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# Odor-Related Bulbar EEG Spatial Pattern Analysis During Appetitive Conditioning in Rabbits

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Mildly thirsty rabbits were classically conditioned by reinforcement with water to give a discriminative licking response to the presentation of odors. The jaw movement component of the licking conditioned response (JM CR) was elicited only by the reinforced odor; an increase in the relative frequency of sniffing (RR CR) occured to both reinforced (CS+) and nonreinforced (CS−) odors. Oscillatory electroencephalographic bursts of high-frequency (40–80 Hz) potentials were recorded epidurally from the lateral olfactory bulb with 64-electrode arrays (8 × 8, 3.5 × 3.5 mm) chronically implanted. Emphasis was on comparing bursts during odor presentation with bursts preceding odor arrival on each trial. A "detection" burst was characterized as occurring immediately after odor arrival and before the sniff response. "Discrimination" bursts occurred during the RR CR and before the JM CR onset. Significant air–odor burst differences (together with sniffing) occurred through up to six sessions for both CS+ and CS− odors for "discrimination" bursts but not for "detection" bursts.

The olfactory bulb is formed by a laminated tissue of neurons that receives input from receptor axons to all parts of its surface. Olfactory bulb electroencephalographic (EEG) activity typically consists of sinusoidal oscillatory bursts of 40–80 Hz riding on crests of a slower respiratory wave. Experimental evidence suggests that odor quality is not encoded in the temporal firing of single units but in the spatiotemporal activity of neuronal populations which can be analyzed best by recording EEG waves (Freeman, 1975, 1981).

In a study with aversive classical conditioning of rabbits to odors, Freeman and Schneider (1982) found statistically significant changes in the air–odor spatial pattern of olfactory bulb EEG burst amplitude whenever a novel odor was associated with an electric shock on the first or second
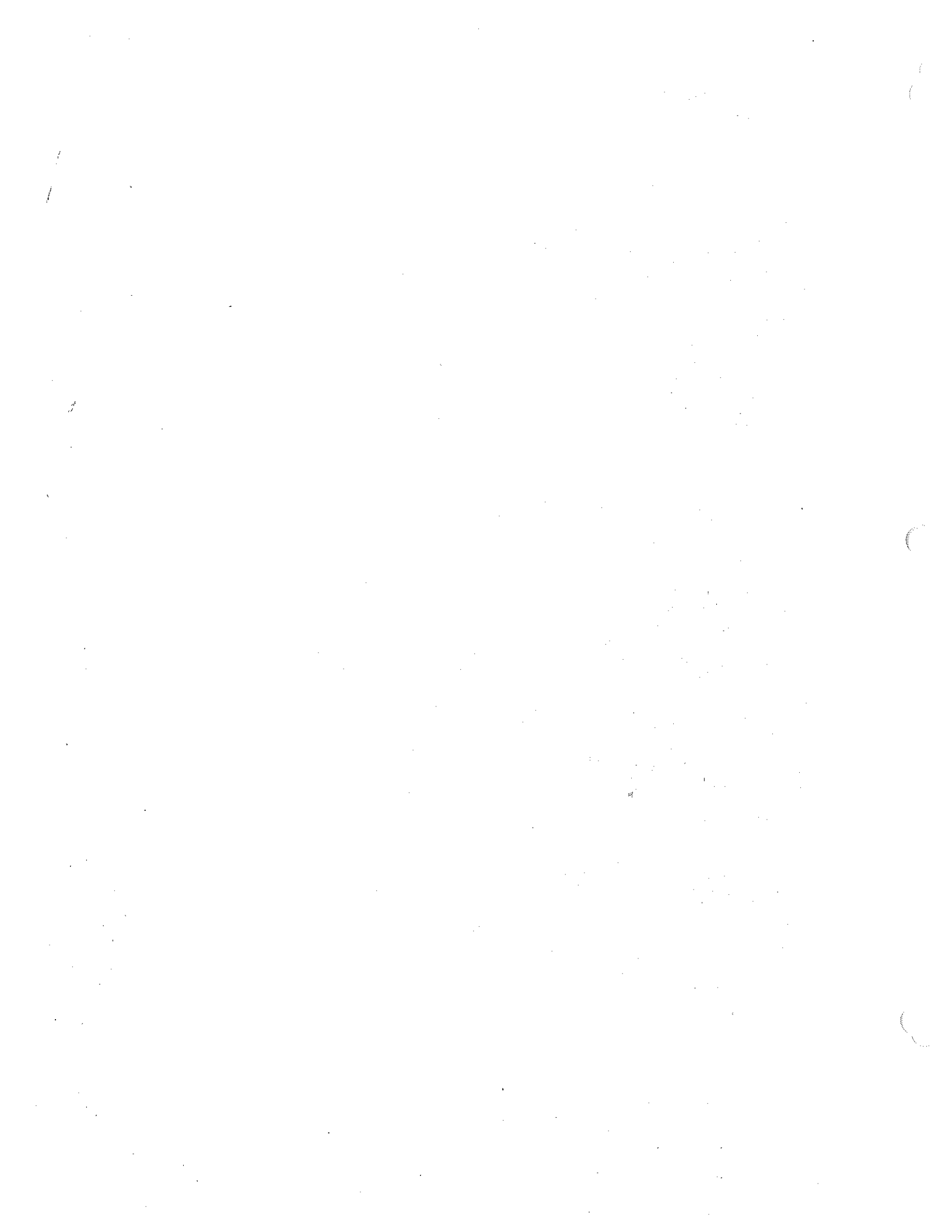
training session but not in subsequent ones. They concluded that this change in burst pattern between control states after training to an expected odor reflected the formation of an expectancy (the disposition to act in response to the odor) rather than the actual physical presence of an odor.

From this and other previous findings (Freeman, 1975), changes in bulbar EEG burst amplitude were attributed to irreversible increases in the synaptic strengths between subsets of mitral cells that were co-excited by the odor; over a series of trials under reinforcement, a template of neurons bound by strengthened synaptic connections would be formed, which would be excited stereotypically or globally thereafter with any inhalation, but more coherently when any subset of neurons within it received the training odor (Freeman, 1979). The prior activation of a neuronal template by contextual cues and centrifugal controls would be manifested in the stabilized EEG burst activity that would correspond to a "search image" for an expected odor (Freeman, 1981). Further shaping of the image was postulated to take place by habituation, which was inferred to be mediated by rapid but reversible weakening of the strength of mitral synapses onto inhibitory interneu-

rons as well as other mitral cells. This hypothesis was formulated in coupled nonlinear differential equations called a KII model (Freeman, 1979). It is this model that formed the basis on which EEG measurements in the present study were predicated.

Working with more precise EEG measurements, we extended the prior studies to an appetitive paradigm in order to investigate further correlates of odor detection and discrimination, with emphasis on what changes took place when an expected odor arrived.

From psychophysical studies, the time required for an animal to identify an odor is less than 0.5 s. Karpov (1980), for example, found that the reaction time to an olfactory cue for rabbits is on average 250 ms. From this it can be concluded that the process associated with odor detection takes place during the time of one respiratory cycle and without temporal averaging over successive inhalations. As a consequence, unlike most EEG work that involves extensive averaging across trials, the time duration for observation of the olfactory EEG must conform to that of a single inhalation. Hence, we sought the crucial information in the "detection" burst (T1) that coincides with the inhalation following the control burst (C3).

