FUNCTIONAL CONNECTIVITY BETWEEN THE RED NUCLEUS AND THE HIPPOCAMPUS SUPPORTS THE ROLE OF THE HIPPOCAMPAL FORMATION IN SENSORIMOTOR INTEGRATION

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ABSTRACT

Experiments were carried out in urethane-anesthetized rats in order to evaluate the hypothesis that the red nucleus has functional connections with the hippocampal formation. Depth profiles of electrical stimulation in Experiment 1 confirmed that stimulation administered to the red nucleus elicited theta field activity in the hippocampal formation with a linear relationship between stimulus intensity and theta frequency. Experiment 2 demonstrated that microinfusion of local anesthetic procaine hydrochloride into the medial septum resulted in a reversible blockade of theta field activity elicited by electrical stimulation of the red nucleus. In Experiment 3, the discharge activity of red nucleus cells was recorded during the field conditions of hippocampal synchrony (theta) and hippocampal asynchrony (LIA- large amplitude irregular activity). Analysis revealed that 26/46 (56%) of red nucleus cells were theta-related while the remaining 20 (44%) were non-related. The majority of theta-related cells were classified as tonic theta-ON. A brief increase above the basal discharge rate of tonic theta-ON red nucleus cells during LIA predicted the transition from LIA to theta with 400 – 500 ms latency. Furthermore, higher frequency transitional discharges predicted higher theta frequencies, while higher discharge rates during theta predicted shifts to higher theta frequencies. The results supported the conclusion that the red nucleus, traditionally associated with motor functions, is functionally connected with the neural circuitry involved in the generation of theta band oscillation and synchrony in the hippocampal formation, in agreement with the predictions of the sensorimotor integration model of hippocampal function (Bland and Oddie 2001).
INTRODUCTION

The red nucleus, or nucleus ruber, is a conspicuous brainstem structure of limb-using vertebrates (Massion, 1988). The mammalian red nucleus is a “distinct cell mass in the tegmentum mesencephali at the level of the oculomotor nerve, rostral to the decussation of the brachium conjunctivum” (Ten Donkelaar, 1988), partitioned into two variably sized subdivisions depending on phylogeny. In the rat, the rostral parvicellular section (RNp) consists mainly of medium and small cells, while the more caudal magnocellular section (RNm) consists of the largest neurons (Ruigrok & Cella, 1995). There does not appear to be a clear distinction between the magno- and parvicellular divisions of the red nucleus in the rat (Keifer and Lustig 2000). The red nucleus is considered to play a role in both locomotion and grasping (Muir and Whishaw 2000; Kuchler et al. 2002; Van Kan et al 1994; Van Kan and McCurdy 2001).

Our laboratory became interested in directly examining relationships between the hippocampal formation and motor structures such as the basal ganglia (Hallworth and Bland 1999) and the RN. The rationale for this work arose from predictions made by the sensorimotor integration model of hippocampal formation function (Bland 1986). In a recent update of the model Bland and Oddie (2001) reviewed data supporting the case that neural circuitry underlying theta band oscillation and synchrony in limbic cortex and associated structures functions in the capacity of providing voluntary motor systems with continually updated feedback on their performance relative to changing environmental (sensory) conditions. A crucial aspect of this performance is the intensity with which motor programs are initiated and maintained. The model posits that the structures comprising the ascending brainstem hippocampal synchronizing pathways make the primary contribution in this regard. Originating in the reticularis pontis oralis (RPO) and the pedunculopontine tegmental nucleus (PPT), pathways ascend to nuclei of the midline posterior
hypothalamic nucleus (PH) and suprammillary nucleus (SUM) (Vertes and Koscis 1997; Bland and Oddie 1998). The pathways then ascend through the medial forebrain bundle to the medial septal nucleus/vertical limb of the diagonal band of Broca (MS/VDBB) (Vertes 1992; Vertes et al. 1995). The medial septum functions as the node of the ascending pathways, sending inputs to the hippocampal formation, cingulate cortex, and entorhinal cortex (Bland 2000). In the updated sensorimotor model the PH is ascribed the major role in relaying movement-related information from motor systems to the hippocampal formation via the MS/vDBB. Although based on animal studies, the sensorimotor integration model was recently supported by a recent study (Caplan et al. 2003) on human theta oscillations recorded during a virtual maze task.

Connectivity between the divisions of the red nucleus, the spinal cord, cerebellum and cortex has been well studied but less is known about their connectivity with subcortical regions. In rats there is evidence for inputs to the red nucleus from the hypothalamus (Bernays et al. 1988; Keifer and Lustig 2000), thus allowing the possibility of the red nucleus interacting with the hippocampus via the ascending brainstem synchronizing pathways.

