next four days, the rats were trained on a continuous reinforcement schedule. On this schedule, the first response that occurred after the passage of 60 s was rewarded with one pellet and then the interval was reset. Each rat was run at the same time each day for one 30-min session.

DRL schedule

After FI testing, the rats rested for 3 days, then were placed directly on the DRL schedule (as they did not need pretraining). This schedule required them to withhold a response for at least a preset criterion interval (e.g. 5 s; DRL5) after their last lever press in order to earn a reward. A premature response reset the clock to zero. For the first four days the rats were trained on a DRL5 schedule, and this was followed by four days training on DRL10 and 8 days on DRL15.

Water maze

The water maze consisted of a rigid black plastic pool (150 cm in diameter, 35 cm high), and was placed in a fixed position in the testing room surrounded by various extramaze visual cues, such as a
After the first behavioural test, in the open field, the rats were allowed to have 3 days’ rest. Then the EEG for each rat was recorded for 1 min during swimming in the water maze, but without the platform in the pool. On the following day, the rats started 4 days’ testing with a total of 16 training trials (four trials each day). The rat was placed in the pool facing the wall and allowed 60 s to find the escape platform. If it found the platform within the given time it remained there for 15 s, otherwise it was guided to it and stayed there for 15 s. Then the next trial started. Note that there was no intertrial interval as we wished to match our previous single-day test in this respect (Pan & McNaughton, 1997). On the last day, after the last training trial, a probe test was given in which the rat was required to swim in the pool without the escape platform for 60 s. All rats were released from the north location on the first trial of the first training day and then followed a counterbalanced sequence (NSWE, ENSW, WENS, SWEN). For the probe test, rats were released from the north location.

The swim path was recorded by a video cassette system. The swim distance, speed and escape times were analysed later by a computer image analysis system (HVS IMAGE Ltd, UK).

Data analysis

For the open field, the average theta frequency in each min during movement, the total ambulation in each min and total rearings over the entire 10 min were each submitted to separate analyses of variance for the effect of lesion (and except for rearing) its interaction with time. The ambulation data were first normalized with a square root $\sqrt{x + 0.5}$ transform. The rearing data were normalized with a logarithmic $\log_{10}(x + 1)$ transform. For fear conditioning, the total freezing and average theta frequency in contextual fear and CS fear were each submitted to analysis of variance.

For the FI schedule, the responses of each rat on the last training day were divided, depending on the time of their occurrence within the 60-s fixed interval, into 10-s bins (six bins totally). That is, all responses occurring within the first 10 s of each interval were summed over the entire session to give a response score for the first bin, and similarly for successive 10-s periods. The raw data were then subjected to a logarithmic transformation $\log_{10}(x + 1)$ to normalize the distribution (Zar, 1974), and submitted to ANOVA. The theta frequency in the 1 s before each bar press was measured, and the results were averaged for each bin so that each theta average matched the summed responses for the same time intervals. Then the data were submitted to ANOVA.

For the DRL schedule, the data for the last training day were analysed. The responses were divided on the basis of inter-response time (IRT) into 2-s bins with a total of 15 bins, and with the last bin including all responses greater than 2 s interresponse time. For example, the first bin contained the sum of all responses that had followed a previous response by between 0 and 2 s. The raw data were then subjected to a logarithmic transformation $\log_{10}(x + 1)$ to normalize the distribution (Zar, 1974), and then submitted to ANOVA. The theta frequency in the 1 s before each bar press was measured, and the average of each rat was submitted to ANOVA.

For the water maze, the average swim distance per day, the percentage time spent in the correct quadrant of the pool (the percentage data were submitted to angular transformation to normalize the variance) and number of times passing through the target area during the probe tests, and the average frequency of theta, were each submitted to analysis of variance (ANOVA). The total theta frequency for every second in the 1-min pretraining swim was measured using the interval between the peaks of successive theta waves, and the average frequency for each rat over the entire minute was submitted to ANOVA.

Results

Effects of AMPA injection in the supramammillary region

To our knowledge the neurotoxic effects of AMPA in the supramammillary region have not previously been reported. The pattern of lesioning we observed is significant both for the assignment of animals to groups in the present study (see section 2.5) and for its implications for histochemical division of nuclei in this region.

