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Hippocampal Response Patterns during Discriminative Eyeblink/Jaw Movement Conditioning in the Rabbit

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Abstract

Rabbits were given concurrent training in eyeblink (EB) and jaw movement (JM) conditioning in which one tone predicted an airpuff and another tone predicted water. After 10 days of discrimination training, the animals were given 10 days of reversal training. In the discrimination phase, acquisition of the two conditioned responses was not significantly different; however JM discrimination errors were much more frequent than EB errors. In the reversal phase, correct performance on EB trials increased gradually, as expected, whereas there was immediate behavioral reversal on JM trials. Differences in size and topography of dorsal CA1 multiple unit responses reflected the ability of the hippocampus to discriminate between stimuli in trained animals, corresponding to the performance of the behavioral discrimination. During jaw movement trials, the rhythmicity of the neural response was further modulated by the type of the prior trial, suggesting the coding of sequential events by the hippocampus. Thus, hippocampal conditioned activity can rapidly change its magnitude and pattern depending on the specific trial type during a concurrent EB/JM discrimination task and its reversal.

Keywords/Phrases

eyeblink; jaw movement; discrimination; rabbit; hippocampus

Classical conditioning, as a paradigm for neurobiological analysis of associative learning, offers the distinct advantage of precise specification of the nature and timing of the relevant sensory inputs, motivational states, and motor responses to be conditioned. A comprehensive and systematic series of studies has documented the classical conditioning of several responses in the rabbit, including both appetitive and aversive training paradigms that elicit distinct reflexive motor responses and presumably opposing (reward and fear or aversion) affective reactions (Gormezano, Prokasy, & Thompson, 1987). In classical conditioning of the eyeblink (EB) response, a tone predicts a corneal airpuff, triggering a defensive eyeblink response (Gormezano, Schneiderman, Deaux & Fuentes, 1962; for review, see Woodruff-Pak & Steinmetz, 2000). In contrast, jaw movement conditioning (JM) pairs the tone with a rewarding

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intraoral injection of water, resulting in a very rapidly acquired rhythmic conditioned movement of the jaw (Mitchell & Gormezano, 1970; Colman, Patterson, & Gormezano, 1966; Sheafor & Gormezano, 1972; Oliver, Swain, & Berry, 1993).

Behavioral studies directly comparing EB and JM paradigms in the same individual rabbits have typically not included electrophysiological recording and have been limited to transfer of training procedures designed to evaluate sequence effects. In those studies, the same CS was used to predict an aversive US on early trials and predict an appetitive US on later trials (or vice versa; Scavio, 1974; Scavio & Gormezano, 1980). Asymmetries in transfer have been reported, such that learning JM (appetitive) first accelerates subsequent EB (aversive) learning, but that aversive learning in the first phase retards acquisition of a subsequent appetitive task. Within each session, the trials were of the same motivational valence (appetitive or aversive) and the transfer phase necessarily included simultaneous extinction of the prior CS-US contingency and acquisition of the new association. Thus, apparent sequence differences in learning rate are difficult to interpret because they were confounded by motivational differences between sessions and the extinction of old conditioned responses to the same CS. This literature does not address important questions such as (i) the ability of the organism to respond adaptively during quickly changing (within session) drive states triggered by conditioned stimuli, (ii) differences in learning rate when the two paradigms are acquired simultaneously within a single animal and session (though see Smith et al., 2004), and (iii) the neural encoding of such incentive discrimination learning. These issues and their neurobiological correlates can be addressed only if the paradigms are trained simultaneously rather than transferred. In a within-subject appetitive/aversive discrimination task, a water-deprived animal receives an equal proportion of both EB and JM trials. During this type of discrimination training, one animal differentially responds on a trial–by-trial basis to stimuli signaling two distinct biologically-significant events. Behaviorally, the animal is required to both initiate the correct response and simultaneously suppress the incorrect response. While the existing literature (see above plus Steinmetz, Logue, & Miller, 1993 for an appetitive/aversive paradigm in rats) is adequate for comparisons of different states between subjects and stages of training, a stronger behavioral and neurobiological analysis could benefit from a within-subjects, simultaneous discrimination design.