The purpose of the present work was to evaluate the hypothesis that the neural activity of the red nucleus is functionally connected to theta band oscillation and synchrony in the hippocampal formation. Functional connectivity could be revealed several ways: 1) demonstrating that electrical stimulation of the RN resulted in systematic changes in the field activity of the hippocampal formation; 2) demonstrating that the activity of RN cells varied systematically in relation to changes in hippocampal field activity; 3) the finding of significant cross correlations between RN cellular activity and hippocampal field activity. Experiment 1 showed that electrical stimulation applied directly to the RN elicited theta field activity in the hippocampal formation.

Experiment 2 demonstrated that this effect was mediated by inputs to the hippocampus arising from the medial septum. Experiment 3 demonstrated precise relations between the discharge rates of RN cells and simultaneously occurring field activity in the hippocampal formation.
Materials and Methods

Animals and surgery

A total of 48 male Long Evans rats (5 in Experiment 1, 5 in Experiment 2, and 38 in Experiment 3) were used, weighing between 200 and 350 grams obtained from the Animal Care Facility at the University of Calgary. Tracheal and jugular cannulations were performed after the rats were anesthetized with a mixture of halothane (M.T.C. Pharmaceuticals, Cambridge, Ontario) and oxygen (1.5% minimum alveolar concentration). When halothane was discontinued, urethane (ethyl carbamate, 0.8 gm/Kg) was administered via the jugular cannula for maintenance of an appropriate level of anesthesia during the remaining surgical and experimental procedures. Rats were secured in a stereotaxic apparatus and prepared for surgery with the plane between bregma and lambda leveled to horizontal. The rat’s core body temperature was maintained at 37 ° C (Harvard Instruments heating pad), and heart rate was monitored constantly throughout the experiment. An indifferent electrode, consisting of an uninsulated tungsten rod, was inserted in the cortex anterior to bregma, and the stereotaxic frame was connected to ground. A tungsten microelectrode (0.1 to 0.5 megohms) for recording hippocampal field activity was placed in the right hippocampal formation in the dentate molecular layer, between 2.6 and 2.8 mm ventral to the dural surface, at coordinates 3.3 mm posterior to bregma and 2.2 mm lateral to the midline. A diagrammatic representation of a sagittal brain section showing the procedures carried out in the three experiments is shown in Figure 1. Experiment 1 utilized a bipolar stimulating electrode constructed of insulated stainless steel wires (each 250 um in diameter) twisted together with the ends attached to a male subminiature connector. In order to determine whether stimulation of the RN elicited hippocampal theta field activity, depth profiles of electrical stimulation were carried out in 5 rats in the following manner: at the level of 6.0 mm posterior to bregma and 1.0 mm lateral to the midline, electrical stimulation at 5 current levels (100, 200, 300, 400 and 500 microamps) was administered at each of 5 depths, starting dorsal to the RN, through the RN and ending ventral to it. This corresponded to depths of 6.5, 7.0, 7.5, 8.0, and 8.5 mm ventral to the
dural surface, respectively. In Experiment 2, the RN site with the lowest threshold for eliciting theta was used. For each rat, the stimulating electrode was cemented into place with dental acrylic at the optimal coordinates. Electrical stimulation was carried out using a Grass 4678 stimulus isolation unit and a Grass CC UIA constant current unit connected to a Grass S44 stimulator. Stimulation consisted of biphasic square waves at a frequency of 100 Hz, with the current varying from 100 to 500 microamps, for duration of 5 s. Both normal and reverse polarity was tested to determine optimal effect of stimulation. Procedures for experiment 2 were the same as those described for Experiment 1, with the addition of a microinfusion cannula located in the medial septum. A hole was drilled for the coordinates of the MS, 0.0 mm lateral and, 0.5 mm anterior to bregma. A 23-gauge tube, lowered 5.5 mm ventral to the dural surface and cemented in place, served as a guide for a 30-gauge injection stilette. The injection stilette was connected to a Harvard Apparatus infusion pump via a 10 µl Hamilton gas tight syringe. Procaine hydrochloride (20% by volume) (Sigma Chemicals, St Louis, MO) was infused into the medial septum at a flow rate of 0.5 µl /min. Infusion procedures were as follows: (1) the RN was stimulated in the range of 100-500 microamps to determine optimal effect, (2) the cannula lowered and 1 µl of the procaine solution infused, (3) following a five-minute wait, the RN was stimulated to check for absence or presence of theta generation, (4) when necessary, additional infusions were administered in .5 µl steps, up to a maximum of 5 µl stimulating each time one minute after infusion. During the maximal procaine effect and subsequent recovery, electrical stimulation at intervals of five minutes was administered in order to test for the recovery of theta. In Experiment 3, using a Kopf microdrive, single cells were recorded from the RN region (6.0 mm posterior to bregma, 1.0 mm lateral to the midline, and 6.4 – 8.0 mm ventral to the dural surface) with a glass microelectrode (7-15 megohms) filled with 0.5 M sodium acetate mixed with 2% Pontamine sky blue. After isolation and a 5-10 minute stabilization period, recordings of RN discharges were made during each of the 2 simultaneously occurring hippocampal field conditions: (1) spontaneous theta, (2) LIA.
Data Acquisition