The preparation we used was the classical conditioning of rabbit's jaw movement (JM) developed by Gormezano and associates in their search for a preparation comparable in frequency and latency determinations to the traditional nictitating membrane paradigm (Gormezano, 1972), but with positive instead of aversive reinforcement. In a preliminary experiment, we found that the JM in the rabbit could be used equally well for classical appetitive conditioning to odors (Viana Di Prisco, Freeman, & Davis, 1982). This preparation has been used successfully to study changes in deprivation levels (Mitchell & Gormezano, 1970), the effect of the magnitude of reinforcement (Sheafor & Gormezano, 1972), pseudoconditioning (Sheafor, 1975), reinforcement omission (Prokasy & Gormezano, 1979), and hippocampal neuronal activity (Berry & Oliver, 1982). In all those experiments, a tone was used as a conditioned stimulus (CS).

The purpose of the present experiment was twofold. Our first objective was to detect burst pattern changes during conditioning to odors and to determine whether they were related to the odor given or to an expectation. With improved precision in EEG measurement, we asked whether it was possible to show a difference in burst spatial patterns between the T1 detection burst and the preceding control burst upon the arrival of an expected odor. Previous results that suggested a positive answer (Freeman, 1979) were drawn from studies in which each novel CS+ or CS− odor was introduced to subjects sequentially at intervals of three or more training sessions. In the present study, however, in order to force an early discrimination and to make comparisons between CS+ and CS− odor bursts from adjacent trials, both the CS+ and CS− were introduced in the same initial session on randomly interspersed trials.

The second aim was to determine whether the sequence of changes over sessions in air–odor burst differences was the same under classical appetitive conditioning as under aversive conditioning. A preliminary study, in which one CS odor was introduced within a training session, had indicated that the sequence of change over sessions was the same (Viana Di Prisco et al., 1982). However, under the new regimen of concomitant introductions of CS+ and CS−, the pattern of air–odor burst differences appeared changed. Differences between the T1 detection and the C1–C3 control bursts did not consistently appear during the first one or two sessions as they had in previous studies, but significant differences between control bursts and subsequent "discrimination" bursts (T2, T3) for both CS+ and CS− did appear and persisted. In order to establish normative data given these new circumstances, the number of sessions for each stage of discrimination was increased from 3 to 6, and the number of bursts taken in each trial was increased from 2 to 6.

The purpose of this report is to describe these EEG burst pattern differences and their relation to behavior and to offer some speculative interpretations on their significance for sensory and perceptual processing.

## Method

### Surgery and Recording

Square electrode arrays (8 × 8) of wires (250-μm-diameter stainless steel coated with Formvar) at a spacing of 0.5 mm were built according to the method described by Eastman (1975). Five adult male New Zealand rabbits weighing 2.5–3.5 kg were intubated and kept under anesthesia with halothane. After orbital exenteration a window was opened on the medial wall of the orbit with a high speed drill, with an air jet used for cooling. The face of the electrode array was placed on the intact dura covering the lateral aspect of the left olfactory bulb. The techniques for surgical placement of the electrode arrays have been described elsewhere (Freeman, 1978). Reference and ground electrodes were placed on the back of the skull, and the orbit was then filled with agar and closed with dental cement.

Monopolar recordings with respect to one orbital electrode were amplified with a bank of 64 fixed-gain (10 K) amplifiers with FET inputs. The output of each channel was filtered (3-dB fall-off at 10 and 300 Hz) and digitized at 500 samples/s (2.0-ms digitizing interval/channel) with 12 bits/sample. The most significant 8 bits were stored. Six EEG periods, 76 ms long, containing a burst superposed on a slow inspiratory

wave were sampled in each trial by visual inspection or by an automated procedure. Three bursts were sampled sequentially from the point of the real CS onset (3.3 s after the start of the trial) forward into the test period (T1, T2, and T3 bursts) and another three from the same point backward into the control period (C1, C2, and C3 bursts; see Figure 1).

Each data set, consisting of 64 time series (38 points in 76 ms), was corrected for the multiplexed time sampling by linear extrapolation between successive points (13 μs) and for amplifier gain differences by multiplying a correction factor. Bad channel values (usually 2–4 and always fewer than 10) were replaced with the average values of two adjacent channels (Freeman, 1978). The low-frequency component due to the respiratory wave was removed by a detrending algorithm, and the root mean square (rms) amplitude was computed for each channel.

In order to record jaw movements, a bipolar stainless steel electrode was implanted subcutaneously into the masseter muscle to record electromyographic activity. The signal was amplified (10 K), filtered (3-dB fall-off at 0.1 and 1000 Hz), full-wave rectified, and smoothed.

### Behavioral Procedures

One week after recovery from surgery, coinciding with the beginning of the familiarization stage (see
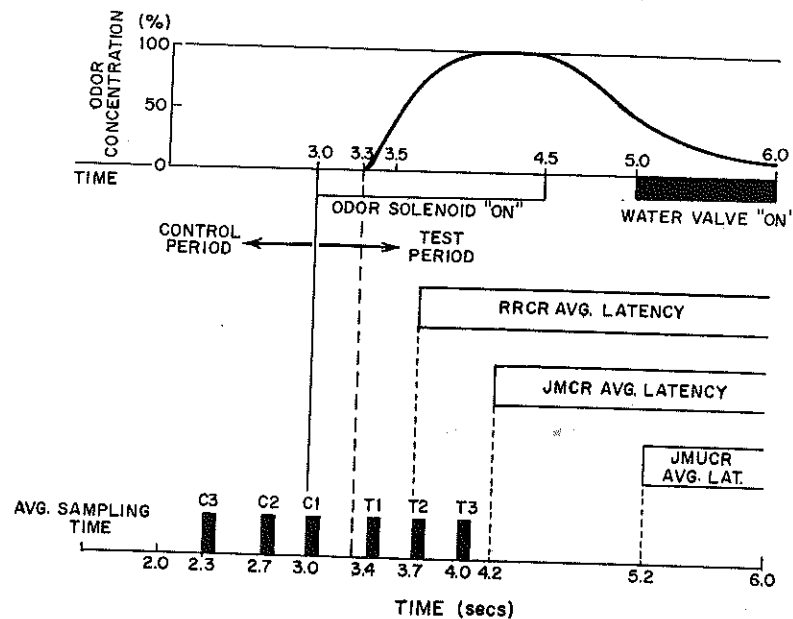


*Figure 1.* Time course schematized for an average trial. (Ten CS+, ten CS−, and ten blank [air] trials were randomly interspersed in each session. Trials consisted of a 6-s recording with a 3-s control and a 3-s test period. Average onset of the concentration rise was 3.3 s (0.3 s after odor solenoid "ON"). Upper trace: Rise to maximum and fall of odor concentration. Lower trace: Average time sampling for each burst. Middle: Average latencies for the sniff [RR] and jaw movement [JM] CRs and for the JM unconditioned response [UCR]. For electroencephalographic analysis, six periods were sampled sequentially from odor onset: forward, T1, T2, and T3; backward, C1, C2, and C3 as shown on bottom.)