Studies of hippocampal unit activity during EB conditioning (Thompson, 1986) and JM conditioning (Griffin & Berry, 2004; Oliver et al., 1993) indicated similarities, yet clear differences, between the two paradigms. In both conditioning tasks, the pattern of hippocampal activity approximated the time-course of the behavioral response, with the increase in unit activity initially appearing in the UCS period and then preceding UCS onset as learning developed. Importantly, control groups receiving explicitly unpaired CS and UCS presentations did not show increases in hippocampal unit activity (Berger & Thompson, 1978; Oliver et al., 1993). Thus, these patterns of hippocampal activity represented associative processes rather than merely reflecting sensory input, motivational reactions, or performance of motor responses. Although the relationship between the pattern of hippocampal activity and the behavioral response was similar in the two paradigms, the actual patterns of unit activity were very different. In EB training, the behavioral and neural responses were monophasic with a single peak occurring before the onset of the UCS (Berger & Thompson, 1978); whereas, in JM training using the same tone CS, the response patterns were cyclical with an approximately 5 to 7 Hz sinusoidal waveform of neural firing and jaw opening and closing (Oliver et al., 1993, see also Huff, Asaka, Griffin, Berg, Seager, & Berry, 2004). Thus, in trained animals, the two types of unconditioned stimuli--airpuff and water--elicited clearly different patterns of increased hippocampal neural activity that could be elicited by conditioned stimuli in their respective paradigms.

Employing a within-subject appetitive/aversive discrimination task would allow comparison of neural responses to two or more motivational types (valences) of stimuli and their corresponding behavioral responses without the confound of differences in global drive state between animals or sessions. A water-deprived animal that receives an equal proportion of both EB and JM trials should have a relatively stable background motivational state (or states) during a 1-hour testing session. Recordings of hippocampal activity during such a task that provides a stable, session-long motivational state, but includes trial-by-trial changes in stimuli signaling appetitive and aversive events and requiring different specific motor responses, could be used to clarify differential hippocampal involvement in sensory, motivational, motor and mnemonic processing. Prior findings of hippocampal discriminatory activity during paired, but not unpaired, instrumental discrimination learning (Freeman et al, 1996) and a decrement of concurrent discrimination (approach/avoidance) performance due to fornix lesions (Smith et al, 2004) strongly suggest that this type of concurrent discriminative processing may rely on the hippocampus. When combined with the behavioral precision of classical conditioning, simultaneous hippocampal recordings provide the necessary data for rigorous examination of relationships between neural activity, the performance of the correct learned behavioral response, and the inhibition of the inappropriate response on that particular trial type. An important aspect of the current study is that, unlike a go/nogo discrimination, an active and correct motor response is available on every trial so that successful performance does not depend solely upon response inhibition. Moreover, subsequent reversal of the CSs in this newly-developed task could address the issue of hippocampal dependence, since lesions are known to disrupt conditioned behavioral discrimination reversal (See Berger & Orr, 1983; Weikart & Berger, 1986; Steinmetz et al., 1993; Disterhoft & Segal, 1978; Orr & Berger, 1985).

Thus, the current study investigated electrophysiological changes in multiple unit activity in the dorsal hippocampus during an appetitive-aversive discrimination task and its reversal. The two types of trials, JM and EB, were alternated in a pseudorandom sequence to produce concurrent appetitive and aversive training. As in the literature on single response training, the appetitive JM response might be acquired more rapidly than the NM response. On the other hand, the concurrent administration of appetitive and aversive trials might accelerate NM acquisition such that the two responses are acquired at an equivalent rate. The fact that the learning here is based upon two active, correct responses (unlike go/no go paradigms) complicates predictions based upon earlier studies, especially about discriminative performance and reversal. Past findings of different patterns of unit activity during separate EB and JM classical conditioning (Berger and Thompson, 1978; Oliver et al, 1993) and hippocampal generation of differential, trial appropriate, activity during concurrent discrimination tasks (Freeman et al, 1996; Steinmetz), lead us to predict discriminative hippocampal activity. Since each trial requires a specific, active behavioral response and the suppression of the incorrect response, we hypothesize that the hippocampus will generate differential patterns of conditioned unit firing during a single session, organized rapidly (700msec ISI) according to the specific UCS predicted by a given CS.