During recording, brain signals of hippocampal field and single cell RN discharges were amplified and displayed, then stored on FM tape for further off-line analysis. Signals were led into two Grass model P511 preamplifiers. One preamplifier isolated field activity, which was led into a model 7D Grass polygraph with filter settings of 1 Hz and 35 Hz and the unit preamplifier was set at 300 Hz to 3 KHz. From these preamplifiers, the field and cell signals were led to a 55100 series Tektronix oscilloscope and the TEAC cassette recorder. Additionally, signals passing out of the cell preamplifiers were led into a Grass AM5 audio monitor. The glass microelectrode tip location was iontophoretically marked with Pontamine sky blue after completion of recordings by passing a 50 microamp current for a total of 15 minutes (5 minute cathodal, 5 minute anodal and 5 minute cathodal). Subsequent perfusion (10% paraformaldehyde) and fixation of the brain was followed by serial slicing of the fixed brain into 30-micron sections of brain tissue using a Leica CM800 microtome. Histological reconstruction of the recording tracks and verification of recording sites followed examination of the brain sections mounted on glass slides. Upon histological examination, placement of recording electrodes was verified by location of the Pontamine sky blue stain. In cases where blue dots were not located, placement was determined from a combination of the depth of the electrode as measured by the distance of the Kopf micropositioner from the dural surface and a reconstruction of the electrode tracks from the histological sections.

Data Analysis

A minimum of 5 data segments with duration of at least 4 s was selected for analysis. Individual cell discharges were analyzed during the 2 different hippocampal field conditions of spontaneous theta and LIA. Cell activity was digitized through a 12-bit A/D converter and sampled at a frequency of 16 KHz while HPC field activity was simultaneously sampled at a frequency of 133 Hz. Data segments were analyzed using a PC microcomputer and software acquisition program (Data Wave Technologies, Longmount, CO), providing the mean, standard error of the mean and
range of cell discharges. Hippocampal field activity was classified as either theta or LIA based on the frequency spectra produced by fast Fourier transform (FFT) analysis. Auto-correlation analyses were performed on the cell activity and hippocampal field signals in order to delineate periodicities. Additionally, cross-correlation analyses were carried out between the hippocampal field signals and unit activity in the red nucleus to detect the absence or presence of correlation between activity patterns in the RN and the HPC, using a frequency domain algorithm (Press, et al 1992). The classification criteria of Colom and Bland (1987), based on the spike train dynamics of the discharging cell in relation to the simultaneously-occurring hippocampal theta and LIA, was used to classify cells as either theta-ON, theta-OFF or non-related. Non-related cells were designated as such by a lack of significant difference in discharge rate between theta and LIA, whereas theta-ON cells displayed a significantly increased discharge rate during theta field activity as opposed to LIA field activity, and theta-OFF cells discharged more during LIA (significance assessed statistically with paired t-tests, probability set at p< .05). Theta-related cells were also sub classified as phasic cells if they discharged two or more spikes per theta wave and the cell discharges were related to the phase of hippocampal theta field activity. The subclass of tonic theta-ON cells discharged in a regular or irregular pattern with no phase relations to theta field activity. All tonic theta-ON cells were subjected to perievent time histogram analyses (PETH), enabling the calculation of the approximate probability distribution of RN cell discharges related to the occurrence of the first positive peak of theta field activity (i.e., LIA-theta transitions. Statistical analysis of the discharge rates during LIA, the LIA to theta transition, and theta field states were carried out using analysis of variance (ANOVA) and significance was determined by follow-up Scheffe tests (significance set at p< .0001).