The histology showed that, in the lesion areas, there was neuronal cell loss and an increase in small glial cells (Fig. 1B). Lesions were found in three distinct and relatively circumscribed areas (Fig. 2): in mSUM, unilaterally in the lateral hypothalamus (LH), and in a region immediately above mSUM that has no specific designation in Paxinos & Watson (1998). Only one of these areas was lesioned in any individual rat. Importantly, no lesion in any rat extended into the mammillary bodies. The lesions seemed to terminate clearly at the border of the mammillary bodies (Fig. 1C).

Restriction of the size of mSUM lesion so as to be specific to mSUM resulted in the lesions being subtotal with an extent from 30 to 90% of the nucleus across the seven rats with specific mSUM damage.

Open field

Figure 3 shows that ambulation in the first 2-min block is highest, then decreases with time, but there was more activity in the lesion groups than control groups ($F_{1, 29} = 4.05, P < 0.016$). Post hoc analysis found that there were significant increases in ambulation only in the mSUM lesion group ($F_{1, 21} = 11.06, P = 0.003$). The ambulation decreased with time in normal, above-mSUM lesion and LH lesion groups, but did so less in the mSUM lesion group (the linear trends of the mSUM lesion group against other groups are significant; $F_{1, 29} = 9.78, P = 0.004$). There were no significant differences in rearing ($F_{1, 29} = 0.85, P = 0.48$; Fig. 3). There were also no significant changes in theta frequency ($F_{1, 29} = 0.36, P = 0.78$; Fig. 3).

Fear conditioning

As shown in Fig. 4, mSUM lesion impaired the learning of contextual fear conditioning. There were significant main effects of Lesion on contextual fear ($F_{3, 23} = 6.32, P = 0.003$), and a significant interaction of Group × Day ($F_{6, 46} = 2.47, P = 0.037$). Post hoc test (ANOVA) showed that only the mSUM lesion significantly decreased contextual fear ($F_{1, 15} = 12.2, P < 0.003$), and showed an interaction of Lesion × Day ($F_{2, 30} = 4.96, P = 0.014$). The lesion did not impair the learning of CS-fear (main effect: $F_{3, 23} = 1.93, P = 0.15$). An analysis of lesion effect by stimulus type (contextual and CS) proved that there were significant differences between the contextual fear phase and CS phase ($F_{3, 23} = 3.08, P = 0.047$).

The averages of theta frequency were < 6.5 Hz in both lesioned and control groups. There was no significant change in theta frequency (Fig. 4) in either the contextual fear phase ($F_{2, 23} = 2.25, P = 0.11$) or the CS phase ($F_{3, 23} = 0.19, P = 0.90$).

FI schedule

Rats in all groups learnt this task very well. The number of bar pressing responses increased with the interval time in all groups.
However, there were different patterns of the learning curves in the different groups (Groups, $F_{3,28} = 3.3$, $P = 0.035$; Group $\times$ Bins, linear, $F_{3,28} = 3.98$, $P = 0.048$). Post hoc analysis showed that, compared to the control group, the LH lesion increased responses over all time bins (Group, $F_{1,18} = 8.59$, $P = 0.0089$; Group $\times$ Bins, linear, $F_{1,28} = 0.25$, $P = 0.62$), but mSUM lesion only significantly increased responses towards the end of the 60 s (Group $\times$ Bins, $F_{1,24} = 9.72$, $P = 0.004$), not the total number of responses (Group, $F_{1,20} = 1.19$, $P = 0.29$). There was no significant change in the above-mSUM lesion group (Group, $F_{1,18} = 1.14$, $P = 0.30$; Group $\times$ Bins, linear: $F_{1,28} = 0.05$, $P = 0.82$).

The theta frequency (Fig. 5) also changed significantly ($F_{3,28} = 3.18$, $P = 0.039$). Post hoc test showed that only the mSUM lesion significantly decreased theta frequency to 6.7 from 7.0 Hz compared to the control group (Group, $F_{1,20} = 4.34$, $P = 0.05$; Group $\times$ Bins: $F_{5,100} = 0.82$, $P = 0.54$).