Method

Subjects

Subjects were 14 New Zealand White rabbits (*Oryctolagus cuniculus*) obtained from Myrtles Rabbitry, Inc. (Thompson Station, TN). Animals were maintained on a 12:12 light/dark schedule, with training taking place during the light phase. Food was given ad lib, and water was continuously available except as indicated below. Handling and treatment of animals was in accordance with National Institutes of Health guidelines and with Miami University's Institutional Animal Care and Use Committee.

Apparatus

Animals were trained in an electrically shielded, sound attenuating chamber. Behavioral and neural activity were amplified and recorded on magnetic tape for off-line analysis using customized bioamplifiers and a transduction system. Stimulus presentation was controlled by a custom microprocessor and interface system. Synchronization pulses from the computer (which mark stimulus onset and offset) were also recorded on tape for analyzing stimulus-evoked activity.

Throughout each session, rabbits wore headgear mounted to their permanently implanted Amphenol connector. The headgear included (a) first-stage FET amplifiers for neuronal recording; (b) a Minitorque potentiometer to transduce the jaw movement to an electrical voltage, connected to the lower jaw with a semicircular lever and tape (see Oliver, 1993); (c) the water delivery system (see below); (d) a metal tailor's hook, insulated except for 0.5 mm on the inner surface over the rabbit's left upper eyelid for recording EB-related EMG potentials; and (e) the air nozzle (2mm in diameter) mounted to a bracket 5 mm lateral to the rabbit's left eye.

The water US was delivered through a length of Tygon tubing connected to a gravity-feed, water reservoir at one end and terminating in a syringe at the other end which press-fit into the nylon fistula protruding through the rabbit's right cheek. Midway between the reservoir and the syringe was a DC-operated solenoid valve (Skinner Electric Valve) which permitted the pulsed delivery of the saccharin solution. A similar type of tubing arrangement was used for delivery of the airpuff US. A Matheson Air regulator (Model 8L-590) was used to maintain the 210 g/cm2 air pressure, a solenoid pulsed the delivery, and the tubing terminated in a small nozzle directed at the rabbit's left eye. The tone CSs were generated by integrated, controlled oscillator chips (Intersil 8038) with computer-triggered, Grason-Stadler equipment providing shaped rise-fall functions, amplification, and mixing of the two tones and continuous white background noise (60 dB) to a single speaker (8 ohm) placed 15 cm directly in front of the rabbit.

Surgery

After at least 1 week of adaptation to the colony room and routine, animals underwent sterile surgical procedures in an aseptic facility. Animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) administered intramuscularly. A 5 mm fistula was made in the rabbit's right cheek and fitted with a nylon tube (for delivery of the water US).

Stainless steel microelectrodes (size 00 insect pins insulated with Epoxylite except for 50 μm at the tip) were implanted unilaterally in the pyramidal cell layer of CA1 in the left dorsal hippocampus according to stereotaxic coordinates (4.5 mm posterior to bregma, 5.5 mm lateral to the midline, and 3.0 mm ventral to dura, lambda was 1.5 mm ventral to bregma) as well as by monitoring neural activity from the electrode tip during penetration. When the dorsal/ventral location was determined, the electrode was secured in place with dental acrylic. Wires from the electrodes and ground screw were soldered to a nine-pin connector, permanently attached to the rabbit's skull with dental acrylic and stainless steel screws. When the dental acrylic dried, the incision was sutured and the animal placed under a heating pad and monitored until it regained consciousness.

Training

After 7 to 10 days of post-surgical recovery, animals were adapted for 3 days to a water regulation schedule, which free access to water for 1 hour per day in their home cages. On the fourth day of water regulation, each rabbit was adapted to the restraining apparatus and recording chamber for one hour.