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below), the animals were placed on a water deprivation schedule by restricting their daily intake to 100–150 ml. The rabbits were conditioned in a sound-masked, electrically shielded chamber provided with constant illumination and adequate ventilation. The animals were first placed in a restraining box before they were put inside the conditoning chamber. Respiration was monitored by a pneumograph fitted around the chest and connected to tambour. A plastic nose cone was fitted over the muzzle for air and odor delivery, and a mouthpiece was attached for intraoral water delivery to avoid development of an operant.

Three odors diluted in water (1:1000) were used: n-butanol, benzaldehyde, and ethylacetate. The final concentration at the nose cone was estimated to be 1:10,000. The odors were delivered through a dilution three-channel olfactometer (Moulton, Turk, & Johnson, 1975). In order to determine the arrival time of an odor at the nose cone, the olfactometer was calibrated by placing dry ice in each channel and, after solenoid activation, measuring the $CO_2$ concentration. On the average, the onset of the concentration rise was recorded at 0.3 s after the odor valve went on (see Figure 1). Water was delivered through another valve calibrated to give 1 ml of water over a period of 5 s.

Two behavioral responses were used as an index of odor discrimination: exploratory sniffing and the jaw movement component of the consummatory licking response. Automated on-line detection of sniff responses (defined as an abrupt change in respiratory rate, RR) was performed with a sniff algorithm (Davis & Freeman, 1982; Freeman, Viana Di Prisco, Davis, & Whiting, 1983). This algorithm detected statistical variations in RR by comparing 3 s of respiratory activity after solenoid activation with a similar 3 s of baseline record immediately preceding. To detect a sniff response, we tested each respiratory period sequentially by subtracting the control period mean and dividing by the control standard deviation to get a z value. When the first z value was found greater than a threshold (4.0), an RR response was flagged, and its latency was computed from the start of the test period (0.3 s after valve was turned on) to the beginning of the flagged respiratory half-cycle.

In a motivated animal, intraoral administration of water elicited a rhythmic JM response. Smith, Di-Lollo, and Gormezano (1966) defined the unconditioned response (UCR) as a sinusoidal oscillation at 5–7 Hz. We defined it, using an absolute double threshold detection algorithm operating on the digitized trace. The first ("sufficient") threshold was set slightly over noise level, and the second ("necessary") threshold was set at a relatively high level in order to reject transient artifacts. This absolute double threshold algorithm operating on the digitized trace allowed rejection of transient artifacts and better estimation of latencies. The JM conditioned response [CR] latency was computed from the start of the air–odor pulse onset and the moment when the signal crossed a lower threshold set slightly over noise level (0.1 V). From measurements in naive animals, the latency for the UCR was approximately 0.2 s.

Both RR and JM CRs were expressed as the mean ± SE of the percentage of responses over the number of trials in each session for all rabbits.

## Training Schedules

After recovering from the surgery, the animals were familiarized with the box and associated apparatus. Familiarization sessions lasted 20–60 min twice a week for four or more sessions. Our objective was to habituate the rabbits to the experimental setup, with the exception of odor and water presentations, and to collect baseline data for sniff frequency, spontaneous JMs, and EEG spatial pattern stabilization evaluation. The experimental training procedure consisted of three stages. Stage 1: For six weekly 1-hr sessions, the animals were trained to respond to the presentation of odor A as CS+, and not to respond to odor B as CS−, by reinforcing only odor A presentations with water. Stage 2: For the next six sessions, the animals were shifted to a new reinforced odor C as CS+, while odor B continued as CS−. Stage 3: In the last six sessions, odor C continued as CS+ and odor A was reintroduced, but as CS−.

For 3 rabbits, odor A was ethylacetate, odor B benzaldehyde, and odor C n-butanol; for the remaining 2 animals, odor A was benzaldehyde, odor B ethylacetate, and odor C n-butanol. Ten CS+, ten CS−, and ten blank (air) trials were randomly interspersed in each session. Each trial consisted of a 6-s recording with a 3-s control period before CS onset (actually 3.3 s due to the dead time in odor delivery). The UCS onset occurred 2 s after the end of the control period (1.7 s after odor onset and 1–2 s before odor clearance). The intertrial interval (ITI) varied randomly between 60 and 120 s.

## Statistical Computations

For each session and subject, bursts were sorted into sets of 10 according to trial type (CS+, CS−, or blank), sequence, control burst sets (C3, C2, C1), and test burst sets (T1, T2, T3). Spatial differences between burst sets were displayed in density plots of the means and standard deviations of set patterns and of their differences.

A quantitative evaluation of the difference between burst sets was obtained by applying a statistical chi-square (Freeman & Schneider, 1982). Each burst pattern was normalized by transforming its set of 64 rms amplitudes to 64 z scores with zero mean and unit standard deviation. This was done in order to remove the variation in overall burst amplitude so as to emphasize the spatial structure of each burst. A small-sample paired t test was performed on the z scores from each of the 64 channels across the 10 pairs of values of two burst sets at a time. The distribution of 64 t values, representing the difference between each pair of control burst sets, was demonstrated to conform to the theoretical t distribution (Viana Di Prisco, 1983). With the 64 absolute t values, a histogram was made of the number of channels observed to fall in eight t intervals determined by the following values of p: $0 < .5 < .3 < .2 < .1 < .05 < .02 < .01$, with $df = 7$. This observed distribution was compared with the expected random one by computing a Pearson's goodness-of-fit chi-square value. For convenience the results are expressed by the logarithm to the base 10.

Large chi-square values could arise either by a deficit of large absolute $t$ values due to patterns that were more similar than expected by chance or by an excess of absolute $t$ values due to patterns more different than expected by chance. In order to distinguish between these two cases, a "sign correction" step was included: The tail of each of the $t$ distributions was examined, and the log chi-square value was multiplied by $-1$ if there were no absolute $t$ values greater than 2.0.

In order to explore the inherent structure of pattern variability, a classification scheme known as nonlinear mapping (Sammon, 1969) was applied to subsets of the data (see Appendix). This method, based on multidimensional scaling, has been used successfully in electrophysiology for classification of evoked potentials from behaving cats by Schwartz, Ramos, and John (1976). The algorithm mapped the 64-dimensional burst pattern into a 2-dimensional surface on which each burst pattern was represented by a single point while preserving the generalized Euclidean distance between burst patterns in the set. The output was a set of points or groups of points (clusters) that had a higher degree of similarity to each other than to any pattern in another cluster. In most cases, in order to visualize the result better, the bivariate mean and standard error were computed and plotted. The core size of the laboratory computer, a Perkin-Elmer 3220 with 512K, limited the set of bursts in each map to 120 at most.

## Results

### Behavioral

During Stage 1 of conditioning, the JM CR was acquired in the first session with an average of 44% which increased to 68% by the third session (see Figure 2). The overall average for Stage 1 was 55.3% ± 9.9%; this was comparable to the results of Sheafor and Gormezano (1972), who reported a 50%–60% level of acquisition (rabbits on a 100 ml/day water deprivation schedule with 1-ml reward and tone as a CS). The level of JM activity during familiarization and training with blank trials was below 5%. During Stages 2 and 3, the overall response level was 41.1% ± 6.0%. The reason for this small drop in performance was not clear but possibly could be attributed to some degree of tolerance to chronic water deprivation.