**DRL schedule**

In this task, all groups showed learning of the requirements of the schedule, with a peak of responding at intermediate IRTs. However, even the control groups had not learned very well by the last training day, with peak IRTs in the region of 12 s on a 15-s DRL schedule. The results of the experiments are shown in Fig. 6. The lesions produced premature responding (Groups $\times$ Bins, $F_{14,238} = 2.16$, $P = 0.0001$). The response peak shifted to 9 s in the mSUM and LH groups. Post hoc analysis showed that, compared to the control groups, significant change occurred in the mSUM group (Group $\times$ Bins, $F_{14,238} = 2.50$, $P = 0.0024$) and in the LH group (Group $\times$ Bins, $F_{14,224} = 4.15$, $P < 0.0001$).

The average theta frequency before nonreinforced responses differed between groups ($F_{2,25} = 3.33$, $P = 0.036$), but theta frequency decreased significantly only in the mSUM lesion groups.
compared with the control groups, as shown by post hoc test ($F_{1,17} = 5.83, P = 0.027$). The average theta frequency compared to the control groups decreased by 0.4 Hz, and dropped from 7.2 to 6.8 Hz (Fig. 6). In contrast, the average theta frequency before reinforced responses showed no significant change between groups ($F_{3,25} = 2.94, P = 0.53$).

Water maze

Learning over days in the water maze was good in all groups (Fig. 7). An ANOVA on path length showed there were no significant group differences (Group, $F_{3,29} = 0.44, P = 0.73$; Group $\times$ Days, $F_{9,87} = 0.67, P = 0.74$). The mSUM and above-mSUM groups may have shown a slight deficit in learning over trials within days but the differences did not achieve acceptable levels of significance. (Group $\times$ Trials, $F_{9,87} = 1.60, P = 0.13$; Fig. 7).

During the 60-s probe trial, a bias for the target quadrant was shown in all groups (compared to chance level 25%, Control, $t = 8.73, P < 0.001$; mSUM, $t = 4.55, P < 0.01$; Above-mSUM, $t = 16.3, P < 0.001$; LH, $t = 5.34, P < 0.01$). There were no significant group differences ($F_{3,29} = 1.61, P = 0.21$) in percentage time

FIG. 4. The effects of the different lesions shown in Fig. 2 over 3 days of fear conditioning as assessed by number of seconds freezing within the 20-s period before stimulus presentation (context) or within the 20-s presentation of the stimulus (CS). The bottom panel shows the frequency of theta in these 20-s periods measured on day 3. Lesions of mSUM impaired fear conditioning to context but not CS as demonstrated by decreased freezing. There were no significant changes in theta frequency. The bars represent 2 SEM for between-group comparisons based on the residual error term of the ANOVA.
spent in the correct quadrant (Fig. 7). The target area crossings were also not significantly different ($F_{3,29} = 0.40, P = 0.76$; Fig. 7).

Theta frequency during the 1-min swimming test (Fig. 7) was significantly different between groups ($F_{3,29} = 4.83, P = 0.0075$). Post hoc test showed that theta frequency was significantly reduced (by 0.4 Hz) only in the mSUM lesion group ($F_{1,21} = 13.36, P = 0.0015$). The swimming speeds during the 1-min test were not significantly different between groups ($F_{3,29} = 0.76, P = 0.53$).

**Summary of behaviour in relationship to changes in theta frequency**

The mSUM lesion-induced changes in theta and the relationship of these changes to the measurement of theta across the various experiments is summarized in Table 1. In the open field and fear conditioning experiments, defensive behaviour decreased as indicated by increased movement relative to control rats. In the same experiments theta frequency (uncorrected for the amount of movement) was not measurably changed. In the operant tests of behavioural inhibition the amount of movement associated with a barpress can be presumed to be relatively unchanging as bar pressing is stereotyped. In these experiments, nonrewarded bar presses were associated with reduced theta frequency, but (in DRL) the rewarded bar presses were not associated with any change in frequency. During swimming in the water maze, theta frequency was reduced but the speed of swimming was unchanged.

**Summary of behaviour in relation to effects of hippocampal lesions**

A comparison of activity changes in the different tasks produced by mSUM lesions with reported effects of hippocampal lesions is given in Table 2. In general the effects are similar, although smaller and less consistent.