RESULTS

Experiment 1

Histology verified the location of the stimulating electrode depth profiles in all 5 experiments. (See photomicrograph in top panel in figure 2 for a representative stimulating track through the
red nucleus). At the lowest current level of 100 microamps hippocampal theta field activity was only elicited when the stimulating electrode was located in the dorsal, middle and ventral regions of the RN. In addition, maximal theta driving at the lowest stimulation current was determined to result from stimulation in the middle of the RN at 7.5 mm ventral from the dural surface (See bottom panel in Figure 2). The results of electrical stimulation of the RN were consistent, resulting in a clear transition from hippocampal LIA to theta. Furthermore, as shown in Figure 3A, increasing the intensity of the stimulating current resulted in an increase in both amplitude and frequency of hippocampal theta field activity. In this example, 100, 200, 300, and 400 microamps resulted in mean amplitudes of 0.85, 1.1, 1.2, and 1.5 mV, and mean frequencies of 3.8, 4.4, 4.8, and 5.2 Hz, respectively. Figure 3A also shows that as stimulus intensity increased, theta field activity persisted for longer durations following termination of the stimulus. The group frequency data for the 5 RN stimulation experiments are shown graphically in Figure 3B. For stimulation levels of 100, 200, 300, 400, and 500 microamps the mean frequencies were 3.40 ± 0.4, 4.3 ± 0.3, 5.1 ± 0.2, and 5.2 ± 0.2 Hz, respectively.

**Experiment 2**

Histology verified the location of the stimulating electrodes in the RN and the microinfusion cannula placements in the medial septum in all 5 experiments. The top panel in Figure 4 shows a micrograph taken at the level of the medial septum. The arrow indicates the tip of the procaine microinfusion cannula in the medial septum. The microinfusion of 1.0 – 1.5 µl of procaine hydrochloride into the medial septum consistently blocked theta field activity in all 5 experiments. During the period of procaine blockade (approximately 1 hr) of the medial septum, electrical stimulation of the RN was no longer capable of inducing hippocampal theta field activity. The bottom panels in Figure 4 show representative examples of a blockade experiment. Figure 4 (top panel) shows the FFT generated from a sample of pre-procaine hippocampal theta field activity elicited by electrical stimulation of the red nucleus (see insert), revealing a peak frequency of 5.7 Hz. Figure 4 (middle panel) shows the equivalent manipulation 10 min post-
infusion of procaine hydrochloride into the medial septum. The insert reveals the lack of theta, corroborated by the lack of a peak frequency in the FFT. Figure 4 (bottom panel) shows the equivalent manipulation 45 min post-procaine infusion. The insert shows that electrical stimulation of the RN again produced hippocampal theta field activity, although the amplitude and frequency (4.9 Hz) had not fully recovered.

**Experiment 3.**

**Histology**

From a total of 64 cells recorded, 52 cells were held long enough to complete the experimental protocol. Of these 52 cells, data are shown for 46 cells recorded from the RN region (see Figure 5), based on the localization of blue dots (n=34) or by reconstruction of electrode tracks (n = 12) in the histological material (Table 1).

**Cell classifications**

Twenty-six out of forty six (56%) of the cells recorded in the RN region were theta-related while the remaining 20 cells (44%) were classified as non-related. The sub classification of the theta-related cells was as follows: tonic theta-ON (n=20), phasic theta-ON (n=3) and tonic theta-OFF (n=3) (Table 1).

