Cognitive, Affective, & Behavioral Neuroscience
2002, 2 (1), 76-83

Similar effects of medial supramammillary or systemic injection of chlordiazepoxide on both theta frequency and fixed-interval responding

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The frequency of theta activity may be important for hippocampal function. Anxiolytic drugs reduce theta frequency and have behavioral effects that are similar to those of hippocampal lesions. The effect of the anxiolytic benzodiazepine chlordiazepoxide (CDP) on theta frequency is partially mediated by the medial supramammillary nucleus (mSuM), part of an ascending theta-activating system. Rats were trained on the hippocampal-sensitive fixed-interval 60-sec schedule (FI60). CDP (5 mg/kg i.p.) released responding suppressed by nonreward, seen as increased leverpressing, and reduced theta frequency concurrently. Microinfusion of CDP (20 μg in 0.5 μl saline) into mSuM had as large effects on both frequency and behavior. Other nuclei mediate the benzodiazepine reduction of theta frequency in the open field and the water maze. But the mSuM appears to be the major, if not sole, nucleus controlling theta frequency and, so, hippocampal-mediated behavioral inhibition in the FI60 lever task.

Rhythmical slow activity, or theta rhythm, is a high-voltage extracellular waveform present in the hippocampal formation of a number of mammalian species (Black & Young, 1972; Kemp & Kaada, 1975; Sainsbury, 1970; Stewart & Fox, 1989b; Winson, 1972), including humans (Sano, Mayanagi, Sekino, Ogashiwa, & Ishijima, 1970). In the rat, theta is prominent during voluntary movements (Slawinska & Kasicki, 1998; Vanderwolf, 1969) and has been associated with basic psychological functions, such as arousal (Green & Arduini, 1954), orienting (Grastyan, Lissak, Madarasz, & Donhoffer, 1959), attention (Bennett, 1975), sensorimotor processing (Bland, 1986), and sensory inhibition (Sainsbury, 1998). Numerous studies have also linked theta with higher order cognitive processes (Ammassari-Teule, Maho, & Sara, 1991; Destrade, 1982; Elazar & Adey, 1967; Givens & Olton, 1994; Grastyan, Karmos, Vereczkey, & Kellenyi, 1966; Landfield, 1977; Landfield, McGaugh, & Tusa, 1972; Wetzel, Ott, & Matthies, 1977; Winson, 1978), and with emotion (Graeff, Quintero, & Gray, 1980; Gray, 1972; Montoya, Heynan, Faris, & Sainsbury, 1989; Snape, Grigoryan, Sinden, & Gray, 1996; Williams & Gray, 1996). What is often common to these latter two perspectives is the idea that the specific frequency of theta is critical for the behavioral output. Several major theories identify the frequency at which theta occurs as important for the overall functioning of the hippocampus (Bland, 1986; Miller, 1991). In particular, it has been proposed that the capacity of anxiolytic drugs to alter the control of theta frequency (Gray & Ball, 1970; McNaughton, Richardson, & Gore, 1986; McNaughton & Sedgwick, 1978; Zhu & McNaughton, 1994) implicates the hippocampus in the behavioral effects of these drugs and, hence, in the control of anxiety (Gray & McNaughton, 2000).

High-frequency stimulation of brainstem sites elicits theta (Green & Arduini, 1954; Petsche, Stumpf, & Gogolak, 1962), the most effective site being the nucleus reticularis pontis oralis (RPO; Vertes, 1980). Theta is paced by cells in the medial septum/vertical limb of the diagonal band of Broca (MS/vDBB). A population of MS/vDBB neurons fire in a constant phase relationship with theta (Apostol & Creutzfeldt, 1974; Brazhnik & Vinogradova, 1986; Gogolak, Stumpf, Petsche, & Sterc, 1968; Petsche et al., 1962; Stewart & Fox, 1989a), and lesions of the MS/vDBB or its main afferent pathway, the fimbria/fornix, abolish theta (Buzsaki, Leung, & Vanderwolf, 1983; Green & Arduini, 1954; Rawlins, Feldon, & Gray, 1979; Sainsbury & Bland, 1981; Stumpf, 1965). It was once thought that the MS/vDBB also encoded the frequency of theta. However, it has been demonstrated that the septal region receives only sparse input from the RPO (Vertes, 1986), and anatomical and physiological evidence suggests that information received by the MS/vDBB has already been coded for frequency elsewhere.

