BEHAVIORAL BIOLOGY, 14, 295-308 (1975), Abstract No. 4305

Temporal Relationships Between Hippocampal Slow Waves and Exploratory Sniffing in Hamsters

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Temporal relationships between inhalation, vibrissal protraction, and hippocampal slow wave activity during exploratory sniffing were studied in freely moving hamsters. Subjects exhibited stereotyped sniffing bouts during which respiration rates routinely were faster than 5 Hz, and inhalations were accompanied by protractions of the mystacial vibrissae. Sniffing invariably was accompanied by rhythmical slow wave activity (RSA) in the dorsal hippocampus. During presentations of various odorants, RSA generally appeared prior to the commencement of sniffing and continued for several minutes beyond the cessation of sniffing bouts. Sniffing and RSA rates were similar, but rarely identical for the full duration of a bout. Bouts typically contained more than 15 sniffs and occasionally consisted of more than 200 successive sniff cycles. During the course of bouts, sniffing and RSA often assumed identical repetition rates for periods of four to ten successive sniff cycles. These recurring periods of entrainment generally were preceded by brief accelerations or decelerations in sniffing rate, or a skipped inhalation cycle or vibrissae twitch. Except for such single skips, inhalation and vibrissal protraction were coordinated regardless of whether the sniffing and limbic rhythms were entrained. For individual subjects, there appeared to be preferred temporal relationships between inhalation cycles and hippocampal waves during entrainments. Analyses of 3000-11,000 sniff cycles and 6000-12,000 slow waves per subject indicated that the repetition rates for hippocampal waves were not altered during sniffing, and that each subject consistently exhibited a preferred temporal relationship between sniffing and RSA in the presence of different odorants. It is suggested that the limbic rhythm does exert a modulating or facilitating influence on the sniffing rhythm but does not directly drive it, and that recurring entrainments with a similar temporal relationship may provide a basis for correlating odor-induced changes in the timing of olfactory unit activity relative to rhythmic unit activity in the limbic system.

[1]This work was supported by NSF Grant GB-36070. The author thanks Drs. William B. Forbes and William M. Youngs for their helpful suggestions during the preparation of this manuscript, and Mr. Stephen Schneider for his assistance with all phases of the research.

INTRODUCTION

The appearance of rhythmical slow wave activity (RSA, theta rhythm) in the limbic system of awake mammals generally is associated with a heightened state of vigilance (Adey *et al.*, 1960; Douglas, 1967) or higher level control of voluntary movement (Whishaw and Vanderwolf, 1973). In an attempt to relate the behavioral concepts of arousal and attention to underlying neuronal mechanisms, Komisaruk (1970, 1973) has noted that units in various regions of the limbic-hypothalamic system tend to fire in phase with RSA. He further observed in the rat that exploratory sniffing and the hippocampal RSA are repetitive at about the same rate. In this species, behavioral arousal is typically accompanied by a sequence of movements (sniffing bouts) in which fixation of the head and neck, protraction of the mystacial vibrissae, and inhalation are precisely coordinated in a one-to-one fashion (Welker, 1964). During exploratory sniffing, vibrissae movements often were observed to bear a one-to-one correlation with hippocampal slow waves (Komisaruk, 1970). Komisaruk therefore suggested that the temporal characteristics of sensory inputs consequent to exploratory movements may be important for neural processing of such inputs to the limbic-hypothalamic system. In particular, he speculated that temporal relationships between sensory inputs and rhythmic limbic unit activity may serve to signal changes in the environment, or to shunt or gate afferent inputs to the limbic-hypothalamic system, thus regulating environmental influences on behavior and endocrine activity. However, a statistical assessment of the degree of correlation between sniffing movements and RSA was not provided. Since sniffing and RSA have similar frequency spectra, on occasion they might assume identical repetition rates simply by chance.

Komisaruk and Beyer (1972) later found that unit populations throughout the rostrocaudal extent of the lateral hypothalamus, the dorsal posterior periventricular diencephalic region, and the region of the dorsomedial thalamic nuclei responded to electrical stimulation of the olfactory bulb. Many of these populations responded to stimulation with odor and some fired in perfect synchrony with the inhalation cycle. Many of these responses were clearly not secondary to general arousal but their magnitude and temporal characteristics were markedly altered during periods when hippocampal RSA was present in EEG records.