The average response level to the non-reinforced odor B during Stages 1 and 2 was 8.3% ± 3.5%. When in Stage 3 the initially reinforced odor A was reintroduced as a CS-, the level of response was relatively high in the first session (28%) but then
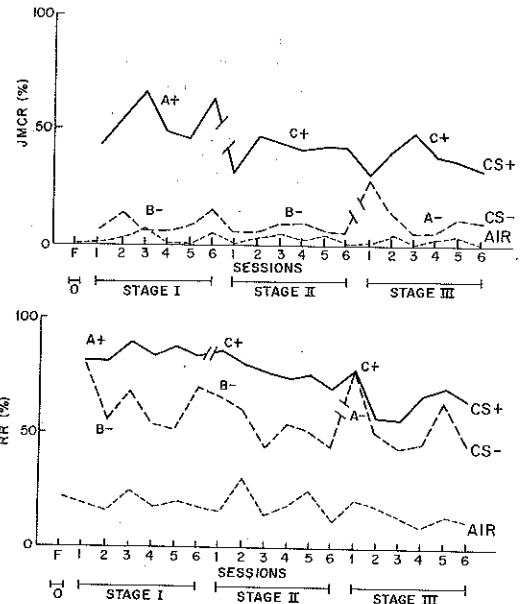


*Figure 2.* Mean percentage of jaw movement conditioned response (JM CR; top) and respiratory rate (RR) CR (bottom) over a number of trials for odor CS+ (solid line), odor CS- (dashed line), and blank (air) trials (dotted line). (F = familiarization stage. In Stage 1, odor A was reinforced and odor B was not; in Stage 2, a novel odor C was reinforced, odor A was discontinued, and odor B continued as CS-; in Stage 3, odor C continued to be reinforced, odor B was discontinued, and odor A was reintroduced but as CS-. Breaks in the curves indicate the change from A+ to C+ [note the small drop in performance, possibly attributable to the change of stimuli or adaptation to water deprivation] and from B- to A- at the start of Stage 3 [note the high response rate for the CS- on the first session that declined in later sessions].)

dropped to 14% by the second session and remained below that level for the rest of the experiment (see Figure 2).

The relative frequency of the RR CR to CS+ presentation was higher than for CS- (see Figure 2), but the latter was above baseline levels in contrast with results of previous experiments (Freeman, Viana Di Prisco, Davis, & Whitney, 1983). This difference was attributed to the circumstance that the two odors were introduced together from the beginning of training.

The RR CR frequency detected during familiarization and training with blank trials was 18.1% ± 4.9%, which was the same as observed with the aversive paradigm (Freeman et al., 1983). Similarly, the

results for average level of RR CR during Stage 1 to the CS+ (85% ± 3.3%) were comparable to the 87% ± 11% reported by Freeman and Schneider (1982). The RR response to the CS− in the first session was equal to the CS+ level (82%), then dropped to 56.8% ± 9.2%, which was near the level for pseudoconditioned responses (61%) previously found (Freeman et al., 1983) and above the level previously found for CS− (16%; Freeman & Schneider, 1982).

During Stages 2 and 3, the response level for the RR CR for the CS+ showed a decline (70.4% ± 8.5%) less rapid than the one observed for the JM CRs. With the reintroduction of odor A as CS− in Stage 3, the RR CR frequency was equal to the CS+ RR CR level (78%), but then dropped to 50% by the second session. The RR CR frequencies for CS+ and CS− both declined over the three stages, but the ratio was relatively constant, which indicates that discrimination occurred in all three stages.

The average latency for RR responses measured from the beginning of odor onset was 363 ± 100 ms. There was no difference in the latency for RR responses to the CS+ and the CS−. The group mean respiratory rate prior to the RR CR was 5.5 ± 2.1 Hz, with an interval of 182 ms. Hence the RR CR occurred typically after one or two inhalations of the CS odor.

The latency for JM CRs measured from CS+ odor onset was on the average across sessions 879 ± 210 ms, a value longer than the 250–445 ms reported by Sheafor and Gormezano (1972), using a tone as a CS. In individual trials the median time interval between the RR CR and the JM CR, when both occurred, was 430 ms, with minimal intervals as short as 100–150 ms and JM always following RR.

*Electrophysiological*

The outstanding feature of the bulbar EEG in these rabbits was a decrease in overall burst amplitude following arrival of a CS. This developed during the first three sessions of each stage and persisted thereafter. The average rms amplitude for each set of 10 bursts is shown in Figure 3 by burst sequence, subject, and stage, for CS+
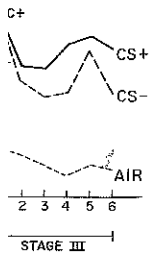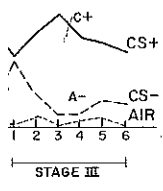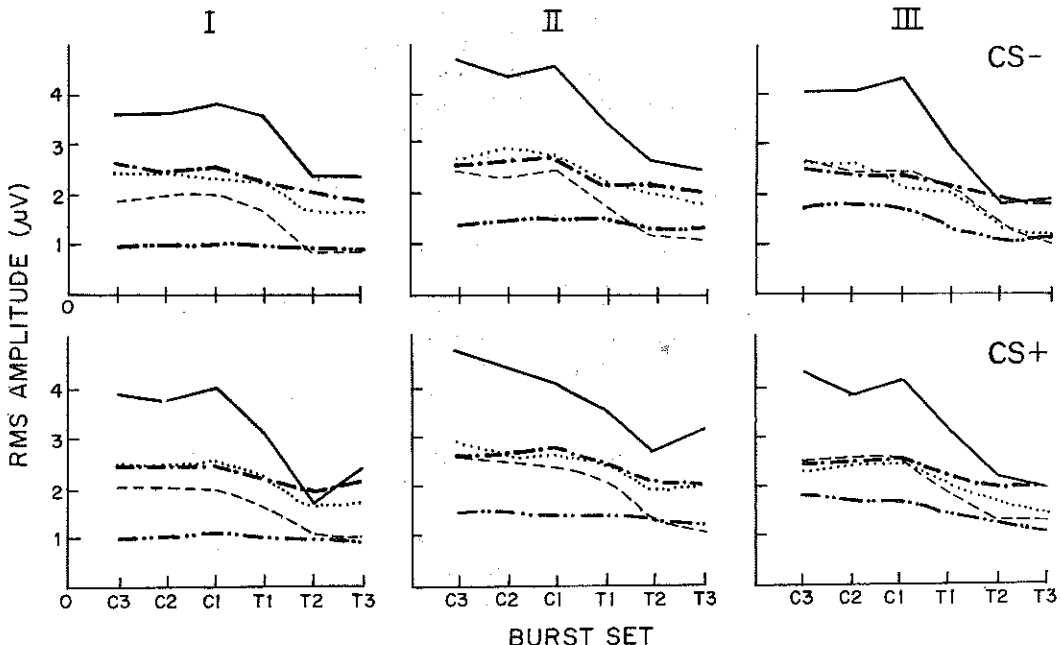


Figure 3. Mean root mean square burst amplitude (in microvolts) for each subject across all sessions and stages, with CS− trials at top and CS+ trials at bottom. (Note the tendency for burst suppression for test burst sets.)