Kirk and McNaughton (1991, 1993) proposed that the medial supramammillary nucleus (mSuM) is one site where tonic (intensity) information from the brainstem is transduced to phasic (frequency) information. They found that, during RPO stimulation, cells in the mSuM of anesthetized rats fire rhythmically and in phase with ongoing theta and persist in firing rhythmically after the MS/vDBB is inactivated (Kirk & McNaughton, 1991). With procaine mapping and RPO stimulation (Kirk & McNaughton, 1993), theta amplitude, but not frequency, was reduced by procaine

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rostral to the mSuM, whereas frequency was reduced by procaine in the pathways from the RPO to the mSuM. Procaine in the mSuM itself reduced both the frequency and amplitude of theta. A transduction role for the mSuM of brainstem impulses bound for the medial septum is consistent with tracing studies that have identified projections to the mSuM from the RPO (Vertes & Martin, 1988) and other brainstem structures (Hayakawa, Ito, & Zyo, 1993) and from the mSuM to the medial septum via the median forebrain bundle (Borhegyi, Magloczky, Acsady, & Freund, 1998; Shepard, Mihailoff, & German, 1988; Vertes, 1988, 1992).

In awake rats, the anxiolytic benzodiazepine chlordiazepoxide (CDP) injected into the mSuM reduced the frequency of theta elicited by RPO stimulation, but to a lesser degree than it did under anesthesia (McNaughton et al., 1995). These findings have two important implications. First, the neural mechanism of theta frequency control by the mSuM is likely to be GABAergic inhibition, which benzodiazepines enhance (Olsen, 1982). Second, frequency is not determined solely by the mSuM in the awake rat, whereas it appears to be in the anesthetized preparation. Another nucleus or other nuclei must augment frequency control by the mSuM under normal behavioral conditions, and recent results from our laboratory suggest that the specific sites involved may vary according to conditions (Woodnorth, Kyd, Logan, Long, & McNaughton, 2002). Nonetheless, according to the view that theta frequency is important for hippocampal functioning, the mSuM would be expected to exert some influence over behaviors under hippocampal control.

To date, only one mSuM study has directly compared performance on a hippocampal-sensitive task with ongoing theta. Pan and McNaughton (1997) found that in the Morris water maze, intracranial injection of CDP into the mSuM produced only a small reduction in the frequency of theta and a similarly modest impairment of spatial learning. They argued that the broad degree to which theta is affected will be associated with the level of behavioral dysfunction in tasks requiring the hippocampus, since in the same and previous experiments (McNaughton et al., 1995; McNaughton & Morris, 1987), the size of treatment effects on theta frequency matched the size of their effects on spatial learning.

The aim of the present experiment was to investigate the effects produced on behavior and theta frequency by injecting CDP into the mSuM in a task involving nonspatial behavioral inhibition (Gray & McNaughton, 2000), as opposed to a task requiring spatial memory or exploratory movement. One such task is acquisition of a fixed-interval (FI) schedule, where animals typically learn to suppress their responding during periods of frustration generated by nonreward. FI responding is released from suppression after systemic injection of both classical anxiolytic drugs such as CDP and novel anxiolytic drugs such as buspirone (Panickar & McNaughton, 1991; Zhu & McNaughton, 1995). Both classes of anxiolytics reliably reduce theta frequency (McNaughton & Coop, 1991; McNaughton et al., 1986). FI responding is also released in rats with septal or hippocampal lesions (see Gray & McNaughton, 1983). Since the mSuM is involved in emotion (Beck & Fibiger, 1995; Sandner et al., 1993) as well as in the control of theta frequency, it was our prediction that CDP would act specifically at the mSuM during FI and that a systemic injection of the drug would thus produce similar effects on theta frequency and behavior.

GENERAL METHOD

Animals

Forty-one adult (250–550 g) male Sprague-Dawley rats were used for this study. They were obtained from the University of Otago Department of Laboratory Animal Sciences. The rats were housed individually in hanging wire-mesh cages. Ten days post surgery, the animals were gradually introduced to a 23-h food deprivation schedule over 4 days. Subsequent to this, they were maintained on 23-h food deprivation for at least 10 days before the commencement of training. During training and testing, the animals were fed in their home cages for 1 h immediately after their daily session.