**Discussion**

**Lesions**

Our main target, mSUM, is a relatively small nucleus. The use of silica capillary tubing (140 μm external diameter) as an injection needle minimized physical damage to the mSUM and structures in the path of the needle. There was no observable trace of a needle-induced lesion. Lesions were restricted to the mSUM when this structure was damaged and appeared to terminate clearly at the border of the mammillary bodies. In other unpublished experiments using ibotenic acid rather than AMPA, we found that damage extended into the mammillary bodies. This suggests that AMPA is particularly suitable for producing mSUM lesions. It also confirms the placement, in Paxinos & Watson (1998), of the lower border of mSUM on a line joining the centres of the bilateral mammillothamic tracts.

AMPA also produced quite distinct small lesions of two adjacent nuclear areas: unilaterally in the lateral hypothalamus (LH), and in a region immediately above mSUM that has no specific designation in Paxinos & Watson (1998). The LH lesions were in an area that is classified as the lateral part of the supramammillary area, rather than lateral hypothalamus, by Swanson (1982) and Risold & Swanson (1997). There was no obvious gliosis between these areas and mSUM, suggesting that our three groups have quite distinct damage to each of three different nuclear areas. The lack of damage between the areas is also consistent with the view that AMPA is destroying neural cells without affecting fibres of passage (Coyle et al., 1978). The lack of damage to the mammillary bodies rules out the possibility that the behavioural effects of mSUM lesions made by AMPA in the experiments reported below resulted from lesions of the mammillary bodies.

While restriction of the lesions to mSUM allows us to be reasonably sure that the behavioural and electrophysiological effects obtained were specific to mSUM, failure to obtain effects must be treated with caution. The requirement for specific lesions resulted in the mSUM lesions being subtotal with an extent from 30 to 90% of the nucleus across the seven rats with specific mSUM damage. A failure to obtain a large behavioural effect (as in the water maze experiment) could therefore be due to insufficient damage.

**Defensive behaviour**

Previous data (Silveira et al., 1993; Beck & Fibiger, 1995; Silveira et al., 1995) have suggested that mSUM might play an important role in emotional behaviour. The present experiments have obtained the first direct evidence of such a role. mSUM lesions changed exploratory activity (ambulation) and contextual fear conditioning (but did not affect fear conditioning to an explicit CS).
consistent with the changes in fos-like immunoreactivity in open field
(Wirtshafter et al., 1998), in the elevated plus-maze (Silveira et al.,
1993) and in fear conditioning (Beck & Fibiger, 1995) tasks, so we
can conclude that mSUM is involved in controlling defensive
behaviours, at least for the first and second levels of defence defined

The mSUM lesions increased ambulation in the open field, but
changes in rearing did not reach significance in the present
experiments. A reason for this may be that the EEG cable connected
to the head of the rats appears to have resulted in very low levels of
rearing in control rats. As well as decreasing rearing, lesion of the
septo-hippocampal system or temporary chemical interruption of
hippocampal function increase activity in the open field (Cannon
et al., 1992; Rossi-Arnaud & Ammassari-Teule, 1992; Stefanski et al.,
1993), so the effects of mSUM lesion in the open field were broadly
similar to the effects of hippocampal lesion.

The effect of mSUM lesion in the fear-conditioning task was also
similar to that of the septo-hippocampal lesions. The amygdala
complex is a key structure in controlling both contextual and CS
conditioning of fear (LeDoux, 1993), but the hippocampal system
appears to be involved in controlling contextual fear conditioning
only (Phillips & LeDoux, 1994). Thus, the behavioural deficits
following mSUM lesions in both the first and second levels of
defence are essentially the same as those following septo-hippocam-
apal lesion. Both an increase in ambulation and a decrease in freezing
mean that movement is increasing, i.e. there is hyperactivity, which is
a common effect of hippocampal dysfunction (Cannon et al., 1992;
Rossi-Arnaud & Ammassari-Teule, 1992; Stefanski et al., 1993;
Phillips & LeDoux, 1994).