In several species, olfactory bulb units have been found to fire in synchrony with the inhalation cycle (Walsh, 1956; Macrides, 1972). Macrides and Chorover (1972) have found that the timing of olfactory bulb unit discharges with respect to nasal airflow often is dependent on the identities of odorants in the inhaled air. That is, different odorants may produce similar changes in the average firing rate of single bulb units but differential changes in the relative timing of bursts during the inhalation cycle. In recordings of

responses to complex animal odors in male hamsters and deermice, single bulb units showed differential timing of bursts according to the sex of odor donors, and these odor-dependent changes in time of firing tended to persist for as long as the odorants were present in the inhaled air. The object of the present study was to characterize the temporal relationship between inhalation and hippocampal RSA during exploratory sniffing in hamsters, and to generate a statistic for testing their degree of correlation. The results indicate that during exploratory sniffing hamsters exhibit entrainments between inhalation and hippocampal RSA, and that during successive entrainments there is a consistent temporal relationship between inhalation and individual hippocampal slow waves despite the presence of different odorants. It is therefore suggested that during entrainments between sniffing and hippocampal RSA different odorants may produce differential timing of olfactory unit bursts relative to rhythmic unit activity in the limbic system.

METHODS

Experiments were performed on 11 adult male Syrian golden hamsters, *Mesocricetus auratus*. For five subjects, qualitative assessments of the relationship between sniffing and hippocampal RSA were made from polygraph records. This report concentrates on the last six animals studied (HM-42 to HM-47). For these subjects, tape recordings containing 3000-11,000 sniff cycles and 6000-12,000 hippocampal slow waves per subject were subjected to retrospective computer analyses. Electronic methods for characterizing and quantifying the temporal relationships of exploratory sniffing and RSA directly from tape-recorded data were developed so as to eliminate selection biases which might have accompanied manual analyses of polygraph records.

Nasal air flow was monitored with a thermocouple imbedded in the tip of a hypodermic needle (Beckman UNT-050) and inserted into the nasal passage through a stainless steel tube implanted vertically through the nasal bone. The thermocouple output was inverted so that inhalation (cooling of the probe) produced a positive voltage swing. The depth of the thermocouple in the nasal cavity was adjusted for maximum voltage swing during inhalation. Vibrissae twitching was monitored with bipolar stainless steel electromyographic (EMG) electrodes sewn through the mystacial musculature, ipsilateral to the thermocouple.

Slow potentials were recorded from the dorsal hippocampus and the olfactory bulb, ipsilateral to the thermocouple, with bipolar stainless steel electrodes. Each pole was insulated to 250 μm from its tip. Hippocampal electrodes had a vertical tip separation of 1.0 mm. The vertical tip separation for olfactory bulb electrodes was 1.75 mm. Subjects were positioned in a stereotaxic instrument with the bite bar 3.0 mm below the intraaural line.

Hippocampal electrodes were implanted 3.0 mm anterior and 2.0 mm lateral to lambda, 2.5 mm below the skull surface. Bulb electrodes were placed 3.5 mm below the skull surface, 12.2 mm anterior and 0.75 mm lateral to lambda. The electrocorticogram (ECoG) over the posterior neocortex was recorded with stainless steel skull screws bilaterally inserted 2.0 mm lateral to lambda.

Following a postoperative recovery period of at least 2 wk, subjects were transferred to a cylindrical Plexiglas chamber (d. 23 cm; h. 23 cm). The chamber had an open top and a perforated floor. Clean air continuously flowed up through the floor at a rate of 1.8 liter/min. To elicit sniffing behavior, a swab saturated with odorant was moved about the chamber by the experimenter. Subjects typically pursued the swab and exhibited rhythmic sniffing toward it for 2-5 min. Thereafter, subjects continued sniffing around the chamber for several more minutes. Prolonged periods of sniffing also could be evoked by blowing cigarette smoke into the chamber. In recording sessions lasting 4-6 hr, cigarette smoke or swabs containing amyl acetate, cineole, linalool, or hamster vaginal discharge were presented to subjects in counterbalanced orders at intervals of 15-20 min. Recording sessions were terminated when stimuli failed to evoke sniffing behavior for longer than 2 min.