Surgery

All the rats were implanted with recording and stimulating electrodes. Those rats that were originally assigned to receive intracranial injections ($n = 30$) were also implanted with cannulae. Atropine sulphate (25 mg/kg) was administered prior to surgery in order to reduce respiratory congestion. Electrodes and cannulae were stereotaxically implanted under anesthesia with sodium pentobarbital (60 mg/kg). Recording electrodes consisted of two strands of Teflon-coated stainless steel wire (70-μm outside diameter), which were twisted together, with the tips separated by 2.0 mm, and implanted in the subicular region of the dorsal hippocampus (bregma A-P 6.0, M-L 2.0, D-V 5.0). A stimulating electrode with a tip separation of 0.5 mm was implanted into the region of the nucleus pontis oralis (RPO) of the rostral pons (bregma A-P 7.0, M-L 1.6, D-V, 8.5). For those animals used for intracranial testing, a 25-gauge guide cannula was lowered either 8.0 or 8.5 mm toward the mSuM, using coordinates 4.5 mm posterior to bregma and 0.9 mm lateral to the midline at an angle from the vertical of 6°. An obdurator (modified insect pin) was inserted into the guide cannula to prevent occlusion. All coordinates were determined with reference to the skull surface and were obtained from the stereotaxic atlas of Paxinos and Watson (1998). A ground electrode consisting of a length of uninsulated silver wire (0.25-mm diameter) was wound around a stainless steel skull screw. All the electrodes were inserted via Amphenol gold pins into a McIntyre miniconnector that was secured to the skull with dental cement.

Drug and Injection Procedures

Systemic injections. Rats receiving intraperitoneal (i.p.) injections were injected 10 min prior to testing. Injections were either saline (0.9% NaCl; 1 ml/kg) or CDP hydrochloride (5 mg/kg, dissolved in saline, 5 mg/ml; Roche Laboratories).

Intracranial injections. Ten minutes prior to testing, the obdurator was removed from the guide cannula, and the injection cannula was inserted. The injection cannula consisted of silica capillary tubing (VS-140-40, Scientific Glass Engineering, U.K.; 140 μm external diameter and 40 μm internal diameter) with one end glued into a stainless steel collar. When the collar was flush against the guide cannula, the injection cannula extended 1 mm into the brain beyond the tip of the guide. Polythene tubing connected the injection cannula to a 10-μl Hamilton syringe, which was driven by an electrical microdrive. Drugs were infused at a volume of 0.5 μl over a period of 2.5 min, after which the injection cannula was removed and the obdurator was reinserted. The rats received either saline or CDP (40 mg/

ml, dissolved in saline). The movement of, and the lack of compression of, an air bubble in the polythene tubing was used to monitor the success of the infusions.

Owing to the brittle nature of the silica tubing and the difficulty of inserting this into the very small aperture of the guide cannula in a fully conscious rat, the silica injection cannulae occasionally snapped off in the guide, rendering further intracranial injections impossible. When this occurred before a rat had received a drug on the 1st day of FI training ($n = 3$), the animal was immediately reassigned to one of the systemic groups. When a cannula breakage occurred after Day 1 of FI training, the rat was retired from the study.

Apparatus and Procedure

Training and testing took place in a Campden Instruments Operant Chamber (24.5 × 22.5 × 23 cm), modified so that 45-mg food pellets (Campden Instruments, U.K.) were delivered to an aluminum dish secured to the grid floor and a recording cable could extend through the ceiling into the chamber. The chamber was controlled by a BBC-B microcomputer programmed in BBC BASIC with SPIDER real-time commands (Paul Fray, Cambridge, U.K.). Individual rats had their operant sessions at the same time on each day of the procedure.

Pretraining. Following 10 or more days of 23-h food deprivation, the rats were trained on a noncontingent random time 30-sec schedule, wherein single food pellets were delivered at intervals of between 1 and 60 sec after the previous delivery. The rats received four 30-min pretraining sessions on consecutive days.

Continuous reinforcement. During 30-min continuous reinforcement (CRF) sessions, a lever was extended into the chamber, and each leverpress made by the rat was rewarded with a food pellet. On the 4th day of CRF and on every subsequent day of the procedure, the cable was suspended into the chamber and connected with the rats' head connectors. The rats received four CRF sessions on consecutive days unless, on the 4th day, their leverpress responding had dropped by more than 30% from the previous session. In these cases, a fifth CRF session was run the following day.