Rabbits were assigned to one of two training paradigms, paired or unpaired. Ten of the rabbits were assigned to the paired, experimental group and trained in a delay conditioning, appetitive-aversive discrimination task and reversal. Daily sessions consisted of six blocks of paired trials, 16 trials per block, to form a total of 96 trials per day. The intertrial interval (ITI) varied randomly between 25 and 35 seconds. Trial-types were presented in a quasi-random order from a tabled sequence that repeated every 16 trials, with no more than 2 consecutive trials or ITIs the same. The quasi-random order of the stimuli ensured that the probability of a particular stimulus following any previous stimulus was as close to equal as possible with the 16 trial block format and the above constraints (for the 8 water trials in each block, 5 were followed by air, p=.625; for the 8 air trials, 5 were followed by water, p=.625). Thus, simple behavioral strategies such as alternation or "win-stay" would be unsuccessful. Half of the trials in each block were EB trials with an 800-ms tone conditioned stimulus (CS) of either 1 kHz or 8 kHz preceding a 100-ms corneal airpuff unconditioned stimulus (US). The other half of the trials were JM trials with the other tone frequency predicting delivery of an intraoral water solution US (200 ms). The interstimulus interval (ISI) was 700 ms. Half of the rabbits (n = 5) had the 1-kHz tone paired with the airpuff and the 8-kHz tone paired with the water solution; the other half had the opposite pairings of stimuli. Each rabbit underwent daily discrimination conditioning for 10 consecutive days. On the eleventh day, a discrimination reversal procedure was implemented such that the tone that had previously predicted the occurrence of the airpuff now predicted the water solution delivery and vice versa. The reversal training lasted for 10 consecutive days. Therefore, the training in the paired group provided a within-subject comparison for the neural and behavioral effects of discrimination and reversal training.

The remaining four rabbits served as control subjects, receiving explicitly unpaired presentations of the four stimuli used in the experimental group, so that each "trial" or event consisted of a single stimulus with no contiguous relationship between the stimuli. Each control rabbit received the same number of presentations of each of the four stimuli as those received by the experimental group (that is, 48 presentations of each of the four stimuli in a quasi-random order with approximately equal probability of any stimulus given any prior stimulus). An ITI varying randomly between 12.5 and 17.5 sec (average 15 sec) in the unpaired group equated the two groups in terms of total time spent in the conditioning chamber. Since there is little evidence of effective conditioning of skeletal responses beyond t=ISIs of 5 to 10 sec (Fishbein & LeBlanc, 1967; McAllister, 1953; Schneiderman & Gormezano, 1964; Spooner & Kellogg, 1947), the unpaired group was intended to control for performance factors while not supporting behavioral conditioning. Thus, the explicitly unpaired control group provided a between-subjects comparison for the associative effects of the discrimination training in the paired group.

Behavioral Analysis

Both the JM and integrated eyelid EMG were recorded on magnetic tape and polygraph (Grass Instruments, Model 7) with a 100-mm/sec paper speed during trials to allow visual inspections of the behavioral response during training. The polygraph was calibrated so that ½ mm of jaw movement produced 1mm of pen deflection. For JM, a response was required to exceed a minimum amplitude criterion corresponding to 0.5 mm of movement. For EB EMG, a CR was scored when the output of the integrator reached a value doubling the largest pre-CS event prior to UCS occurrence on a given trial. Pilot testing with both EMG and potentiometer transduction of EB ensured the functional equivalence of this measure to the traditional 0.5 mm EB criterion. It should be noted that, while individual differences existed, amplitude differences between EB and JM responses were not striking and were judged not to contribute to differences in apparent learning rates (see Huff et al, 2004 for JM kinematics).

Analysis of the behavioral data was essentially twofold, consisting of (a) the percentage of correct, CS-elicited responses (e.g., EB responses occurring prior to airpuff onset) and (b) the percentage of intrusion errors during an inappropriate CS (e.g., JM responses occurring during the CS period of an airpuff trial or EB responses occurring during a water trial). In the unpaired group, percentage of reflex responses to airpuff stimuli or water stimuli, respectively, were determined as was the percentage of pseudoconditioned or sensitized responses to each of the tones.