Retrospective analyses of tape-recorded data were performed with a modified Nicolet 1072 Instrument Computer and a Hewlett-Packard 9810A calculator. Pulses which denoted the onsets of inhalation were obtained by processing the amplified signal from the thermocouple through a Schmitt trigger circuit. The trigger level was adjusted so that output pulses occurred approximately at the middle of each positive voltage swing (see Fig. 2). Pulses denoting the onsets (zero crossings) of hippocampal slow waves similarly were obtained. The various pulses were used to calculate the periods of individual sniff (ISI) or wave (IWI) cycles, and for triggering computer sweeps during correlational analyses (see below). Bandpasses during recording were 150 Hz-10 kHz for the mystacial EMG, and 1.5-100 Hz for the ECoG, the signal from the thermocouple and the slow waves from the dorsal hippocampus and olfactory bulb. On the basis of initial, wideband analyses of repetition rates for inhalations and hippocampal slow waves (see Results) our computer system was programmed to define a *sniff* as an inhalation cycle with a period of 74-200 msec. This would correspond with inhalation rates of 5.0-13.5 Hz for repetitive sniffing. RSA was analyzed over the same frequency range, and playback bandpasses were compressed to 5-15 Hz for the thermocouple, hippocampal, and olfactory bulb signals. Such filtering improved the reliability of Schmitt trigger pulses, and reduced the amplitudes of higher frequency induced waves in the olfactory bulb (Adrian, 1942, 1950; Baumgarten *et al.*, 1962) which otherwise obscured lower frequency evoked components in the bulb record (Gault and Leaton, 1963).

To determine whether individual subjects maintained temporal relationships between sniffing and hippocampal RSA more consistently than would be expected by chance, the Nicolet 1072 was operated as a multichannel computer of average transients (CAT) with sweeps initiated at the onsets of sniffs (see Fig. 4). Sweep durations (epochs) were 200 msec long, so that each epoch corresponded with one full sniff and part of the next inhalation cycle. Averages were based on 128 epochs, corresponding with 256 sniff cycles. Since our concern was for consistency over long periods of time, no effort was made to restrict averages to separate sniffing bouts. The computer continuously monitored the recorded data and initiated sweeps whenever the criteria for sniffing were met. Whenever 128 sweeps had occurred, a new average was begun. In this way, 16-40 sets of averages for all recordings from each subject were obtained.

The CAT averages provided a statistic for assessing the consistency of a temporal relationship between sniffing and RSA over intervals encompassing equal numbers of sniff cycles in the presence of different odorants. The separate averages were normalized by calculating the time between the first and second positive peak in the channel which processed the thermocouple output (Snf) and equating this time to 360°. The location of the first positive peak in the Snf channel was arbitrarily defined as 90°. It was compared to the location of the first positive peak in the hippocampal channel (Hip) to determine the phase difference between each pair of Snf and Hip averages. The distributions of such differences were plotted for all pairs of averages from individual subjects (see Fig. 5) and were tested against an expected random distribution.

For histological confirmation of electrode positions, brains were fixed in 10% formalin. Serial sections were cut 40 μm thick and stained with cresyl violet. The relative A-P position of the thermocouple in the nasal cavity was determined with calipers for subjects HM-42 to HM-47.

RESULTS

In wideband analyses of thermocouple output, inhalation rates during resting conditions were found to be in the range of 1-3 Hz. Inhalation rates during sniffing (coordinated inhalations and mystacial contractions) were rarely less than 5 Hz and never more than 13.5 Hz. Wideband analyses of RSA showed it to be principally in the range of 6-11 Hz with a second peak of components above 20 Hz. The latter, higher frequency components were excluded from subsequent analyses (see Methods). Figure 1 shows the distributions of frequencies for all sniffs (squares), all hippocampal RSA (circles), and the subset of RSA which was accompanied by sniffing (triangles), recorded from each of the six subjects and meeting our criteria for