**Tonic theta-ON red nucleus cells**

**Discharge properties during LIA, theta, and LIA-theta and theta-theta field transitions**

According to Colom and Bland’s (1987) theta cell classification system, tonic theta-ON cells discharge at significantly higher rates during theta than during LIA, with a regular or irregular, discharge pattern. As noted above, 20 RN cells met these criteria, with significant differences between the discharge rates during the LIA and theta conditions (mean difference = -3.330, degrees of freedom 42, t = -5.847, p<. 0001). (See Table 1 for mean discharge rates for all 20 tonic theta-ON cells for the three conditions). In addition, all 20 cells displayed another very dramatic discharge property that has not been previously documented. The transition from hippocampal LIA to theta field activity was signaled by a brief and very pronounced increase in
discharge rate. Figure 6A shows examples of a RN tonic theta-ON cell discharging in relation to the simultaneously recorded hippocampal field activity. The upper panel (Figure 6A) shows an LIA-theta-LIA transition while the lower panel (Figure 6B) shows an LIA-theta-LIA-theta transition. The increase in discharge rate just prior to the transition to theta is visible in all three cases. Cross correlation analyses (not shown) carried out on all RN tonic theta-ON cells during theta field activity were not significant. However, the same analyses carried out at LIA-theta transitions revealed significant relationships with RHO values ranging from .2 to .6. The upper panel of Figure 7A shows the discharges of the same cell related to a longer period of LIA. The lower panel (Figure 7B) reveals that, similar to the finding of increased cell discharge rates occurring at the transition from LIA to theta field activity, the cell’s discharge rate also signaled the transition from a lower frequency of hippocampal field activity to a higher frequency of theta field activity. These discharge properties were quantified by carrying out perievent time histogram (PETH) analyses on the cell discharges, in relation to the transition points between hippocampal LIA and theta field activity. For the LIA to theta transition analysis this was achieved by marking the “0” point of the analysis at the positive peak of the first theta wave and analyzing 2000 ms in front of this point and 2000 ms behind this point. For the theta to theta transitions, the “0” point was placed at the positive peak of the faster theta wave marking the shift to a higher frequency. Figure 8A shows a representative PETH analysis carried out on 8 LIA-theta transitions of a tonic theta-ON RN cell. The PETH graph shows that cell discharges increased 400 – 500 ms during the LIA just prior to theta onset and continued at a lower rate during theta field activity. This lower discharge rate was still significantly higher (see below) than the discharge rate accompanying the LIA occurring prior to the transition point. Figure 8B graphically illustrates the results of 45 LIA-theta transitions measured by PETH analysis, for the same cell. Analysis of variance carried out on the discharge rates during the LIA, the LIA-theta transition, and theta field conditions revealed a significant difference, $F(43,2) = 262.582, p< .0001$. Follow up Scheffe tests showed there were significant differences in discharge rate
between hippocampal LIA and the LIA-theta transition (p < .0001), between the LIA and theta (p < .0001), and between the LIA-theta transition and theta conditions (p < .0001). The same analyses were carried out on all 20 tonic theta-ON cells and the data reported above were representative of all cells.

**Discharge properties of tonic theta-ON cells related to increasing theta frequencies at the LIA-theta and theta- theta transitions**

With the demonstration that tonic theta-ON RN cells signaled the imminent transition from the hippocampal LIA to the theta field condition, it became of interest to determine whether these cells also coded for the frequency of the theta field activity that was to occur. Subsequent analysis confirmed that this was the case. Figure 9 (A, B, C) shows a representative RN tonic theta-ON cell during 3 LIA-theta transitions, with 3 different theta frequencies occurring at the transition. In panel A the transition from LIA to 3.8 Hz theta was signaled by a discharge rate of 8.0 Hz, in panel B the transition from LIA to 4.3 Hz theta was signaled by a discharge rate of 13 Hz, and in panel C, the transition from LIA to 4.8 Hz theta was signaled by a discharge rate of 24 Hz. The results of a quantitative analysis of a representative RN tonic theta-ON cell, comparing discharge rates during the field conditions of hippocampal LIA, LIA transition, and theta, at 4 different theta frequencies, are presented graphically in Figure 9D. Analysis of variance (ANOVA) carried out over the 3 hippocampal field conditions at each theta frequency revealed that there was a significant difference between the field conditions and cell discharge rate at the 4.0 Hz theta frequency condition \(F (2, 36) = 159.802, p < .0001\). Follow up Scheffe tests for the 4.0 Hz theta frequency revealed a significant difference between cell discharge rate and the LIA and LIA transition conditions \(p < .0001\), between the LIA and theta conditions \(p < .001\), and between the LIA transition and theta conditions \(p < .0001\). The ANOVA carried out over the 3 hippocampal field conditions revealed that there was a significant difference between the field conditions and cell discharge rate at the 4.3 Hz theta frequency condition \(F (2, 36) = 100.800, p < .0001\). The Scheffe tests for the 4.3 Hz theta frequency were also all significant at \(p < .0001\)
for the three field conditions. The ANOVA for the 4.8 Hz theta frequency condition again revealed significant differences between the 3 field conditions and the cell discharge rate (F (2,12) = 54.197, p< .0001). Scheffe tests revealed significant difference between the LIA and LIA transition conditions (p< .0001) and the LIA and theta field conditions (p< .0001), but not between the LIA transition and theta field conditions. Similarly, the ANOVA for the 5.0 Hz theta frequency condition again revealed significant differences between the 3 field conditions and the cell discharge rate (F (2,6) = 10.419, p< .05), while the Scheffe test revealed significant differences between the LIA and LIA transition field conditions and the LIA and theta conditions (both at p< .05). The difference between the LIA transition and theta field conditions was not significant. The same analyses were carried out on all 20 tonic theta-ON cells and the data reported above were representative of all cells.