**Behavioural inhibition**

According to Gray (1982) and Gray & McNaughton (2000), the
septo-hippocampal system is the main substrate of ‘behavioural
inhibition’, a neuropsychological process, which is important in
anxiety. Of particular relevance to the present experiments, hippo-
campal lesions increase the response rate in the FI task
(Manning & McDonough, 1974), and especially in the DRL task
(Jarrard & Becker, 1977; Acsciá et al., 1986; Woodruff et al., 1987).

In the present DRL test, rats with mSUM lesion showed an
increase in nonrewarded response rate relative to controls and a shift
in the peak of the DRL curve to earlier interresponse times. This is
similar to the effects of hippocampal lesions. The fact that the curve
retained its shape is important as it indicates that the rats were still
responding to the task requirements but were less able to withhold
responding. This result suggests that the effects of mammillary body
lesions on DRL reported by Tonkiss & Rawlins (1992) may have
been the result in whole, or at least in part, of the extensive damage
they produced to mSUM. Further experiments will be required to
determine whether there is a specific role of the mammillary bodies in
DRL.

In the present FI task, mSUM lesions increased responding only in
the later part of the interval. This effect is only partially like that
reported for hippocampal lesions and systemic injections of
chloridiazepoxide. However, it is similar to the effects of chloridiazep-
oxide in rats treated with naloxone (Tripp & McNaughton, 1992) and
in a later experiment we found that injections of chloridiazepoxide in
mSUM produced similar sized effects on FI and theta frequency to
those of systemic chloridiazepoxide (Woodnorth & McNaughton,
2002). It seems likely therefore that the current result is due to the
small size of the lesions (30–90% of mSUM). Nonetheless, the fact
that the earlier parts of the schedule were not affected, unlike the later
parts, goes against the idea that the effect is due to a loss of
behavioural inhibition, which might be presumed to be greatest in the
earlier parts of the interval.

LH lesion also produced increased responding in both the FI and
DRL tasks, but did not change theta frequency. Because the pattern of
hyperactivity in the FI task was different for LH and mSUM lesions,
the behavioural effects of mSUM lesions are unlikely to be due to
incidental damage to LH, and *vice versa*.

mSUM lesion produced hyperactivity in the DRL task, which is
consistent with the effects of mSUM lesions in the open field and fear
conditioning tasks, and similar to the effect of hippocampal
dysfunction in these tasks. This result suggests that mSUM is
involved in the behavioural inhibition function of the hippocampus.
The results with the fixed interval schedule are less consistent with
this hypothesis.

**Spatial learning**

We previously reported (Pan & McNaughton, 1997) that infusions of
CDP into the mSUM region produced very modest, but statistically
significant, impairments in learning in the water maze. However, the
learning impairment did not become evident until about the 10th trial
in the 20 trials of continuous training. Because the rats were exposed to the water for a relatively long time (> 10 min), the decrease in body temperature in the later stages of training will have made a significant contribution to the observed learning impairment. The present experiments avoided this problem because there were only four trials per day, which should not have produced a level of cooling sufficient to alter theta rhythm or learning (Pan & McNaughton, 1997). The results showed that neither lesions of mSUM, lesions of LH nor lesions immediately above mSUM impaired spatial learning over training days or on the probe trial. This lack of behavioural effect in the mSUM group was despite the fact that mSUM lesion modestly decreased theta frequency in the pretraining swimming test. This may represent a true lack of involvement of mSUM in spatial reference memory. It is consistent with reports (Sziklas & Petrides, 1993; Sziklas et al., 1995) that lesions restricted to the mammillary and supramammillary nuclei did not impair performance in a radial maze. Thus CDP injected into mSUM may have been effective by facilitating the decrease of body temperature in the cold water exposure during the training. This would be consistent with the fact that mSUM is activated by cold and swim stress (Kiyohara et al., 1995; Miyata et al., 1995; Cullinan et al., 1996). Another alternative is that mSUM is more involved with spatial working memory (which would have had a major role with 20 trials per day) than with consolidation of spatial reference memory (which should have occurred with training over 4 days). There is a hint of an effect on working memory in our present results but, if present, it is not specific to mSUM. There is no evidence from any of these results that mSUM per se is involved in spatial reference memory or its formation. However, the reduction produced in theta frequency was not as great as is obtained with systemic injections of CDP or cooling that clearly impair water maze performance. It may be therefore that larger lesions (the present ones damaged between 30 and 90% of the nucleus) would have had both a larger effect on theta and a substantial effect on water maze learning. A difficulty with testing this hypothesis is that large lesions specific to mSUM would require multiple injections and be likely to have a high failure rate.