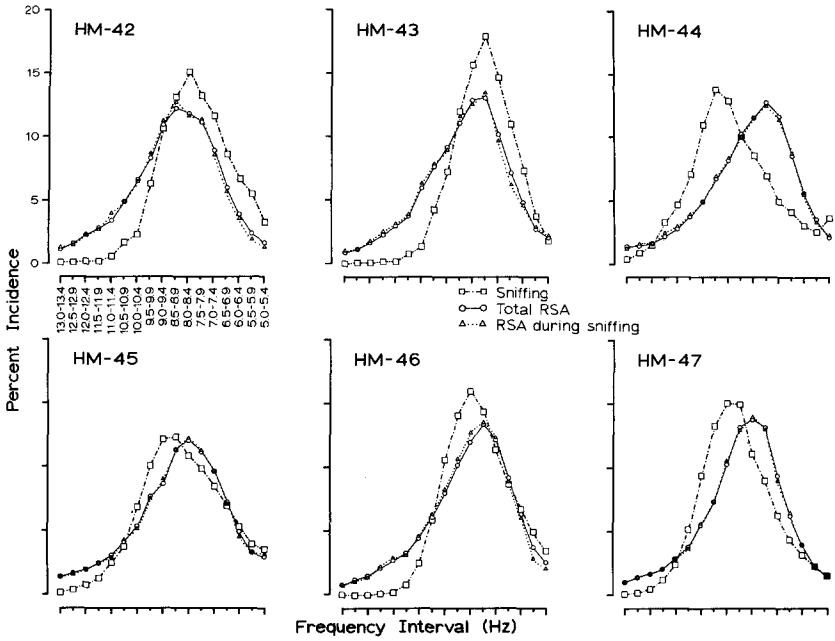


Fig. 1. Frequency distributions of sniffing and hippocampal RSA. See Table 1 for measures of central tendency and spread. To avoid the need for calculating fractions of cycles, repetition rates (frequencies) were determined as reciprocals of periods. Distributions of ISI's and IWI's were computed, and the portions of these distributions over the range of 74-200 msec then were converted to frequency distributions over the range of 5.0-13.5 Hz. Frequency distributions for the subsets of RSA accompanied by sniffing were obtained by electronically excluding data for waves which did not commence within 200 msec of the onsets of sniffs. To ensure that analyses were of sniffs or hippocampal waves occurring in repetitive trains, data were not accepted by the computer unless the preceding cycle also had a period of 74-200 msec. As a consequence of this criterion the first sniff or hippocampal wave in each train was excluded from all final analyses. It was expected that exclusion of first cycles would have negligible effects since trains rarely contained fewer than 15 cycles. Note that for each subject the frequency distribution of sniffs is similar to, but distinguishable from, the distributions of RSA whereas the distribution of RSA during sniffing is almost perfectly superimposed on the distribution of total RSA. Thus the repetition rates for hippocampal slow waves do not appear to have been altered appreciably during exploratory sniffing.

sniffing or RSA. Numerical data associated with these distributions are in Table 1. The median frequencies for hippocampal RSA were rather similar across subjects (range 8.13-8.70 Hz), whereas the median sniffing frequencies were more variable (range 7.75-9.17 Hz).

Sniffing bouts tended to be quite prolonged. They rarely contained fewer than 15 sniffs and occasionally consisted of more than 200 successive sniff cycles. Sniffing bouts invariably were accompanied by hippocampal RSA,

TABLE 1

Number of Sniff or Wave Cycles (N), Median (M), and Semiinterquartile Range (Q) for Frequency Distributions of Sniffing and Hippocampal RSA

Subject		HM-42	HM-43	HM-44	HM-45	HM-46	HM-47
Sniffing	N	4254	3022	6690	9452	6887	11,387
	M (Hz)	8.00	7.75	9.17	8.47	8.00	8.85
	Q (Hz)	0.93	0.78	1.08	1.11	0.86	0.92
Total RSA	N	8321	6049	10,495	10,187	12,086	11,460
	M (Hz)	8.62	8.33	8.20	8.40	8.13	8.47
	Q (Hz)	1.11	1.06	1.12	1.16	1.03	1.00
RSA During Sniffing	N	5818	3908	8161	9419	7264	10,990
	(% of Total RSA)	(69.9)	(64.6)	(77.8)	(92.5)	(60.1)	(95.9)
	M (Hz)	8.70	8.40	8.20	8.40	8.13	8.47
	Q (Hz)	1.08	1.08	1.16	1.16	1.03	1.00

although RSA often was observed in the absence of sniffing (see Table 1). Following presentations of odorants, RSA typically appeared approximately 10-30 sec before the appearance of sniffing, and tended to continue for several minutes beyond the cessation of sniffing bouts. Periods during which individual sniffs, vibrissae twitches and hippocampal waves were correlated in a one-to-one fashion for as many as 20 or more successive inhalation cycles occasionally were observed, but such periods were rare. They never occurred more than twice during any recording session. However, during the course of sniffing bouts, inhalation and vibrissae twitching often appeared to be well correlated with individual hippocampal waves for four to ten successive cycles. Such periods of close correlation generally were preceded by brief accelerations or decelerations in sniffing rate, or other discontinuities in sniffing pattern such as a skipped inhalation cycle or vibrissae twitch.