**Phasic theta-ON and tonic theta-OFF red nucleus cells**

**Discharge properties during LIA, theta, and LIA-theta and theta-theta field transitions**

As mentioned above, only three cells meeting the criteria of phasic theta-ON cells were recorded in the RN. Due to the low number, extensive analyses were not carried out. However, the analyses that were carried out indicated some similarities to the discharge properties of tonic theta-ON RN cells (See Table 1). Figure 10 (A, B, C) show representative examples of the discharges of a phasic theta-ON cell in the RN, accompanying hippocampal field conditions. Figure 10A shows the very low discharge rate associated with hippocampal LIA field activity, while Figure 10B shows that the transition from LIA to theta was signaled by an increase in discharge rate, with an irregular pattern, that then became phasic (rhythmic) during theta field activity. Figure 10C shows the transition from lower frequency theta to higher frequency theta was also signaled by a non-rhythmic increase in discharge rate, followed by an increased number of rhythmic discharges. Cross correlation analyses (not shown) carried out on all 3 RN phasic theta-ON cells during theta field activity were significant with Rho values ranging from .4 to .6.
The three tonic theta-OFF cells discharged at a similar rate during all LA conditions and either ceased discharging or reduced their discharge rates to near zero during theta (See Table 1).

**DISCUSSION**

In this study we investigated the hypothesis, based on predictions arising from the sensorimotor integration model of hippocampal function, that neural activity of the red nucleus was functionally related to the neural activity underlying mechanisms of theta band oscillation and synchrony in the hippocampal formation. The data provided strong support for the hypothesis. The results of Experiment 1 demonstrated that activation of the red nucleus with electrical stimulation elicited transitions from hippocampal LIA field activity to hippocampal theta field activity. Furthermore, theta amplitude and frequency increased as a function of increases in the intensity of stimulation. The depth profiles of electrical stimulation demonstrated that the elicitation of hippocampal theta field activity was well localized to the red nucleus. However, these experiments could not distinguish whether the effects of electrical stimulation were a result of activating RN cells or fibers of passage through the nucleus. Vertes (1981, 1982) demonstrated that electrical stimulation of the medial longitudinal fasiculus (MLF) resulted in the generation of hippocampal theta, and these axons are in close proximity to the RN. Experiment 2 demonstrated that microinfusions of procaine hydrochloride into the medial septum reversibly abolished the ability of electrical stimulation of the red nucleus to elicit hippocampal theta field activity. This finding supported the conclusion that the red nucleus contributed ascending inputs to the hippocampal formation via a septohippocampal pathway, similar to other brainstem nuclei comprising the ascending brainstem hippocampal synchronizing pathways (Bland and Oddie 1998). In future experiments it will be important to determine exactly what nuclei of the ascending brainstem synchronizing pathways receive inputs from the RN. Experiment 3 revealed that the discharge profiles of twenty six out of forty six (56%) of the cells recorded in the red nucleus region met the criteria for classification as theta-related cells while the remaining 20 cells (44%) were classified as non-related. The majority of the theta-
related cells (20/26) were tonic theta-ON, while three of the remaining cells were phasic theta-ON and three were tonic theta-OFF. In addition to being theta-related by virtue of increases in overall discharge rates during theta, tonic red nucleus theta-ON cells were shown to display several other precise relations to hippocampal field activity that have never been previously described. A brief increase above the basal discharge rate during LIA predicted the transition from hippocampal LIA to hippocampal theta with a latency of 400 – 500 ms. Furthermore, the rate of increase above the basal discharge rate during the LIA transition period was related to the frequency of theta that followed, such that higher discharge rates predicted higher theta frequencies. Similarly, the occurrence of a brief increase in discharge rate during theta field activity predicted shifts to higher frequencies of theta. Although fewer in number, the data also suggested that phasic theta-ON cells may display similar properties.