### Table 1. Changes in theta frequency produced by mSUM lesions and the associated changes in movement during the periods that theta was measured

<table>
<thead>
<tr>
<th>Theta frequency</th>
<th>Amount of movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open field</td>
<td>=</td>
</tr>
<tr>
<td>Fear conditioning</td>
<td>=</td>
</tr>
<tr>
<td>Bar pressing (nonrewarded)</td>
<td>=</td>
</tr>
<tr>
<td>Bar pressing (rewarded)</td>
<td>=</td>
</tr>
<tr>
<td>Swimming</td>
<td>↓</td>
</tr>
</tbody>
</table>

The symbols show that theta frequency or amount of movement decreased (↓), increased (↑), or was not changed (=) relative to control rats.
The role of mSUM in modulating theta frequency in behaving rats

Previous data have shown that mSUM influences the frequency of theta elicited by stimulation of the reticular formation in anaesthetized rats (Kirk & McNaughton, 1993; Kirk & McNaughton, 1991; Oddie et al., 1994) to a very large extent. However, it does so to a lesser extent in freely moving rats (McNaughton et al., 1995) and may not be at all involved in controlling theta elicited by some classes of movement (Thinschmidt et al., 1995). In the present experiments, the effects of mSUM lesions on theta frequency varied across tasks (Table 1). However, mSUM lesions and CDP in mSUM (Pan & McNaughton, 1997) do decrease theta frequency during swimming both prior to and during training, respectively, without changing swimming speed. This suggests strongly that mSUM may modulate some but not all classes of movement-elicited theta as well as some, and perhaps not all, classes of nonmovement theta. These data are consistent with the fact that mSUM controls reticularly elicited theta to a very large extent in anaesthetized animals but only partially in freely moving animals.

The failure of mSUM lesions to produce observable reductions in theta frequency in defensive tasks is not conclusive evidence of a lack of contribution from mSUM to theta in such tasks. During the measurement of theta, the amount of movement differed substantially between the experimental and control groups in the open field and fear conditioning tasks. Theta frequency increases linearly with the amount of movement (Vanderwolf, 1969; McFarland et al., 1975; Morris et al., 1976; Rivas et al., 1996; Slawinska & Kasicki, 1998). It is likely therefore that the greater movement of mSUM rats in the defensive tasks produced an increase in theta frequency that counteracted the decrease in frequency produced by mSUM lesion. This suggests that mSUM could be involved in modulating the frequency of movement theta even if the contribution of mSUM to theta frequency appears modest in behaving rats. Equally, mSUM cannot be the only nucleus controlling theta frequency because theta activity never totally disappears in freely moving rats after mSUM lesions even with large lesions (Thinschmidt et al., 1995; McNaughton et al., 1995). It seems likely that movement activates some pathway in addition to mSUM and that this additional pathway plays a dominant role in controlling theta frequency in behaving rats.

In contrast, the amounts of movement during the measurement of theta were the same in the two groups in the water maze where mSUM lesion decreased theta frequency by 0.4 Hz during the 1-min pretraining swim. We also previously found that CDP injected into mSUM decreased theta frequency by 0.35 Hz on the first training trial. In all these cases, different treatment groups swam at a similar speed. Likewise, bar pressing is likely to be a fairly stereotyped response and the speed of movement should be similar in the experimental and control groups although the force, which we did not measure, could have been different. Theta frequency changes are related to speed rather than force and occur immediately in advance of the movements with which they are related (Morris & Hagan, 1983). Therefore, in the FI and DRL tasks, theta frequency was measured for the 1 s before bar pressing occurred. It was found that mSUM lesion decreased theta frequency before nonrewarded bar pressing in both the FI and DRL tasks. All of the above data are consistent with the idea that incomplete lesions of mSUM tend to reduce theta frequency in all of the tasks tested once changes in movement are controlled for. They leave open the question of whether, in defensive tasks, countervailing changes in behaviour are the result of the changes in theta control.