Record A in Fig. 2 shows a period when inhalation, protraction of the vibrissae, and hippocampal waves were correlated in a one-to-one fashion. A slow potential associated with each inhalation can be seen in the olfactory bulb record. No inhalation related activity is apparent in the ECoG. Records B-E show some of the discontinuities (brackets) in sniffing pattern which were typically followed by a resumption of the temporal relationship between inhalation and hippocampal waves as observed more consistently in Record A. Vibrissae twitches corresponded with inhalation regardless of whether inhalation in turn was synchronized with RSA. The same was true for the slow potential in the olfactory bulb, although occasionally it was obscured by higher frequency induced waves.

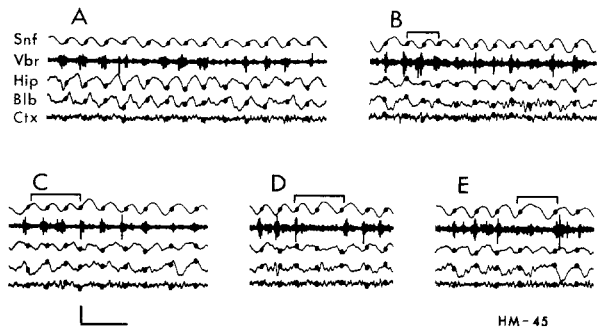
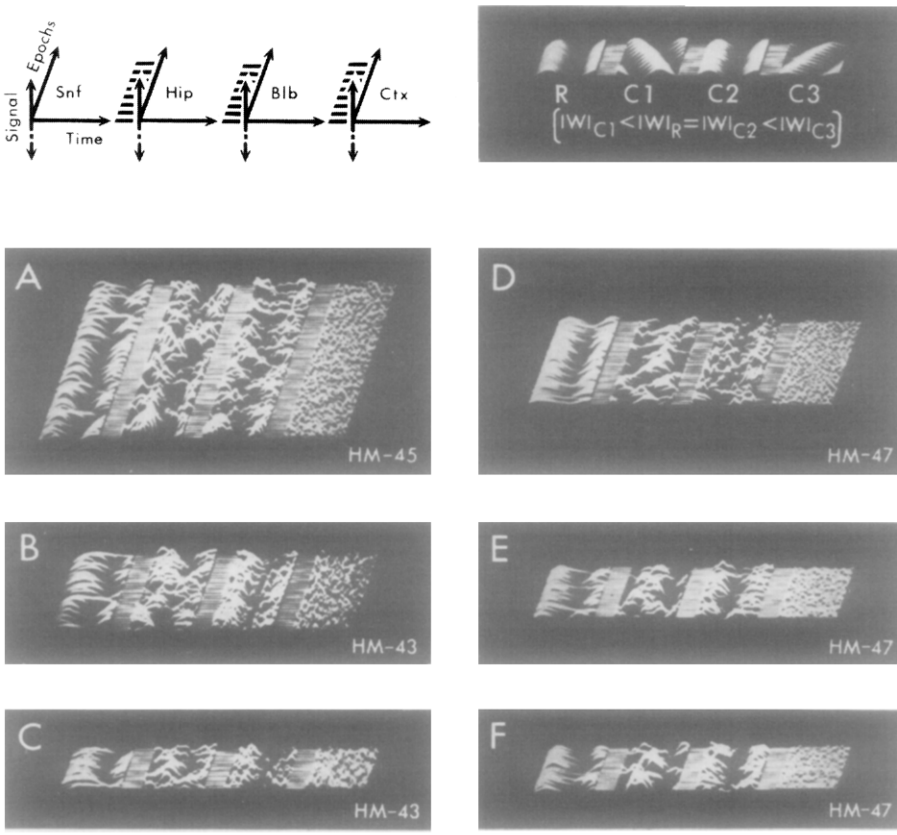


Fig. 2. Photoreversed oscilloscope records of sniffing (Snf), mystacial EMG (Vbr), and gross potentials recorded from the dorsal hippocampus (Hip), olfactory bulb (Blb), and posterior neocortex (Ctx). Brackets indicate discontinuities in sniffing pattern followed by a resumption of identical repetition rates for sniffs and hippocampal waves with the temporal relationship observed more consistently in Record A. Note in Record E that on one sniff cycle the subject withheld exhalation, then rapidly exhaled and inhaled with a more robust contraction of the mystacial musculature. During this discontinuity the slow potential in the olfactory bulb followed inhalation whereas the oscillatory potential recorded in the hippocampus was not interrupted. The absence of parallel discontinuities in Snf and Hip records indicates that the oscillatory potential from the hippocampus does not represent volume-conducted olfactory evoked responses or cable artifacts from sniffing movements. Note that mystacial contractions and inhalations remain coordinated regardless of their moment-to-moment relationship with RSA. Vertical calibration: 100 μV for Vbr, Hip and Ctx; 180 μV for Blb. Horizontal calibration: 250 msec.