Interestingly, although there did not appear to be significant relationships between the discharges of RN tonic theta-ON cells to sustained hippocampal theta field activity (such a relationship was demonstrated for RN phasic theta-ON cells), there were significant relationships between these two activities during the transition period from LIA to theta. This is the first investigation of theta-related cells in the red nucleus. Theta-related cells comprising two distinct populations termed theta-ON and theta-OFF were first described in acute preparations using extracellular recordings by Colom et al. (1987), followed by a detailed cell classification paper by Colom and Bland (1987), subsequently used to classify theta-related cells in the HPC in a number of studies (Bland and Colom 1988, 1989; Mizumori et al. 1990; Colom et al. 1991; Smythe et al. 1991; Konopacki et al. 1992; Bland et al. 1996). Theta-ON and theta-OFF cells have also been recorded in the medial septal nucleus and nucleus of the diagonal band of Broca (MS/vDBB) (Ford et al. 1989; Bland et al. 1990; Colom and Bland 1991; Bland et al. 1994), the entorhinal cortex (Dickson et al. 1994, 1995), cingulate cortex (Colom et al. 1988), caudal diencephalon (Bland et al. 1995; Kirk et al. 1996), rostral pontine region (Hanada et al. 1999),
the superior colliculus (Natsume et al. 1999), the basal ganglia (Hallworth and Bland 1999),
and the neocortex (Lukatch and MacIver 1996).
The sensorimotor integration model of hippocampal function is based on the assumption that
neural circuitry underlying theta band oscillation and synchrony functions in the capacity of
providing voluntary motor systems with continually updated feedback on their performance
relative to changing environmental (sensory) conditions. A crucial aspect of this performance is
the intensity with which motor programs are initiated and maintained. Data from single cell
experiments in monkey has been interpreted as reflecting a role of the red nucleus in the accurate
coordination of distal movements (Gibson et al. 1985; Houk et al. 1988), further supported by
experiments demonstrating the dominance of red nucleus inputs to distal extensor muscles in the
demonstrated that monkey red nucleus cells in the magnocellular division were not activated
strongly during coordinated, whole-limb reaching movements that did not include hand use.
These same cells discharged more strongly related to metacarpi-phalangeal extension than to
movements of the wrist and more proximal forelimb joints. Van Kan and McCurdy thus
provided strong support for the role of primate magnocellular red nucleus cells in controlling
hand preshaping during reaching to grasp movements. However, sensory feedback specifically to
the red nucleus from the hippocampus may not be necessary. Sarrafizadeh, et al. (1996)
demonstrated that rubral responsiveness to sensory stimulation was found to be significantly
diminished during active limb movements, thereby suggesting that sensory inputs to the RN were
not used for the on-line modification of motor commands.
Lesion studies of red nucleus in rat have also demonstrated specific deficits in the regulation of
coordinated distal limb and paw movements involved with grasping (Whishaw and Gorney 1996;
Whishaw et al. 1990). Support for the lesion studies was recently provided by Jarratt and Hyland
(1999). These authors studied the relationship between red nucleus cell discharges to the onset of
wrist movement and the end of a movement (defined as the arrival of the paw over food) in a
reach-to-grasp motor task. The study demonstrated that while changes in red nucleus cell activity occurred in all phases of the task, a large proportion of excitations occurred during the reach. There have also been suggestions in the literature that the red nucleus may be important in less skilled movements, such as correcting and adapting ongoing locomotion (Armstrong 1986). A recent study, utilizing excitotoxic lesions of the red nucleus of rats provided evidence for this suggestion. Muir and Whishaw (2000) demonstrated that 24 – 48 hr after such a lesion, rats moved with an asymmetric gait during which abnormal braking and propulsive forces were produced during the dual contact time of the forelimb contralateral to the lesion and the ipsilateral hindlimb. The deficit lasted for the 55 day duration of the study and demonstrated that the red nucleus plays a role in ongoing over ground locomotion of the rat.

Motor activities that appear to be regulated by the red nucleus, such as the onset of limb movement, manipulatory movements of the paw, and locomotion, are also classed as type 1 movements associated with the onset of hippocampal theta field activity (Vanderwolf 1988). The present work is the first to demonstrate functional connectivity between the neural activity of the red nucleus and the neural circuitry involved in the generation of theta band oscillation and synchrony. Furthermore, the study demonstrated that the synchronizing effects on the hippocampus produced by electrical stimulation of the RN occurred via a septohippocampal pathway, similar to other nuclei of the ascending brainstem hippocampal synchronizing pathways. In their updated sensorimotor integration model Bland and Oddie (2001) suggested that activity of the ascending brainstem synchronizing pathways provides the hippocampus with sensory information relevant to the initiation of voluntary movement. This information is relayed from the hippocampus to motor structures (such as the red nucleus), which in addition to initiating movements send inputs to the posterior hypothalamus signaling that these movements have been initiated. As movement continues, the combination of sensory inputs from the ascending brainstem synchronizing pathways and movement related inputs from motor structures such as the red nucleus, ascend back to the hippocampus, allowing the hippocampus to integrate
sensory and motor information necessary for the initiation and maintenance of voluntary motor behavior. The present findings support the role of the hippocampal formation in sensorimotor integration since they were predicted by the sensorimotor integration model (Bland 1986; Bland and Oddie 2001).
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<tr>
<th>Cell Location</th>
<th>Cell Classification</th>
<th>Discharge rates (Hz)</th>
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<td></td>
<td></td>
<td>LIA</td>
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<td>Red Nucleus (n = 46)</td>
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<td></td>
<td>Phasic Theta-ON (3)</td>
<td>17.3 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>Tonic Theta-OFF (3)</td>
<td>25.2 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>Non-Related (20)</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