Fixed interval. For the remainder of the experiment the rats were trained on an FI 60-sec (FI60) schedule. In this schedule, the first leverpress response of the session is rewarded, and this response starts the first 60-sec interval. The rats received a reward only after 60 sec had elapsed since the last rewarded response, and each rewarded response reset the interval. Each FI session was 30 min long, and training continued for 8 days or until cannula breakage prevented further drug infusions.

Theta Recording and Data Collection

During the last CRF session and throughout FI training, EEG data were recorded from the hippocampus. Electrodes were connected via the McIntyre connector to a cable and a dual field effect transistor, preamplifier (Grass P511K, 1–30 Hz bandpass filter), and extracellular field activity was digitized at 100 Hz for subsequent analysis by an Acorn A5000 computer. The leverpress data were collected and analyzed through a parallel EEG channel. Both the EEG and the behavioral data were displayed on the Acorn computer during FI sessions, the first as a continuous waveform and the second as a series of discrete bars with rewarded responses represented at twice the height of nonrewarded responses.

Histology

The 27 rats that had received intracranial injections were deeply anesthetized (sodium pentobarbital) and were perfused transcardially with saline and then with 10% formalin. The brains were removed and kept in 30% sucrose-formalin for 7 days. Frozen coronal sections (60 μm) were mounted and stained with thionin. The positions of the cannula tips were reconstructed according to the atlas of Paxinos and Watson (1998). The animals were classified on the basis of the distance of tip location from the mSuM (see Figure 1). The radius of diffusion of drugs injected in a volume of 0.5 μl is approximately 500 μm (Myers, 1966), and this also appears to be the extent of the functional diffusion of drugs in the region of the mSuM (McNaughton et al., 1995). The animals were classified as *mSuM* if the tip was in or within 500 μm of the mSuM (4.16–4.80 mm posterior to bregma) or *outside the mSuM* when the tip was remote from the boundary of the mSuM by >500 μm (see McNaughton et al., 1995). These outside the mSuM animals were included in the analyses

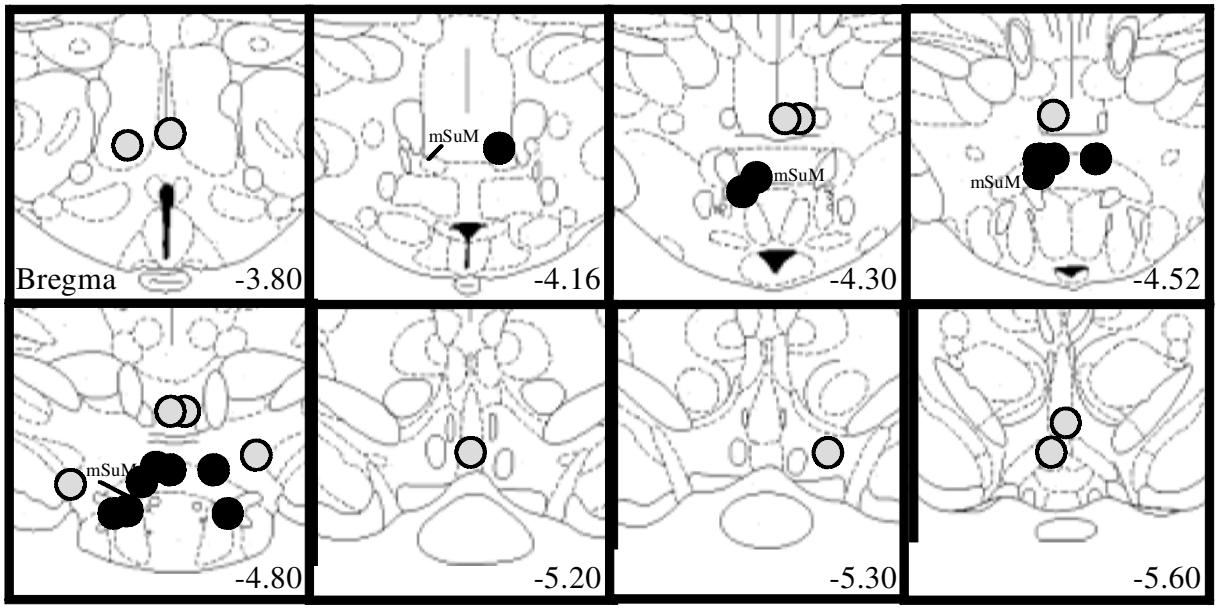

Figure 1. Cannula tip locations for 27 rats that had received one or more intracranial injections of CDP (20 μg in 0.5 μl vehicle) or saline. Stained coronal sections (60 μ) were compared with the stereotaxic atlas of Paxinos and Watson (1998). Dark circles, placements in or <500 μ from the mSuM; light circles, placements >500 μ outside of the mSuM.