The cycle-to-cycle variability in entrainments between sniffing and RSA was monitored with Response Surfaces (Macrides and Chorover, 1972). The separate traces in Fig. 2 are segments from a series of approximately 140 sniff cycles illustrated in Response Surface A of Fig. 3. The calibration surface at upper right in Fig. 3 was constructed with an 8 Hz sine wave (R) as the sweep-triggering variable. Sine wave C2 had an IWI identical to R, C1 had an IWI 4 msec shorter, and C3 had an IWI 4 msec longer. These slight differences in period (±3%) may readily be discerned. Surfaces A-F support the impression that the slow potential in the olfactory bulb consistently is correlated with inhalation, unless the potential is obscured by induced waves. The correlation between sniffing and individual hippocampal waves is clearly less consistent. If these two oscillatory activities were completely independent, and apparent entrainments (identical repetition rates) in fact were chance occurrences, the temporal relationship between inhalation and peaks or troughs in the hippocampal waves would not be consistent from one entrainment to another. In contrast, the Response Surfaces indicated the presence of preferred temporal relationships for individual subjects.

Fig. 3. Response Surfaces for three subjects. These displays accentuate slight differences in the repetition rate and temporal relationships among recorded signals. They are constructed by operating a storage oscilloscope in the X-Y mode and displaying



individual epochs (sweeps) entering the CAT averages (see Fig. 4). In each surface, successive epochs are displayed with incremental displacements up and to the right, indicated by spacer-bars between the four signals. Thus, for those sections of each surface which represent the signal in the Snf channel, successive epochs display the first and part of a second sniff in a train, then the third and part of a fourth, and so forth. The brightness of signals at each point in time is varied according to their amplitude. The net result appears as a 3-dimensional surface composed of hills and valleys illuminated from above. The hill to the extreme left of each surface represents the sweep-triggering variable (the first sniff in each epoch) and appears to travel upward in parallel with the left and right edges of the surface. Any signal which is perfectly correlated with the sweep-triggering variable (e.g., the second sniff in each epoch when sniffing rate is constant, and the evoked slow potential in the olfactory bulb) will appear as a hill or valley also travelling in parallel with the frame of the surface. When the correlation is less exact, the hill or valley will wander. For example, the hill associated with the second sniff in each epoch wanders to the left when inhalation is accelerating, and to the right when decelerating. The representative Surfaces for three subjects indicate the variability in relative timing of sniffs and peaks of hippocampal waves. For individual subjects, whenever sniffing and RSA assumed identical repetition rates they also had a similar temporal relationship. Note that sniffing is periodic regardless of whether it is entrained to RSA.

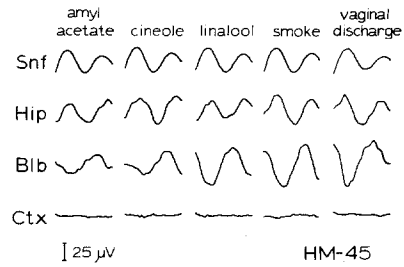


Fig. 4. Sample CAT averages taken at various times throughout a recording session. The odorant being investigated by the subject is indicated above each set of averages.

To determine whether subjects assumed similar repetition rates and temporal relationships between sniffs and hippocampal waves more consistently than would be expected by chance, the individual epochs of Response Surfaces were averaged (see Methods). The CAT averages in Fig. 4 were selected to illustrate our general finding that (i) the potentials recorded over the posterior neocortex, which did not bear any consistent temporal relationship to sniffing, cancelled each other so that their average appeared as a straight line, (ii) the relationship between exploratory sniffing and hippocampal RSA was similar in all averages for individual subjects and did not appear to be related to specific odorants in the subject's environment, and (iii) the peak-to-trough amplitudes for averages of hippocampal RSA did not systematically co-vary with the peak-to-trough amplitudes of average evoked responses in the olfactory bulb. Spearman rank correlation coefficients (r_S) for amplitudes of hippocampal versus bulbar averages were 0.21, 0.02, 0.48, 0.02, 0.21, and 0.05 for subjects HM-42 to HM-47, respectively. Only the correlation for HM-44 was statistically significant ($P < 0.05$). In contrast to the hippocampal averages, the amplitudes of bulbar averages appeared to be related to the concentration of odorants in the inhaled air. That is, during presentations of each odorant, bulbar averages were of larger amplitude for times when subjects were close to the swab bearing the odorant. These various observations indicate that hippocampal averages did not reflect olfactory evoked responses, but rather reflected recurring entrainments with similar temporal relationships between inhalations and hippocampal slow waves.