Neural Analysis

Multiple-unit recordings were used in this study due to their stability over multiple sessions and ability to characterize the ensemble behavior of neurons (local clusters) in a given area. While single-unit recordings might be preferred for their ability to track responses of a single neuron, they are not able to be unequivocally sustained over many days of training as with multiple unit recordings (Miller & Steinmetz, 1997). Issues concerning contamination of the neural records by electronic or behavioral movement artifact were resolved by comparison to recordings from unpaired control subjects, whose responses to conditioning stimuli were uncontaminated by erroneous behavioral or neural responses. In addition, this within subjects design would distribute electronic artifact equally between trial types and subjects' responses. It should be noted that the small, vertical jaw movements elicited by water reward are substantially different from the complex, horizontal and vertical grinding movements elicited by food or stress-induced displacement responses that can produce substantial artifact in the neuronal recordings Huff et al (2004). For the paired group, neural activity from Days 1 and 10 of discrimination and Days 1 and 10 of reversal training were analyzed. For the unpaired group, neural activity from Days 1 and 10 of training were analyzed. Data from intervening days were inspected, but did not vary systematically with training or emergence of conditioned responding and will not be presented here. Hippocampal unit activity was band-pass filtered (500-5000 Hz, Krohn-Hite Model 3700) and passed through a window discriminator (Haer Electronics) set to trigger on the largest spikes in the unit record (with at least a 3 to 1 signal-to-noise ratio). Output of the window discriminator was sampled in 20-ms bins and averaged over the entire session. From the averaged data for each day, standard scores were computed by subtracting the average bin value of the pre-CS period from the value of each of the 100 bins and dividing by the standard deviation of the pre-CS period.

Histology

At the conclusion of training, animals were given an overdose of sodium pentobarbital. A small marking lesion was made at the recording site by passing a DC current (200 μA for 10 s, Grass Stimulator Model SD-9) through the tip of the electrode. Animals were perfused intracardially with 0.9% saline, followed by a 10% formalin solution. Brains were fixed in a solution of formalin for at least 1 week before being placed in a 20% ethanol solution for 48 h. Frozen coronal sections were taken at 40-μm intervals and embedded on gelatin-coated slides. A Prussian Blue stain was used to mark the displaced iron from the electrode tip and a safranin counterstain was used to mark cell bodies. Electrode location was verified by microscopic examination and recordings judged not to be in or near the pyramidal cell layer of left dorsal CA1 were excluded from the study.

Results

Behavior

Discrimination Training—In characterizing the development of the learned behavioral discrimination between JM and EB trials, a correct conditioned response was designated as a CS-appropriate response (a blink to the airpuff or jaw movement to the water delivery) that occurred before the US onset. In addition to stimulus-appropriate responses, there were two

types of intrusion (incorrect response) errors, JM intrusions (on an EB trial) and EB intrusions (on a JM trial). In part because the two types of CRs and URs are initiated by different response systems, it was possible to have both a correct CR and an intrusion error on the same trial at the same or a different latency (see Fig. 1).

As expected, at the beginning of training there were relatively few CRs or CS period intrusion errors as shown in Figure 2. During the intermediate days of training, the number of conditioned behavioral responses increased along with the number of intrusion errors. The final days of training showed a sustained discriminative conditioned response rate and a stable, but smaller, rate of intrusion errors. The acquisition rates were not significantly different for correct JM and EB responses, with sharp increases in the percent of correct responses up to Day 4 for JM trials and Day 6 for EB trials. Repeated measures ANOVA tests supported this observation. A 2 (trial-type) × 10 (day) ANOVA on the correct responses indicated no significant difference between JM and EB trials ($F(1,9) = 1.85$ p = n.s.), and no significant interaction between Trial-Type and Day ($F(9, 81) = .07$, p = n.s.). Conditioned response rates remained high across subsequent days of conditioning, with means of 75.3% and 61.1% for the last three days of discrimination conditioning for JM and EB trials, respectively (see Figures 2A & 2B D1-10).