Table 1
Numbers of Rats in Each Condition for the Behavioral and Theta Frequency Analyses

	FI Behavior		FI Theta Frequency	
Condition	CDP	Saline	CDP	Saline
mSuM	7	7	4	5
Outside the mSuM	7	6	7	3
Systematic (i.p.)	7	7	5	5

as anatomical controls for the effects of the drugs in the mSuM (see Table 1).

Data Collection and Analysis

Behavioral data. During FI sessions, the leverpresses were allocated to twelve 5-sec bins according to when they occurred in the interval. The total number of responses in each of the 12 bins was recorded for each rat for each day of FI. These data were transformed ($X' = \mathrm{SQRT}[X + 0.5]$) to normalize the data distribution (Zar, 1974) and were submitted to a repeated measures analysis of variance. The drug received (CDP or saline) and the site of injection (mSuM, outside mSuM, or i.p.) for each animal were extracted as between-subjects factors. Days and interactions with days were extracted as between-days components, and the variation in response rates across bins and all interactions with bins were extracted as between-bins components. Polynomial contrasts were extracted for the site (linear and quadratic) and the days and bins (linear, quadratic, and cubic) factors (Snedecor & Cochrane, 1967). Given the specific ordering chosen of levels of the factor of site of injection (mSuM, outside mSuM, i.p.), the quadratic component estimates variance common to mSuM and i.p. and not shared with outside mSuM, whereas the linear component results from differences between mSuM and i.p., ignoring outside mSuM. "Linear" and "quadratic," therefore, identify the method of extraction of the contrasts but are not the linearity or quadraticity of any underlying unidimensional factor.

EEG data. Only data from rats that produced a reliable EEG were analyzed (29 rats). A computer program selected the first leverpress made in each 10-sec bin of each 60-sec interval of the FI and analyzed 0.75 sec of theta beginning 1 sec before the leverpress. Frequency of theta was determined by an algorithm that detected peaks of successive theta waves and calculated frequency from the number of waves and the interval between the first and the last peaks. The criteria for theta were that the waveform should be sinusoidal, with a frequency of 4–12 Hz. Each sample was visually inspected during analysis. Waveforms that did not match these criteria were excluded. Correct peak detection was also confirmed visually for all the samples. Mean frequency for each of six 10-sec bins for 30 min of FI was calculated for each rat and was analyzed as above, with change in theta frequency across bins and interactions with bins analyzed as between-bins components.

RESULTS

Fixed Interval Barpressing

The effects of CDP at the three injection sites on FI responding are shown in Figure 2. It can be seen that the drug received and the site of injection both affected responding. The three saline groups produced similar rates of responding on individual days (Figure 2A and 2B) and overall (Figure 2C). Responding by the rats that had CDP outside of the mSuM resembled that of the saline groups on Day 1, but, overall, appeared slightly below that of controls. CDP i.p. and CDP in the mSuM produced rates of responding similar to one another and greater than those produced by saline and CDP outside the mSuM. The magnitude of these effects increased over the 4 days [drug × site: × days: dev × quad × lin, $F(1,25) = 9.24$, $p < .05$]. A post hoc analysis restricted to the i.p. and mSuM sites of injection revealed that there was no difference in overall responding between CDP i.p. and CDP in the mSuM [site: dev, $F(1,15) = 0.427$, n.s.]. A post hoc analysis also yielded no difference in overall responding between the CDP and the saline animals with placements outside the mSuM [drug: dev × lin, $F(1,15) = 0.673$, n.s.]. The release of responding seen with CDP i.p. and mSuM/CDP did not involve any change in the shape of the FI curve. Therefore, drug and site did not interact to produce any effect on bins.