(lower panels) and CS− (upper panels). The amount of decrease was related to control amplitude, but the relative decrease was the same across subjects, averaging −15% for T1 and −35% for T2 and T3, with approximately equal decreases for CS+ and CS−.

Analysis of spatial pattern changes was done on normalized and nonnormalized data. Visual inspection of density plots of normalized individual bursts was the best means to see pattern difference independently of burst amplitude. As in previous experiments (Davis, Freeman, & Whitney, 1981; Freeman, 1978, 1981; Freeman & Schneider, 1982; Viana Di Prisco et al., 1982), the spatial patterns of burst rms amplitude z scores were nonhomogeneous, with irregular shape and location unique to each rabbit. The density plot of the mean z scores over a set of 10 control bursts best served to display the stable configuration for each rabbit. The plot of the mean z scores that best displayed this configuration appeared as a single peak or occasionally as a double peak within the mean z score domain. The "active" area for single bursts was most often less than the domain, although an occasional high amplitude burst covered the domain. The sequence of amplitude, size of area, and peak location within the domain for successive single bursts was erratic and unpredictable in the control periods.

The characteristics are summarized in Figure 4 in which sets of 24 control bursts from each of 5 rabbits were classified by using the nonlinear mapping algorithm. The bursts fell into well-separated clusters, one for each rabbit. Maps of up to 120 control bursts for each rabbit showed that each cluster was unimodal resembling a bivariate normal density function. The time order of bursts was not apparent in any structure within the cluster. We concluded that each control burst could be regarded as a random sample from a homogeneous population.

A qualitative estimation of pattern change with CSs was obtained by visual comparison of (8 × 8) density plots of z scores of (a) normalized control bursts, (b) test bursts, and (c) their absolute difference
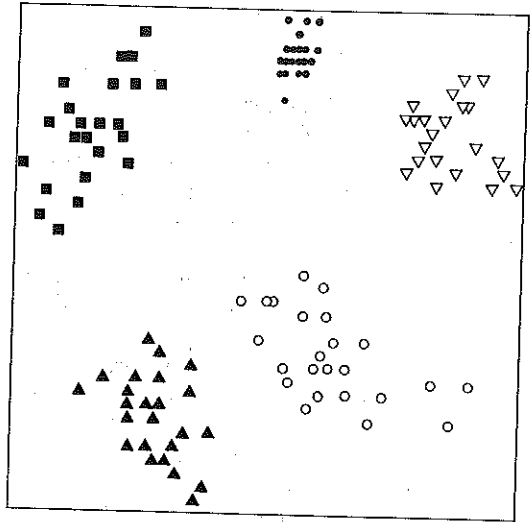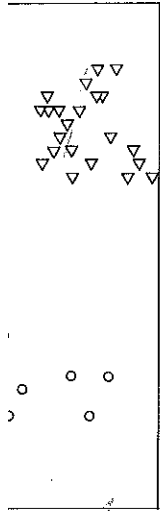


Figure 4. Burst clusters for 24 burst patterns from each of 5 subjects. (Bursts were taken during the last familiarization session for each subject. The nonlinear mapping algorithm was applied to the set, and well-separated clusters, one for each subject, emerged. This finding conformed to the fact that each rabbit had a unique and relatively stable spatial pattern of its EEG root mean square amplitude.)

patterns. Pattern differences were seen of greater or lesser degrees in all subjects and sessions for both CS− and CS+ trials. The differences between air and odor patterns did not show any consistent spatial patterns on the bulb; this agreed with previous results (Freeman & Schneider, 1982) showing that when patterns changed, only a small number of channels were affected but with variable, unpredictable location, that is, differences were global and not localizable. Furthermore, the differences between successive air bursts (air–air) and those between successive odor bursts (odor–odor) were qualitatively indistinguishable by visual inspection from the air–odor burst differences. The average absolute difference over sets of 10 burst pairs ranged from 0.4 to 0.7 standard deviation of the normalized bursts ($SD = 1$) for all three comparison groups, results showing that such odor-related or expectancy-related burst pattern differences as might exist were obscured by the continual background fluctuations in burst pattern.

The first of two statistical evaluations of within-trials burst differences was under-

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taken with the nonlinear mapping algo-
rithm applied to both normalized and non-
normalized burst data. A representative set
of 60 bursts from 1 subject and session is
shown in Figure 5. The upper panel (A)
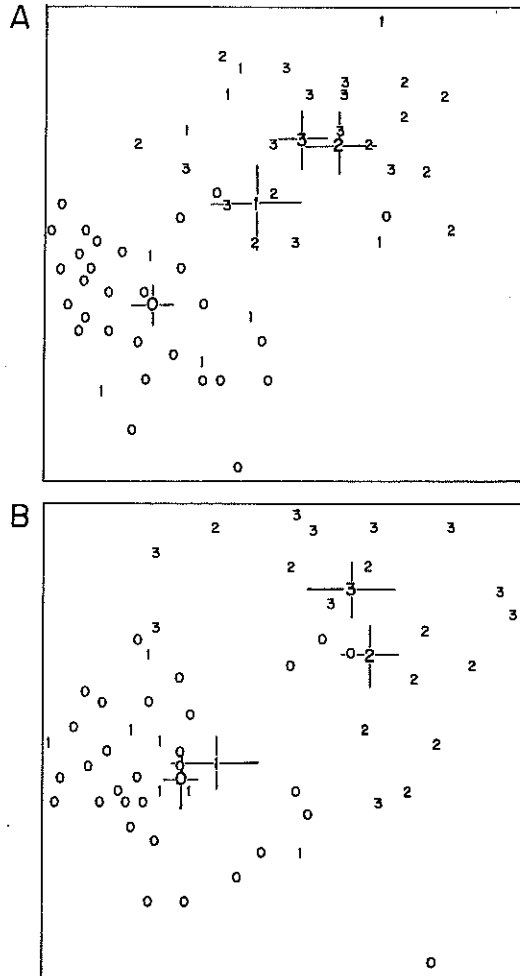shows the 60 points from 10 CS− trials,



*Figure 5.* Results from the nonlinear mapping ap-
plied to a representative session and subject for CS−
(odor A) and CS+ (odor B) trials. (Panel A shows 60
points from 10 CS− trials; panel B shows those from
10 CS+ trials. In each panel, 0 represents 30 control
bursts; 1, the T1 detection bursts; 2 and 3, the discrim-
ination bursts T2 and T3. Control and T1 burst pat-
terns were segregated from T2 and T3 burst patterns;
control bursts were more concentrated, and T2 and
T3 bursts were more scattered. Large numerals and
crosses represent centroids. Bars represent ±1 *SE* on
each dimension. The apparent difference between 0
and 1 in panel A was uncommon. The typical outcome
for both CS+ and CS− was that shown in panel B.)

and the lower panel (B) shows those from
10 CS+ trials. In each panel, 0 represents
the 30 control bursts; 1, the T1 detection
test bursts; 2 and 3, the T2 and T3 remain-
ing discrimination test bursts. The larger
numerals and crosses represent the mean
and standard error of each subgroup. (Com-
parisons between nonlinear maps were not
valid because the coordinates were derived
from the data within each map.)

An empirical statistic $t$ was devised from
the $t$ test by calculating the Euclidean dis-
tance between the means for each pair of
subgroups and dividing this by the pooled
standard error for the two subgroups in
both dimensions. A distribution was con-
structed for the $t$ values of 20 sets of 20
control burst pairs (air–air comparisons),
which gave a 95% confidence range of $0 <
t < 2.21$.