Although comparable conditioning rates were observed for JM and EB responses and a clear discriminative response was learned, the error rates for the two responses were not equivalent. Note, in Figure 1, the occurrence on D10 of trials with the correct response either alone (A) or accompanied by an intrusion error (B) of the other response on the same trial. Overall, JM intrusions during EB trials were more frequent than were EB intrusions during JM trials ($F(1,9) = 39.39$, $p < .001$). Figure 2A (D1-10) shows the pattern of EB intrusions (filled circles) emitted during the CS period across discrimination and reversal training. EB intrusions gradually increased across discrimination training from an average of 2.0% on the first day of training to an average of 21.5% on the last day. As seen in Figure 2B (D1-10), JM intrusions error rates during discrimination training were higher, increasing from an average of 0.9% on the first day to an average of 34.9% on the last day.

In summary, although acquisition rates for correct responses were comparable for JM and EB trials, analysis of the pattern of intrusion errors revealed differences in the discriminative or inhibitory control of conditioned JM and EB responses, with JMs being more intrusive than EBs.

Reversal Training—In reversal training, the CS previously associated with the airpuff was paired with the water, and the CS previously predicting water was paired with the airpuff. Overall, the correct EB responses followed a predictable pattern, dropping significantly on the first day of reversal training ($t(9) = 2.88$, $p < .01$, one tailed) and gradually increasing on subsequent days (Figure 2B, R1-10). However, the reversal pattern of correct JM responses was very rapid, almost immediate. Surprisingly, there was only a slight decrease from day 10 of discrimination training to day 1 of reversal training in the correct responses during JM trials ($t(9) = 1.333$, p =n.s.) and there was little to no increase across the reversal phase (Figure 2A, R1-10).

Although the effect of reversing the two CSs immediately resulted in unexpectedly high rates of correct JM responses, JM intrusion errors were more consistent with predictions. CS period JM intrusions showed the expected increase from Day 10 of discrimination to Day 1 of reversal ($t = -5.02$, $p < .001$, one-tailed). CS period EB intrusion errors showed a similar pattern, increasing from Day 10 of discrimination to Day 1 of reversal ($t = (9) = -1.93$, $p < .05$, one-tailed) and decreasing gradually during the remainder of reversal training (Figure 2A, R1-10). In summary, reversal training precipitated an initial increase in EB error rates and decrease in correct EB response rates, followed by improving performance over days. A high rate of correct

JM responses, however, appeared immediately and the percentage remained high and stable throughout reversal training. JM error rates followed a similar pattern to EB error rates, increasing on the first day of reversal training and decreasing across the remainder of training.

Unpaired Training—Unpaired presentations of the four stimuli assessed the baseline, nonassociative response rate for the two CSs and two USs. The results indicate a low (8.4%) unconditioned response rate to CSs for both JMs and EBs. The percentage of US intrusion errors, either a JM to an airpuff presentation or an eyeblink to a water presentation, was also low. The overall average of US intrusion errors across the ten days of unpaired training was 14.7%. As in paired training, JM US intrusions to airpuff presentation were more frequent (M = 12.0%) than were EB US intrusions to water (M = 2.6%). Thus, the CS- and US-evoked changes observed in behavioral response rates that developed across sessions in the trained group were concluded to be associative in nature.