Theta Frequency

Only data from the first 3 days of FI training could be analyzed, owing to missing values. Figure 3 shows the effects of CDP at the three sites of injection on the frequency of theta recorded 1 sec prior to barpressing. Those rats that received saline in any site or CDP outside of the mSuM produced an average frequency of theta of between 6.8 and 7 Hz. CDP i.p. and mSuM/CDP reduced these frequencies to below 6.2 and 6.4 Hz, respectively [drug × site: dev × quad, $F(1,8) = 11.14$, $p < .05$]. No significant difference in overall theta frequency was found in post hoc analysis of the CDP i.p. and mSuM/CDP groups [site: dev, $F(1,3) = 0.0128$, n.s.]. Frequency was stable for all groups across days and bins.

DISCUSSION

In this experiment, the effects of a systemic injection of the benzodiazepine CDP on both behavior and theta frequency were reproduced in their entirety when the drug was injected into the mSuM. As with previous experiments from this laboratory (Panickar & McNaughton, 1991; Zhu & McNaughton, 1995), CDP i.p. increased the rate of FI barpressing, relative to saline. Concurrent with the effect produced on responding, CDP reduced the frequency of the hippocampal theta rhythm. Although this effect of benzodiazepines on frequency in awake animals is well documented in electrophysiological tests (McNaughton & Coop, 1991; McNaughton et al., 1995; McNaughton et al., 1986), this is the first time, to our knowledge, that it has been demonstrated during an operant task used to model anxiety. When CDP was injected into or near the

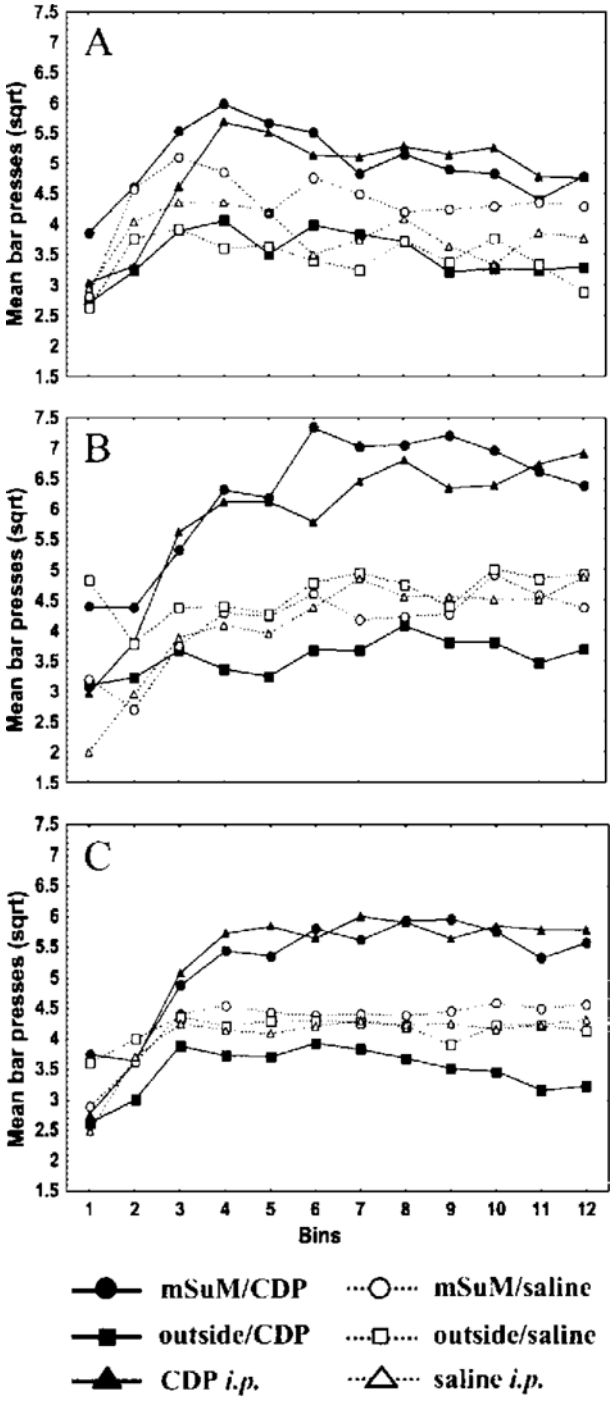

Figure 2. The effects of CDP (in mSuM, outside mSuM, or i.p.) on responding during acquisition of FI60 for (A) Day 1 and (B) Day 4 of training and for (C) the average of Days 1–4. Data are SQRT[X = 0.5] of the mean number of responses binned according to latency since the last rewarded response. Note that the acceleration over the latter bins typically seen with this schedule ("scalloping") is missing on individual days and overall. It is possible that attachment with the recording cable may have interacted in some way with the schedule's control of the rats' behavior. However, these patterns of responding were identical in rats treated with i.p. saline or CDP and in rats treated with intracranial saline or CDP.