The main findings for both normalized
and nonnormalized data were as follows.
(a) Each control cluster was homogeneous,
with occasional outliers; on partitioning in
various ways (e.g., first and last trials, C1,
C2, C3), the submeans did not differ signif-
icantly in location (pooled $t = 0.97$, $p <
.05$). (b) The means for T2 and for T3 did
not differ significantly from each other
(pooled $t = 1.32$, $p < .05$), nor in groups of
both CS+ and CS− bursts did they differ
with respect to CS (pooled $t = 1.54$, $p <
.05$). (c) The means for T2 and T3 differed
from control means for both CS+ and CS−
(pooled $t = 3.23$, $p < .05$). (d) The standard
errors for the T2 and T3 clusters were
larger than those for the controls; the me-
dian ratio of variances was 2.72 by $F(20,
20)$, $p < .001$. (e) Overall, there were no
significant differences between the means
for the T1 detection burst and the controls.
In Figure 5 the apparent difference between
0 and 1 in panel A ($t = 2.24$, $p < .05$) was
unusual. For both CS+ and CS−, the
persistent outcome was that shown in panel
B (pooled $t = 1.78$, $p < .05$).

The mapping algorithm demonstrated a
systematic change in spatial pattern across
the stages of conditioning. An example in
Figure 6 shows the results from one subject
of pooling the 60 control bursts (30 for CS+
and 30 for CS−) from each session into a
grand mean for the 64 rms amplitudes. The

means for the six sessions of each stage fell into nonoverlapping clusters, and there was a clear progression from the pattern during familiarization on through Stage 3. Because there was no corresponding change in the grand mean for burst amplitude over sessions and stages (as there was within trials), the cluster analysis revealed a long-term change in spatial pattern relating to conditioning that was independent of burst amplitude.

Further analysis of burst differences within trials was needed to determine whether the differences revealed by cluster analysis reflected simply the burst suppression with the CS or whether there were also intrinsic pattern changes. The second quantitative measure of the difference between normalized spatial patterns was obtained by applying the chi-square test pairwise to sets of burst $z$ scores. For each animal in a given session, a total of nine air–odor and three air–air comparisons were made between control and test sets for each trial type (CS+ and CS−). All the air–air values were pooled across animals and sessions in order to get an empirical



*Figure 6.* Evolution of EEG spatial pattern revealed by nonlinear mapping. (Cluster analysis showed the evolution of the control average pattern change across stages of training; an example is shown for one subject. (0 = familiarization; I = Stage 1; II = Stage 2; and III = Stage 3. Each point represents the average spatial pattern of 60 control bursts in each session.)

distribution, which was found to have a mean of 1.21 and a standard deviation of 0.60. From the theoretical chi-square distribution, the significance level for $p < .025$ was 1.96 ($df = 63$), but because the degree of independence of each of the 64 channels was not known, an empirical level was required. For the Freeman and Schneider (1982) data, this level was 2.09; in the present case it was 2.32.

An empirical distribution was obtained for air–odor values which suggested a bimodal character (Figure 7). When the sign correction was performed on the log chi-square values (see Method), a bimodal distribution was obtained. The distribution was regarded as the sum of two distributions: One consisted of values from pattern pairs more similar than expected by chance, with a peak on the negative side; the other consisted of values from pattern pairs more different than by chance, with a peak on the positive side. The valley near zero indicated that the differences between patterns were rarely random. The proportion of values belonging to each distribution varied. For the air–air comparison, a total of 29% of the values were on the "different" distribution, for air–odor (CS−) it was 67%, and for air–odor (CS+) it was 69%. The mean values for these subgroups within the peak on the positive side were 1.40 ± 0.78 for air–air, 2.20 ± 0.89 for air–odor (CS−), and 2.16 ± 0.79 for air–odor (CS+). The means for the subgroups air–air and air–odor comparisons were almost identical, subgroups forming peaks on the negative side.

The next step was to locate statistically significant differences ($p < .025$) between patterns of air and odor bursts within sessions and stages. For each subject, session, and trial type, the mean number of significant log chi-square values was calculated and sorted according to sets T1, T2, and T3. The results summarized in Figure 8 showed that the significant differences were between control bursts and both types of odor bursts. The same result held for the mean log chi-square values. The differences were further located by separately averaging over differences between the control and T1 sets in both trial types; the differ-
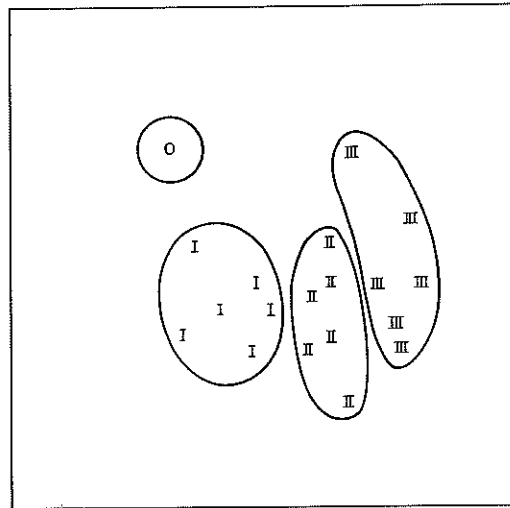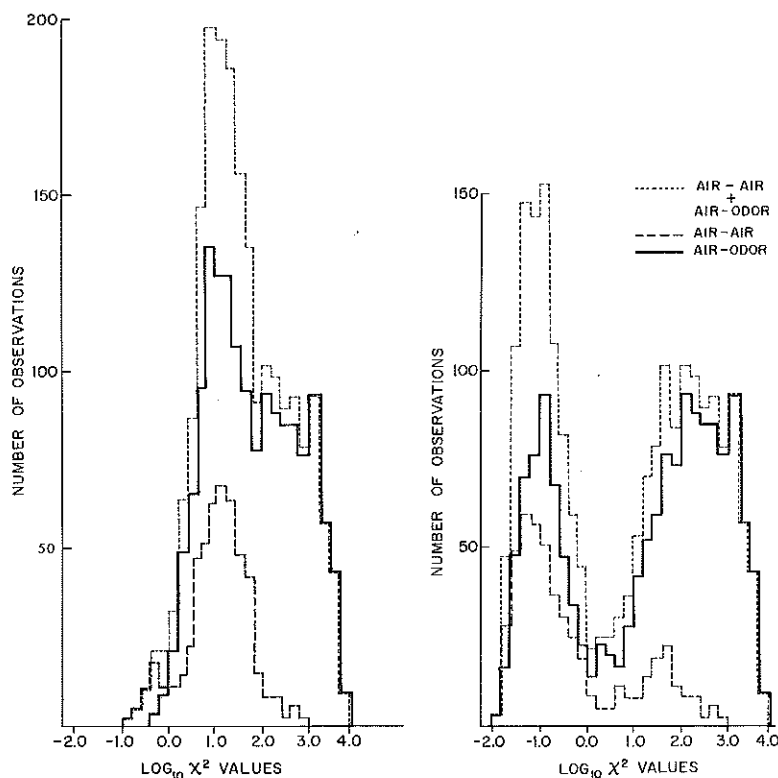
*Figure 7.* Distributions of chi-square values from comparison of burst spatial patterns. (Evaluation of pattern differences was by a statistical chi-square test that required an empirical distribution of control values to establish confidence intervals. (A chi-square value was calculated by comparing the theoretical $t$ distribution with the experimental distribution of $t$ values for each of the 64 channels that resulted from the comparison of a pair of burst sets [10 in each set]. Left: Global distribution of 2,247 $\log_{10}$ chi-square values comparing two sets of 10 bursts within sessions before the "sign correction" step. From the air–air distribution [dashed line], an empirical level of significance of 2.32 [$p < .025$] was obtained. Right: After the sign correction step, two bimodal distributions emerged, each regarded as the sum of two subdistributions: one of values from patterns more similar than expected by chance, with a peak on the negative side; the other from pattern pairs more different than expected by chance, with a peak on the positive side. The results from 627 air–air comparisons [left, dashed line] gave a larger peak on the left of "similar" distribution, whereas the air–odor values [$n = 1,620$, solid line] distribution gave a larger peak on the right side or "different" distribution. This showed that most of the values in the global distribution [dashed line] came from air–odor comparisons.)