Neural Activity

A clear pattern of conditioned unit responses developed over training in the paired group but not the unpaired controls. Figure 3 shows peristimulus time histograms illustrating the average neural activity in successive 20ms time bins beginning 700ms before CS onset and ending 700ms after US onset. Each histogram represents a 48 trial daily average of one trial type for all animals. Several general observations were made from visual inspection of the paired and unpaired average histograms, and supported by statistical analysis (see below). First, the unit activity during the CS and US period in paired animals was greater on the last day of discrimination training than on the first day of training. In addition, the pattern of the neural response to airpuff trials (EB trials) that developed during discrimination training was qualitatively different from the response on JM trials. On Day 1, JM trials showed an excitatory/inhibitory sequence of hippocampal activity that was absent on Day 10 and did not occur at any stage of EB training. Neural responses during EB trials after behavioral acquisition displayed an increase in activity near the end of the CS period followed by a marked increase during the first half of the US period. In contrast, responses during JM trials typically displayed a smaller increase in activity that began earlier in the CS period and was sustained throughout the US period. Figure 3B shows the peristimulus histograms for Day 1 and 10 of the reversal phase, in which the appropriate pattern of hippocampal firing was established on Day 1 and did not show a marked development across days. A three-way ANOVA on neural standard scores was conducted with Trial-Type (air or water), Phase (discrimination days 1, 10 or reversal days 1, 10) and Time Period (CS1,2,3 and UCS1,2,3) as within-subjects factors. Significant effects were observed for Trial-Type (($F1, 9) = 10.25$, $p<.011$), Phase ($F (3, 27) = 3.04$, $p<.046$) and Time Period ($F (5, 45)= 38.75$, $p<.001$). In addition, the three-way interaction term was significant ($F (15, 135) = 1.93$, $p<.026$). Post hoc comparisons (LSD test with df = 1, all differences discussed below were $p<.05$ one-tailed) were used to test for simple main effects. Significant differences between trial types occurred in the first UCS period on all days, with the response to air being significantly larger than the response to water. During the CS period, the only differences were significantly smaller (even inhibitory) responses to the CS for water than for air during CS2 and 3 on day 1 of the discrimination phase and a significantly larger early (CS2) response on water trials on reversal day 10. For both air and water trials, there was a significant increase in the UCS1 response across the discrimination phase, which was maintained on both days 1 and 10 of reversal. CS period 3, the time just before UCS onset behaved differently across training in the two trial types. For water trials, CS3 began as an inhibitory response (firing below baseline pre-CS values) but was a moderate excitatory response by day 10 and, importantly, retained this excitation immediately (R1) during the reversal phase. The CS3 response on air trials increased significantly during the discrimination phase to exceed levels found in baseline and CS1 and CS2. On the first day of reversal, however, unlike water trials, the CS3 air response was not significantly above that of CS1 and CS2,

suggesting that reversal training suppressed the conditioned CS3 neural response. By day R10, however, this CS period 3 response on air trials had recovered to be significantly above the pre-CS, CS1 and CS2 response levels. Thus, neural activity showed distinctly different conditioned response patterns between the two trial types and reacted differently to the initiation of reversal training.

Autocorrelations to characterize differences between airpuff and water trials in neural periodicity were performed on the US period of the averaged hippocampal unit responses. Averages of all EB trials were, as expected, aperiodic. In contrast to Oliver et al (1993), the group average JM histograms were not noticeably periodic. However, the 1993 study had JM trials only, whereas here there was a pseudorandom sequence of EB and JM trials (limited to no more than two consecutive trials of a single type). Because previous studies had demonstrated that conditioned neural responses in discrimination training may encode trial sequences (Deadwyler, West, Christian, Hampson, & Foster, 1985; Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999), the neural responses here were divided into four categories based on trial sequence: (a) airpuff trials preceded by a water trial (WA), (b) airpuff trials preceded by another airpuff trial (AA), (c) water trials preceded by an airpuff trial (AW), and (d) water trials preceded by another water trial (WW). As seen in Figure 4, there is an absence of rhythmicity in the neural response to airpuff trials (both WA and AA). In contrast, a slow rhythmicity (<2 Hz, indicated by a poorly defined peak near 500 msec) was detectable in the water trials (both WW and AW), but there was a clear periodicity at approximately 5.5 Hz when there were two successive JM trials (WW). The autocorrelation indicates this by repeating peaks approximately every 180 msec). This latter JM frequency is typical of that seen (behaviorally and neurally) in studies that do not mix JM and EB trials (Oliver et al, 1993; Seager, Borgnis, & Berry, 1997; Asaka et al., 2000; Griffin & Berry, 2004, Huff et al., 2004). Comparisons of neural responses between discrimination learning and reversal learning revealed no noticeable differences in autocorrelations. For comparison with paired hippocampal activity, Figure 5 illustrates a series of 48 trial histograms of hippocampal unit activity averaged across subjects in the unpaired group. Separate averages were obtained for Day 1 and Day 10 of unpaired stimulation. There were very small excitatory/inhibitory responses to the tone stimuli and unconditioned evoked responses to the UCSs, neither of which grew across the days of stimulus exposure.