mSuM, the effects on responding and on theta frequency were striking in their similarity to those produced by the i.p. injection. The magnitude of the release of FI responding by both groups, relative to controls, increased incrementally over days to a virtually identical degree. Theta frequency was analyzed across fewer days of training than was behavior, but on all of these days, both i.p. CDP and mSuM/CDP produced a stable reduction of frequency. These findings suggest that the mSuM was mediating the effects of CDP on both theta frequency and barpressing during the FI schedule.

When CDP was injected into brain structures remote from the mSuM by more than 500 μm, neither of the effects of CDP i.p. were reproduced. Theta frequencies from animals that had received this treatment were indistinguishable from the saline controls. After 4 days of training, the FI responding of these animals may have been slightly suppressed, as compared with the saline groups, but was certainly not increased.

Consistent with McNaughton et al. (1995), we have found that the mSuM has an important role in determining theta frequency in the awake rat. According to the view that theta frequency is important for normal hippocampal functioning (Kirk, 1998; Gray & McNaughton, 2000; Miller, 1991; Vinogradova, 1995), then, the mSuM is likely to interact with some functions of the hippocampus. Initial proof of this was obtained by Pan and McNaughton (1997), who found that benzodiazepine enhancement of GABAergic inhibition in the mSuM reduced theta frequency and impaired spatial learning in the Morris water maze. However, the size of both effects was much smaller than the size of systemically injected CDP. In the present experiment, the large reduction of theta frequency with mSuM/CDP was associated with a specific change in the rate of FI barpressing, which is also controlled by the septohippocampal system (Beatty & Schwartzbaum, 1968; Ellen & Powell, 1962; Haddad & Rabe, 1969; Manning & McDonough, 1974). Together, these findings suggest that the hippocampus is capable of making functionally relevant distinctions between different frequencies of theta.

However, it is important to rule out the possibility that changes in the theta recorded during FI training were merely dependent on movement, which the hippocampus does not control. Hippocampal theta has a robust association with voluntary movements (Slawinska & Kasicki, 1998; Kramis, Vanderwolf, & Bland, 1975; Vanderwolf, 1969; Whishaw & Vanderwolf, 1973), including leverpressing (Black & Young, 1972). A number of observations support a relationship between movement or movement initiation and theta frequency (Bland & Vanderwolf, 1972; Morris & Hagan, 1983; Oddie, Stefanek, Kirk, & Bland, 1996; Rivas, Gaztelu, & Garcia-Austt, 1996; Slawinska & Kasicki, 1998; Vanderwolf, 1969; Whishaw, Bland, & Vanderwolf, 1972). In general, higher frequencies are associated with greater initial speed of movements and the preparation for such movements. Bland's (1986) sensorimotor model regards movement-related theta as being due to feedback to the hippocampus from systems involved in executing movement.

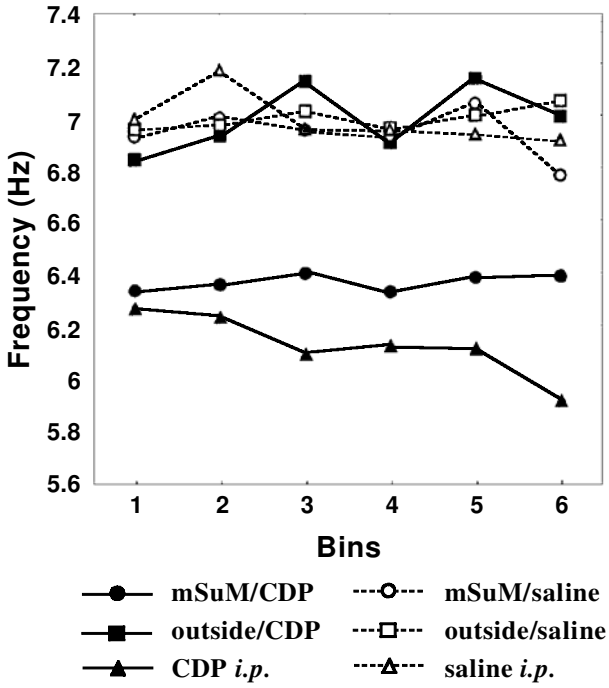

Figure 3. Effects of CDP (in the mSuM, outside the mSuM, or i.p.) on theta frequency during FI60. Data are the mean frequency (in hertz) of theta waves recorded in the second prior to the first barpress in each of six 10-sec bins. Data are averaged over the first 3 days of FI60 training.