ences between the control and T1 sets did not differ materially from the sequence of chi-square values of air–air comparisons (not shown), whereas T2 and T3 bursts differed consistently from control bursts (Figure 9).

Given that the T2 and T3 discriminatory bursts following the T1 "detection" bursts differed from the control bursts on both CS+ and CS− trials, we asked the question, did the T2 and T3 bursts on CS+ trials differ from those on CS− trials? A standard distribution establishing the significance of differences was formed by comparing air–air bursts on adjacent trials rather than on the same trial. This distribution of log chi-square values of air–air intertrial comparisons did not differ significantly from that for intratrial comparisons (see Figure 3). Comparisons were then made between T2 and T3 bursts on adjacent CS+ and CS− trials. Briefly, no significant difference was
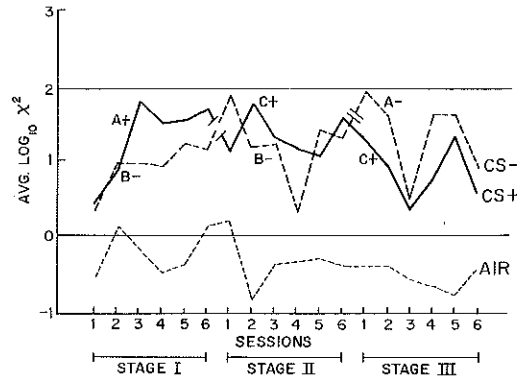
*Figure 8.* The sequence of statistically significant differences over sessions. (The chi-square test was applied for each animal in a given session. Totals of 9 air–odor CS+, 9 air–odor CS–, and 6 air–air values were obtained for each session. The number of those values that reached statistical significance [$p < .025$] was plotted for each session and trial type. On average, between 1/3 and 2/3 of the air–odor comparisons were significantly different for both CS+ and CS– trials. No decrement of the number of values for the last session in each stage was observed.)

found for T2 and T3 sets between CS+ and CS– trials, either in the mean values or in the number of significant log chi-square values from each subject and session. This result corroborated the outcome from the nonlinear mapping algorithm.
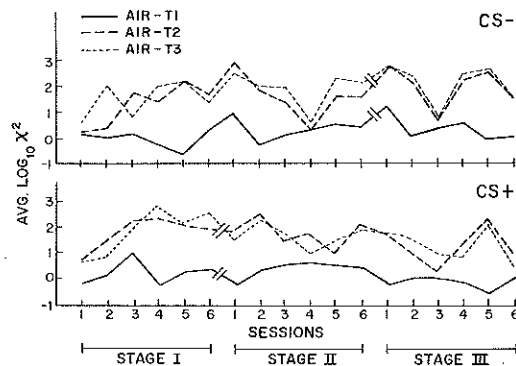


*Figure 9.* Mean chi-square values for control, T1, T2, and T3 burst sets. (The $\log_{10}$ chi-square values for air–odor comparisons were sorted and averaged separately by burst set. The average of these means across subjects was plotted for each session and stage. Differences for air–T2 [dashed line] and air–T3 [dotted line] were higher than those for air–T1 [solid line] for both CS– [top] and CS+ [bottom]. Individual air–T1 differences seldom achieved significance level.)

## Discussion

Adrian (1953) postulated that odor quality is encoded in spatial patterns of neuronal activity, on the basis of his multiunit recordings in different regions of the bulb in rabbits and cats. Recent studies with 2-deoxyglucose have lent support to this hypothesis (e.g., Lancet, Green, Kauer, & Shepherd, 1982). A fundamental assumption of these studies is that information in the olfactory bulb is carried by single neuronal units that respond to a given odor. Work done with the intention of confirming this hypothesis, however, has failed to establish the predicted correlation between single-unit activity in the olfactory bulb and a particular odor (e.g., Lettvin & Gesteland, 1965).

An alternative interpretation of the data holds that the information carried by neurons in the olfactory bulb is in a different form than that assumed in previous studies. According to this view (Coopersmith & Leon, 1984; Freeman, 1981; John, 1972), odor detection involves the formation of neuronal "templates": globally organized patterns of activity among masses of interconnected neurons. This view obviously departs from the "single unit" view of brain functioning, but it also differs significantly from traditional template models of brain functioning in holding that neuronal information is not reducible to the activity of individual neuronal units or circuits of such units but instead is carried by patterned activity across whole populations of neuronal elements. Essential to this view is the fact that neuronal activity, while patterned, is random as to whether a particular neuron is or is not involved in any way.

In an attempt to test this interpretation in relation to olfactory system functioning, Freeman (1978) recorded EEG activity simultaneously from up to 64 channels spatially distributed at regular intervals over the surface of the bulb. EEG burst activity appeared in restricted foci; the position and shape of this burst activity remained relatively stable independently of odor presentations. Only when the significance of an odor was changed by reinforcement was a sustained change in the spatial pattern of

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burst amplitude observed. It was concluded that changes in bulbar EEG burst amplitude spatial pattern were related to changes in expectancy and not to reception of an odor (Freeman, 1981; Freeman & Schneider, 1982). This finding is significant. It says that sensory input through the olfactory system is not only encoded largely in spatial patterns of activity but is not efficacious unless the animal is engaged. In short, perception is not a passive process completely constrained by receptor input; it is constrained by the animal's past experiences, its expectations, and its purposes, which determine what and even whether something happens in the olfactory bulb.

Our conclusion from the present study is that the new data tend to support the original search image hypothesis of earlier studies, which said that burst pattern changes during conditioning to odors are related to the animal's expectations rather than to odor input. As in previous studies, each animal generated a relatively stable EEG image which irreversibly changed after the introduction of each new training odor. The independence of EEG spatial amplitude pattern from odor input was shown by the lack of significant difference between control and T1 detection bursts in this aspect. A state-dependent change in amplitude of the EEG followed presentation of odors previously given in the context of reinforcement.