Variation in amount of movement seems a less likely explanation for the difference between rewarded and nonrewarded pressing in the DRL task. (There are relatively few rewarded responses in the FI task and so we did not separate them from nonrewarded responses nor compare them with the DRL task.) mSUM lesion did not change theta frequency prior to rewarded responses, but decreased the frequency prior to nonrewarded responses. These results suggest a close relationship between learning errors and theta frequency. If, as we have argued, lever pressing is stereotyped, then the amount of movement should not be different between these two. Further, the lever press is completed before the animal can know whether reward or nonreward will be delivered. Theta frequency can be determined by environmental factors quite independent of movement (Sainsbury, 1998). What factors are involved in modulating theta before rewarded and nonrewarded responses remain to be determined.

Theta frequency and behaviours

Given that mSUM lesions modestly decrease theta frequency in behaving rats, the question arises as to whether this modest change is the basis of the observed changes in behaviour. Although a great deal has been learned about the functions of the hippocampal formation over the years, the role of theta activity, particularly theta frequency, in hippocampal function remains controversial. No one has been able to study the function of theta frequency directly because no method has been available to alter theta frequency in isolation. The function of theta frequency has been suggested only by correlations between it and certain behaviours. For example, some suggest that theta is merely an epiphenomenon of movement (Vanderwolf, 1971; Whishaw & Vanderwolf, 1973; Morris & Hagan, 1983). Others believe that theta frequency may modulate memory processes or

<table>
<thead>
<tr>
<th>Tasks</th>
<th>mSUM lesion (this study)</th>
<th>Published hippocampal lesion data (papers cited)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open field</td>
<td>↑</td>
<td>↑ (Cannon et al., 1992; Rossi-Arnaud &amp; Ammassari-Teule, 1992)</td>
</tr>
<tr>
<td>FI (rate of response)</td>
<td>↑</td>
<td>↑ (Manning &amp; McDonough, 1974)</td>
</tr>
<tr>
<td>DRL (rate of response)</td>
<td>↑</td>
<td>↑ (Jarrard &amp; Becker, 1977; Aschádi et al., 1986; Woodruff et al., 1987; Tonkiss et al., 1998)</td>
</tr>
<tr>
<td>Learning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water maze</td>
<td>=</td>
<td>↓ (Morris et al., 1982; Sutherland et al., 1983; Moser et al., 1995; Aznar et al., 1998)</td>
</tr>
</tbody>
</table>

↑, increase; ↓, decrease; =, no change.
spatial mapping (O’Keefe & Nadel, 1978; Vertes, 1986; Miller, 1991; O’Keefe & Recce, 1993), somatosensory integration (Bland, 1986) or sensory inhibition (Sainsbury, 1998). Moreover, the effects of theta in modulating behaviour may be quite frequency specific or depend on particular frequency bands (Gray & Ball, 1970; Gray, 1970; Gray, 1982; Snape et al., 1996; Williams & Gray, 1996).

If theta frequency in the hippocampus is functionally significant, its functions should be hippocampal-dependent. Spatial learning is the best-studied behaviour with a high sensitivity to hippocampal dysfunction. Our studies showed that mSUM lesion or CDP in mSUM (Pan & McNaughton, 1997) decreased theta frequency by ≈0.4 Hz, but had only modest effects on spatial learning working memory and apparently no effect on spatial reference memory or its formation. This does not support the idea that small changes in theta frequency should be enough to modulate all hippocampal-dependent behaviours. However, this does not mean spatial learning is not theta-dependent because large changes in theta frequency by different, albeit less specific, methods (CDP-i.p. or ‘long cooling’) do impair spatial working memory (Pan & McNaughton, 1997).

The present study did not systematically measure the amplitude or power of theta, which is likely also to play a role in hippocampal function and behaviours. However, there were no obvious changes in power unrelated to decreases in frequency and concurrent effects on both are usually seen with anxiolytic drug treatment (McNaughton & Sedgwick, 1978).