This elicited theta, in turn, provides movement systems with an update of their performance. In this experiment, theta was sampled prior to a stereotyped behavior (leverpressing). Because the force required for each press of the lever was equivalent and the range of behaviors possible immediately prior to responding limited, the behavioral response was effectively "clamped" (see Lee, Chrobak, Sik, Wiley, & Buzsaki, 1994) during measurement of theta frequency. Therefore, group differences in theta frequency cannot easily be attributed to any motor hypothesis. Moreover, CDP i.p. and CDP in the mSuM *increased* the overall rate of leverpressing while *reducing* the frequency of theta associated with it. Theta frequency also, unlike movement, did not change noticeably during training.

The present results are more easily explained in terms of behavioral inhibition, rather than free movement. Behavioral inhibition is characterized in many operant tasks by the passive avoidance (suppression) of a prepotent response in the presence of punishment or nonreward (Gray & McNaughton, 2000). Anxiolytic drugs that reduce theta frequency reduce behavioral inhibition in many tasks. Opposite effects on behavioral inhibition have also been obtained with different frequencies of theta produced by septal driving stimulation delivered prior to testing (Gray, 1972; Snape et al., 1996; Williams & Gray, 1996). In our FI60 paradigm, barpressing was released by CDP during nonreward, and individual responses were associated with lower frequencies of concurrently recorded theta than were produced by controls. These findings are consistent with a role for the hippocampus, via theta, in the suppression of behavior during frustration or nonreward and, hence, in the control of anxiety (Gray & McNaughton, 2000). In the case of anxiety as modeled by FI60, our results suggest that the benzodiazepine-sensitive component of theta frequency is determined largely, and perhaps exclusively, by the mSuM. The mSuM is likely, therefore, to be one important site for the clinical effects of anti-anxiety drugs. However, sites that are sensitive to anxiolytic drugs in animal models are widely distributed in the brain, and in addition to the mSuM, anxiolytic effects of intracranially administered benzodiazepines have been observed in dorsal and median raphe, dorsal periaqueductal gray, amygdala, hippocampus, septum, and mammillary bodies (see Menard & Treit, 1999). It is, therefore, very unlikely that the mSuM is crucial for anxiety in any global sense but, during the FI task, it is probably the major substrate for the action of benzodiazepines on behavioral inhibition.

If specific control of theta frequency by the mSuM is related mainly to a behavioral inhibition function of the hippocampus, the mSuM is probably less involved in determining frequency during hippocampal-mediated behaviors for which behavioral inhibition is less critical. The Morris water maze is a hippocampal-sensitive task, that has a behavioral inhibition component (Gray & McNaughton, 2000) but is generally regarded as a test for spatial memory ability. Pan and McNaughton (1997) found only small effects of mSuM/CDP on water maze learning and theta frequency, despite the robust effects on both variables with other treatments (systemic CDP, long cooling). Clearly, theta frequency is critical for correct water maze performance, but the mSuM is not the only site that determines frequency during this task. There is other evidence for more than one nucleus' controlling the frequency of theta in awake rats. Lesions of the mSuM and surrounding areas do not alter the frequency of theta recorded during simple movement (Thinschmidt, Kinney, & Kocsis, 1995), and the frequency of RPO-elicited theta depends only partially on the mSuM in freely moving animals (McNaughton et al., 1995). The involvement of the mSuM in any hippocampal-sensitive task, therefore, may depend on the contribution the mSuM makes to the control of theta frequency in that task, relative to other sites. The site controlling theta frequency appears to depend on the nature of the task: The mSuM is extensively involved in FI theta, uninvolved in simple movement theta, and jointly involved with some other site or sites in the control of theta in the water maze. From our results, as well as from those of Pan and McNaughton (1997), it would appear that, regardless of which specific sites control theta frequency in a given situation, the degree to which any manipulation affects frequency will affect the degree of disruption of hippocampal-mediated behaviors.

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(Manuscript received October 15, 2001;
accepted for publication February 21, 2002.)