The distributions of phase differences for normalized CAT averages of sniffing and hippocampal RSA are illustrated in Fig. 5. Each of them is significantly different from a random distribution (chi-square; $P < 0.001$). The maximum semiinterquartile range observed was 30.3°.

For subjects HM-42 to HM-47 the relative A-P placements of the thermocouple were determined postmortem. More anterior placements of the thermocouple were correlated with larger median phase differences between averaged sniffing and RSA ($r_S = 0.94$; $P < 0.01$). For all subjects, olfactory

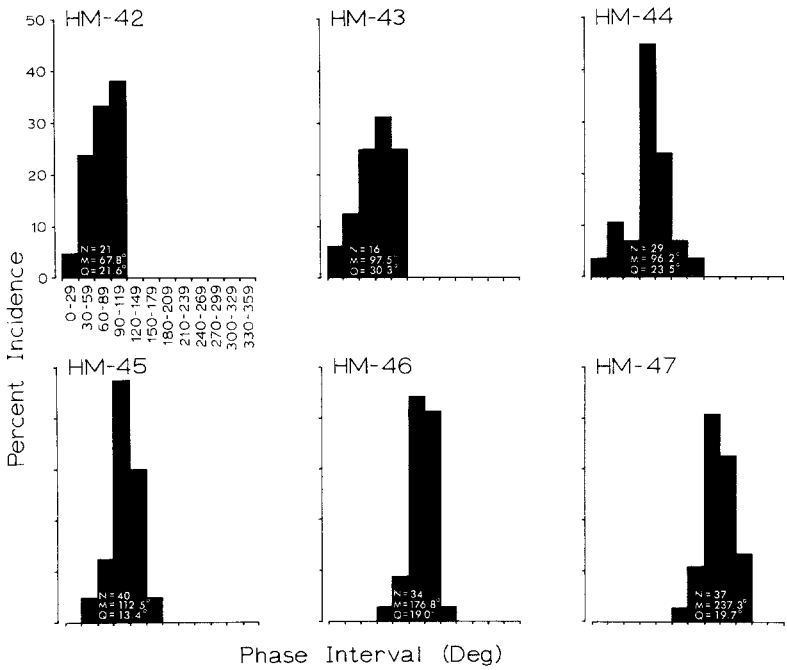


Fig. 5. Distributions of phase differences for all CAT averages of sniffing and hippocampal RSA obtained for each subject. The number of pairs of averages (N), median phase difference (M), and semiinterquartile range (Q) are indicated for each distribution.

bulb electrodes had one pole in the granule cell layer and the other pole in, or superficial to, the mitral cell layer. Hippocampal electrodes straddled the dorsal pyramidal cell layer, and in no case did the ventral pole extend into the dentate gyrus.

DISCUSSION

Our finding that averages of hippocampal RSA exhibited a narrow range of phase relationships to averaged sniffing for individual subjects, supports our impression that these subjects had preferred temporal relationships during entrainments between exploratory sniffing and RSA, and demonstrates that such entrainments are not chance phenomena. However, the relationship between individual sniffs and hippocampal slow waves reflected in these averages need not itself be one of constant phase. It is equally possible that the neural mechanism which underlies the entrainments serves to maintain a narrow range of latencies between some point in the hippocampal slow waves

and the onsets of inhalation. Over the observed range of repetition rates during entrainments, the differences in timing expected from a *phase* versus *latency* relationship approximate the normal variability in timing which we observed.