The role of mSUM in behaviour

The present study showed that mSUM lesions produced hyperactivity in defensive tasks (movement increased) and nonreward schedule tasks (bar pressing increased), which is similar to that of hippocampal lesions, but there was little effect on spatial learning in the water maze task, which is hippocampal-dependent (Table 2). This pattern of changes across tasks suggests that mSUM is more important for the control of emotional behaviours than cognition (as exemplified by spatial learning). This differentiation is consistent with previous data on c-fos activity (Silveira et al., 1993; Beck & Fibiger, 1995; Wirtshafter et al., 1998) and on lesions of the mammillary-SUM area (Jarrard et al., 1984; Tonkiss & Rawlins, 1992; Siklás & Petrides, 1993; Siklás et al., 1995).

But, how did mSUM lesions impair emotional behaviours in ways that are similar to those produced by hippocampal lesion if the effect of the mSUM lesion is not being mediated by dysfunction of the hippocampus in general and theta in particular? The known anatomy of mSUM may provide a provisional answer.

In addition to bidirectional connections with the septo-hippocampal system, mSUM connects extensively with many components of the mesencephalic limbic system. These include the medial preoptic area, anterior hypothalamus, lateral hypothalamus, central medial nucleus of thalamus, central grey (CG), dorsal raphe, median raphe and amygdala (Shibata, 1987; Vertes, 1992; Hayakawa et al., 1993; Thinschmidt, 1993; Risold & Swanson, 1997; Risold et al., 1997). This suggests that mSUM may relay information travelling from the hippocampus to the mesencephalic limbic system, as well as in the other direction. The mesencephalic limbic system has long been considered essential for emotional behaviour (MacLean, 1949; MacLean, 1990). The previous sections focused on how the entire pattern of mSUM lesion impairing behaviours could be due to changes in afferents to the hippocampus that control theta. However, it could equally be due to disconnection of hippocampal efferents to the mesencephalic limbic system via mSUM.

If this is true, mSUM lesion can only partly cancel the output of hippocampus to areas such as the amygdala. The mSUM lesion destroys indirect connections but leaves the direct connections intact. Consistent with this, the effects of mSUM lesion are much smaller than the effects of lesion of the fornix or dorsal hippocampus previously reported on contextual fear conditioning (Phillips & LeDoux, 1994; Phillips & LeDoux, 1995), or on FI (Manning & McDonough, 1974) and DRL tasks (Jarrard & Becker, 1977; Acsádi et al., 1986; Woodruff et al., 1987; Tonkiss et al., 1998).

A number of data suggest that the hippocampus is important for both cognition and emotion. However, the hippocampus need not have dual roles, one in cognition and one in emotion. It may act in a similar manner on structures processing more cognitive or more emotional stimuli (Gray & McNaughton, 2000). While the areas of fos activity in some emotional tasks include many parts of the limbic system, the hippocampus appears surprisingly silent (Sandner et al., 1993; Silveira et al., 1993; Kiyohara et al., 1995; Miyata et al., 1995; Cullinan et al., 1996; Wirtshafter et al., 1998). The amygdala (and its output to CG) is crucially involved in the control of both positive and negative emotions (LeDoux, 1995; Fendt & Fanselow, 1999). However, although it has similar effects in many tasks to hippocampal lesions, it is not involved in water maze learning (Gray & McNaughton, 2000). Thus the hippocampus may be centrally involved in cognitive processing. But this purely cognitive processing may function to produce significant emotional output (McNaughton, 1997) through interactions between the hippocampus and amygdala or central grey while producing more purely cognitive output when the hippocampus interacts with other structures.

mSUM may then be one bridge between the hippocampus and amygdala and/or CG creating a route for cognitive–emotional interactions. It may modulate theta frequency mainly through ascending connections to the medial septum, but have a significant additional role in emotional function through descending connections from the lateral septum.

Acknowledgements

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Abbreviations

CDP, chloridazepoxide; CG, central grey; CS, conditional stimulus; DRL, differential reinforcement of low rates of response schedule; FI, fixed interval schedule; IRT, inter-response time; LH, lateral hypothalamus; mSUM, medial supramammillary nucleus; US, unconditioned stimulus.

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