The new data, however, also revealed a serious deficiency in the KII model for testing the search image hypothesis (Freeman, 1979). In this study we found that the EEG change was an abatement or at times an apparent suppression of burst activity. Prediction from the model had it that bulbar burst amplitude might increase or decrease with the arrival of an expected odor but that on the average the amplitude pattern should not change, there being only a change in the pattern of phase in the burst. There was the further problem that burst suppression, when it occurred, predominated after rather than simultaneously with the T1 detection burst, most clearly in conjunction with the RR response, a result suggesting that it was part of an

exploratory response to odor detection under centrifugal control.

The tendency for olfactory EEG burst amplitude to decrease on presentation of odors is well known for both foods and laboratory chemicals used as CSs (Freeman, 1960) as well as for pheromones (de Boer & Verberne, 1981). The average evoked potentials in both the bulb and the cortex are proportionately attenuated during the EEG reduction with odor (Freeman, 1968), results indicating that the reduction is due to an excitability decrease and not to a reduction in receptor input to the bulb. In a previous study with aversive conditioning, 3 of 15 rabbits showed such dramatic suppression that they were deemed unsuitable for the study of burst activity (Freeman & Schneider, 1982). The other 12 rabbits showed about 15% decrease on the average, although the number of training sessions for each subject seldom exceeded three.

In the present study, the suppression phenomenon developed on the first three sessions and thereafter was often rather more dramatic than the average 35% reduction would suggest. Suppression occurred on different trials with or without sniffing and lasted several seconds, or much longer than the sniff; it was also observed in rabbits with their mouths taped shut, so it could not be attributed to the changes in nasal air flow that accompanied sniffing or to mouth breathing. In the previous study (Freeman & Schneider, 1982), both sniffing and burst suppression occurred only to the CS+ and not to the CS−, whereas in this study they occurred to both CS+ and CS−. These facts indicate that suppression is maximal after odor detection and may be part of a mechanism for an exploratory response or for odor identification prior to a CR.

Several possibilities arise in this connection. If, on detection, some information has been received centrally, further input from the bulb may be suppressed by centrifugal control until the information has been acted upon. Or a neuronal process of identification in the bulb may require reduction in background "noise." Or the identification process may involve null detection. In

any case, from these and the preceding results it is clear that odor detection and its bulbar EEG manifestations can be either quite specific to a CS+ or more broadly tuned to both CS+ and CS− and that detection does not necessarily involve burst suppression.

The fact that RR responses in the present series were to both CS+ and CS− odors is consistent with the search image hypothesis, which states that a template forms under reinforcement to all odors present in the context of the reinforcement. Clearly, this includes the background odor complex as well as the CS+. If a centrifugally induced process of synaptic modification that is initiated by a UCS outlasts the average ITI, the odor context would include the CS− as well. Evidence for this has been provided by measuring the sniff rate under pseudoconditioning (Freeman et al., 1983); the sniff rates to odors given in the context of shock but not paired with it were comparable to those for the CS− in this study.

In both studies, the distributions of unsigned chi-square values were similar in range and were bimodal for each subject, but the average value was higher in the present series. This was consistent with the improvement in measurement of the EEG data, but it did not serve to resolve groups by degree of difference among CS+ and CS− bursts. Although the control–T1 differences were consistently larger than air–air differences, they were not statistically significant in contrast to the control–T2, T3 differences. The previously observed pattern of change (increase in chi-square with each new CS+, followed by decrease) was not found. The persistent elevation of control–T2, T3 chi-square values through each stage and the separation of these bursts by cluster analysis were consistent with a change in spatial pattern following detection of an odor. However, to the extent that spatial structures existed in these data, they were beyond the limits of resolution by existing statistical procedures. Differential conditioning with intertrial interactions may have increased the complexity of spatial events beyond resolution, or it may have pushed the discrimination process centrally to the olfactory cortex (Bressler,

1984), leaving the bulb with some simpler or prior tasks to perform.

In either case, better EEG resolution is needed. Moreover, several critical questions remain unanswered: What happens in the T1 detection burst such that with one inhalation the animal can detect an odor and initiate exploration and discrimination? If the EEG manifests search-related information and is not merely a reflection of nonspecific trophic, regulatory, or developmental phenomena (e.g., Graziadei & Monti Graziadei, 1978), the T2 and T3 discriminatory bursts must differ in a significant way from each other as well as from the control burst. In what way or ways do they differ? Our model (Freeman, 1979) predicted that the difference would not be in amplitude pattern but in some other property of the burst. Analyses of the burst patterns of frequency, phase, and correlation in these new data have thus far failed to reveal such a difference.

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*(Appendix follows on the next page)*

## Appendix

### Nonlinear Mapping Algorithm

The algorithm described by Sammon (1969) makes no a priori assumptions about the structure of the data.

A set of $n$ burst patterns (up to 120) is summarized by a distance matrix D, where each entry is defined as the Euclidean distance $d_{ij}$,

$$d_{ij} = \sqrt{\sum_{k=1}^{64} (x_{ik} - x_{jk})^2},$$

where $(x_i, x_j)$ is any pair of bursts in the set and the index $k$ refers to each channel; therefore $d_{ij}$ is a measure of the similarity between burst $x_i$ and burst $x_j$.

The aim is to map the 64-dimensional burst vectors into a 2-dimensional vector space Y while preserving the distance between burst patterns, that is, the algorithm tries to find a 2-dimensional set of vectors whose distance matrix C would be as close as possible to D, with the use of a steepest descent procedure to search for the minimum error of a function $E$ defined as

$$E = \frac{\sum_{i<j}^{n} \dfrac{(c_{ij} - d_{ij})^2}{c_{ij}}}{\sum_{i<j}^{n} c_{ij}},$$

which is a measure of the least squares fit of the higher and lower dimensional distance matrices.

Initially, the 64-dimensional space is mapped orthogonally onto a 2-dimensional space spanned by the two original coordinates with largest variances. At each iteration $t$, the error $E(t)$ is determined. A new 2-dimensional configuration for each vector component is computed.

$$y_{pq}(t + 1) = y_{pq}(t) - 0.3\,\Delta_{pq}(t).$$

The increment $\Delta$ is the ratio of the first derivative to the absolute value of the second derivative of the error $E$ with respect to the component $pq$.

$$\Delta_{pq}(t) = \frac{\dfrac{\partial E(t)}{\partial y_{pq}(t)}}{\left| \dfrac{\partial^2 E(t)}{\partial y_{pq}^2(t)} \right|},$$

where

$$\frac{\partial E}{\partial y_{pq}} = -\frac{2}{\sum_{i<j}^{n} c_{ij}} \sum_{\substack{j=1 \\ j \neq p}}^{n} \left\{ \frac{(c_{pj} - d_{pj})}{d_{pj} c_{pj}} (y_{pq} - y_{jq}) \right\}$$

and

$$\frac{\partial^2 E}{\partial y_{pq}^2} = -\frac{2}{\sum_{i<j}^{n} c_{ij}} \sum_{\substack{j=1 \\ j \neq p}}^{n} \left\{ (c_{pj} - d_{pj}) - \frac{(y_{pq} - y_{jq})^2}{d_{pj}} \left[ 1 + \frac{(c_{pj} - d_{pj})}{d_{pj}} \right] \right\} \frac{1}{c_{pj} d_{pj}}.$$

A new distance matrix $C(t + 1)$ is computed and the respective error $E(t + 1)$ and so on until an asymptotic error value is obtained. Typically, the mapping error is less than .05.