In contrast to our present findings in the hamster, Komisaruk (1970) has reported that rats do not appear to have a preferred temporal relationship for entrainments between exploratory sniffing and hippocampal RSA, and that sniffing and RSA rates remain identical throughout separate sniffing bouts. However, he did not determine distributions of temporal relationships during entrainments for his subjects, and he used vibrissae twitching as his sole monitor of exploratory sniffing. Due to the variable dispersion of activity in the EMG records, we found in the hamster that the activity of the mystacial musculature often appeared to be correlated with individual hippocampal slow waves at times when comparisons between nasal air flow and RSA clearly showed the timing of sniffs relative to peaks or troughs in the slow waves to be steadily drifting. Our monitoring procedures accentuated these slight differences in repetition rates, and showed that whenever sniffing and RSA did assume identical repetition rates they had a similar temporal relationship. Thus, our findings in the hamster that cycle-to-cycle correlations between inhalation and RSA rarely persist throughout a sniffing bout, and that temporal relationships reflected in averages of exploratory sniffing and RSA computed across sniffing bouts are more consistent than would be expected by chance, may not represent a species difference between the hamster and the rat. Rather, they might be attributed to differences in analytical procedures.

Across subjects, the observed differences in preferred timing of sniffs relative to hippocampal slow waves in part may have been related to thermocouple placement in the nasal cavity. More anterior placements may have reduced the intervals between *actual* onsets of inhalation and cooling of the thermocouple (*measured* onsets of inhalation). Shortening of the intervals between actual and measured onsets of inhalation should have resulted in comparable lengthening of the intervals between measured onsets of inhalation and slow waves. Indeed, a high positive correlation was observed between median phase difference and thermocouple placement. A dependence on electrode placement also might be expected, although placements of hippocampal electrodes were similar for all subjects.

The frequency distributions in Fig. 1 indicate that repetition rates for RSA were not altered appreciably during exploratory sniffing. This observation and the finding that entrainments between sniffing and RSA generally were preceded by discontinuities in the sniffing pattern, suggest that it is the respiratory rhythm which was being entrained to the ongoing RSA. These data also indicate that cable artifacts associated with sniffing movements were not

major components of the recorded signals. The presence of such artifacts would have resulted in parallel discontinuities in Snf and Hip records, and would have caused the frequency distributions for RSA during sniffing to be more like the distributions for sniffing itself, than like those for total RSA. Komisaruk (1970, 1973) has suggested that the neural substrate for RSA acts as a direct motor pacemaker for coordinating the movements of exploratory sniffing. Our findings are incompatible with this interpretation since such direct driving also should have produced more similar distributions for sniffing and the RSA accompanied by sniffing. Furthermore, inhalation and vibrissae twitching remained coordinated during exploratory sniffing regardless of their relationship to RSA. Entrainment to RSA thus is not critical for coordination of these movements (see Fig. 2) or for the occurrence of rhythmical sniffing (see Fig. 3). Our results are compatible with the interpretation that the limbic rhythm exerts only a modulating or facilitating influence on the (independently generated) respiratory rhythm. Lesions of the medial septum in rats have been shown to eliminate hippocampal RSA and produce impairments in rhythmicity, bilateral synchrony, and amplitude of vibrissae twitching in some subjects, but approximately half the subjects with septal lesions and loss of RSA had no apparent impairment in the motor rhythm (Gray, 1971). Thus the limbic rhythm does not appear to be the motor pacemaker for exploratory sniffing in either the hamster or the rat. Further research is needed to resolve the neural basis for entrainments between these two rhythms.

Individual subjects exhibited a narrow range of phase relationships between averaged sniffing and RSA while investigating a variety of different odorants. The timing of inhalations relative to hippocampal slow waves thus does not appear to be dependent on the identity of odorants in the subject's environment. In response to several of these odorants, olfactory bulb units have been found to undergo tonic, differential changes in the timing of discharges relative to the onsets of inhalation (Macrides and Chorover, 1972). Therefore, during periods of entrainment between sniffing and RSA such odorants might differentially affect the timing of olfactory unit activity relative to rhythmic unit activity in the limbic system, since the latter unit activity tends to occur in phase with RSA (Komisaruk, 1970). Our findings thus lend additional plausibility to Komisaruk's speculation that regulation of exploratory movements is important for processing of sensory inputs to the limbic-hypothalamic system, and to the speculation of Macrides and Chorover (1972) that periodic olfactory sampling plays a role in olfactory processing. Since one of these odorants, vaginal discharge, promotes copulatory behavior (Murphy, 1973) and elevates plasma testosterone levels (Macrides *et al.*, 1974) in male hamsters, the hamster is an ideal subject for future studies into the possible importance of these temporal correlations in behavioral and neuroendocrine regulation.

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