**Figure 1.** Diagrammatic representation of a sagittal section of the brain indicating the location of the experimental procedures carried out for Experiments 1, 2, and 3.

**Figure 2.** Top panel. Photomicrograph of a coronal section taken at the level of the red nucleus. The arrow indicates the bottom of a stimulation track that started just dorsal to the red nucleus and ended just ventral to it. Bottom panel. Diagrammatic representation of a frontal section of the brain summarizing the histological reconstruction of stimulating electrode tracts through the red nucleus and the effects on hippocampal theta field activity, in 5 experiments.

**Figure 3.** (A) Representative examples of hippocampal theta field activity elicited by electrical stimulation of the red nucleus at intensities of 100, 200, 300 and 400 microamps, top to bottom panels, respectively. (B) Graph summarizing the group data for 5 rats of the frequencies of hippocampal theta field activity elicited by increasing intensities of electrical stimulation of the red nucleus (bars are standard errors of the mean).

**Figure 4.** Top panel. Photomicrograph of a coronal section taken at the level of the medial septum. The arrow indicates the tip of the procaine microinfusion cannula. Bottom panel. Fast Fourier transform analysis of hippocampal field activity during the conditions of (A) pre-procaine, (B) procaine, and (C) post-procaine of the medial septum. Inserts in each panel are a representative examples of the field activity recorded during the respective conditions.

**Figure 5.** Diagrammatic representation of brain sections (Swanson Maps 39-40) showing the location of recorded cells in the red nucleus and their respective theta-related cell classifications. Four tonic theta-ON cells and five non-related cells are not shown due to overlap with other cells.

**Figure 6.** Relationship between the discharges of a representative red nucleus tonic theta-ON cell (bottom panels) and the transition of hippocampal field activity from LIA to theta (bottom panels in (A) and (B), respectively. (A) An example of the discharge rates associated with one transition from LIA to theta field activity. Note the increase in rate just prior to theta. (B)
Examples of discharge rates associated with two transitions from LIA to briefer occurrences of theta field activity.

**Figure 7.** (A) Relationship between the discharges of a representative red nucleus tonic theta-ON cell (bottom panel) during LIA (top panel), and (B) during the transition from lower frequency theta to higher theta frequency. Note this transition was also signaled by a brief increase in discharge rate just prior to the transition.

**Figure 8.** (A) Perievent time histogram analysis of 8 LIA – theta transitions of the red nucleus tonic theta-ON cell shown in Figure 6. Note the increase in discharge rate in the 400 – 500 ms period of LIA just prior to onset of theta field activity. (B) Graph illustrating the discharge rates of 45 samples of the cell analyzed during LIA preceding the “transition LIA”, the LIA immediately preceding the transition to theta, and during the first few seconds of theta following the LIA (bars are standard errors of the mean).

**Figure 9.** Examples and analysis of the discharge rates of a representative red nucleus tonic theta-ON cell during transitions from LIA to theta of increasing frequencies. (A) sample showing the discharge rate associated with a transition from LIA to 3.8 Hz theta. (B) sample showing the discharge rate associated with the transition from LIA to 4.3 Hz theta. (C) sample showing the discharge rate associated with the transition from LIA to 4.8 Hz theta. (D) Graph showing the results of analysis of this cells discharge rates during the conditions of LIA preceding the “transition LIA”, the transition LIA immediately preceding theta, and the first few second of theta following the transition LIA (bars are standard errors of the mean).

**Figure 10.** Examples of the discharge rates and patterns of a representative red nucleus phasic theta-ON cell during (A) LIA, (B) the transition from LIA to theta, and (C) the transition from a lower to higher frequency of theta
Figure 1
Figure 2

Bottom of red nucleus stimulation track
Figure 3
Figure 4
Figure 7
Figure 8
Figure 9