In summary, the increase in neural responsiveness in the paired group was a conditioned response, as indicated by the differences between the paired and unpaired groups and between Days 1 and 10 within the paired group. There were significant differences within the paired group in the pattern of response to airpuff and water stimuli as well as to the CSs that predicted them. Autocorrelations indicated that the neural responses to the JM trials were modulated by trial sequence, being clearly rhythmic on the second consecutive water trial. In contrast, both behavioral and neural CRs on EB trials were aperiodic. Reversal of unit responses were virtually immediate for JM trials whereas hippocampal CRs on EB trials were significantly reduced on day R1 and redeveloped over the 10 days of reversal training.

Discussion

The major behavioral findings of the present study were: (a) Behavioral acquisition and asymptotic performance rates for the JM and EB trials were similar during discrimination learning. (b) There was a significant difference observed in the EB and JM discrimination error rates, with JM responses being significantly more intrusive than EB responses. (c) Upon the introduction of reversal training, correct EB responses dropped significantly on the first day and gradually improved while correct JM responses reversed within the first session. The important neural findings were: (a) In discrimination training, significant differences developed in conditioned neural responses to the two tone CSs and to the two USs (airpuff and

water delivery), revealing that neural discrimination occurred in the hippocampus between appetitive JM trials and aversive EB trials. (b) The hippocampus was capable of differential, trial-appropriate response patterns on JM and the EB trials that were only a few seconds apart in the same training session. (c) The same neural topography that was observed in the hippocampus during the JM and EB discrimination task was seen during the reversal task but occurred more quickly for JM trials than for EB.

In contrast to transfer of training and approach/avoidance instrumental studies, we found that acquisition rates for JM and EB trials were similar, indicating that the intermixing of aversive and appetitive trials neither differentially facilitated, nor suppressed the acquisition of either response. Performance of the present task required not only correct response selection, but also the simultaneous suppression of an active, alternative response on each trial, unlike previous go/no-go discrimination studies. Intrusion errors, as an index of failure to discriminate and/or suppress the incorrect response, did display significantly different patterns for EB and JM responses. In fact, comparison to very low error rates in unpaired controls suggests that intrusion errors may be associative in nature, especially those that occurred during the CS period. The rate of JM intrusions on EB trials was notably higher than the rate of EB intrusions on JM trials. This behavioral asymmetry is consistent with findings of asymmetrical transfer between sequential JM conditioning and EB conditioning paradigms, with prior JM facilitating subsequent EB but not vice versa (Scavio & Gormezano 1980; Steinmetz et al., 1993). One possible factor in these results is that corneal airpuffs activate trigeminal neurons resulting in small, nonrhythmic jaw movement (Steinmetz, 2000). This jaw movement, then, may be a component of the comprehensive eyeblink response, which could lead to the EB US partially conditioning the JM response to the EB CS. As a consequence, during the initial increase in learning of the appropriate eyeblink response, we might expect to see an increase in the frequency of JM intrusion errors, much like the pattern of CS intrusion errors observed here (Figure 1B). On the other hand, Scavio (1987) has demonstrated functional independence of the EB and JM responses when each is specifically elicited by the appropriate US, which occurred here but would not have occurred in the Steinmetz study. Importantly, our unpaired results suggest that, while more frequent than EB intrusions, the rates of JM intrusion on EB trials in the paired group cannot be accounted for simply by nonassociative links between response systems. Whether partial overlap between response systems is a possible explanation for the asymmetry in the transfer of training experiments remains to be determined.

As predicted, correct EB responses decreased on the first day of reversal and gradually increased throughout the remainder of training. JM correct responses, however, did not show a significant decrease on the first day of reversal training, indicating that the transition to the reversal phase happened much more quickly for JM responses than for EB responses. This finding is similar to that of Disterhoft and Segal (1978) who found that rats in a classical appetitive discrimination reversal task quickly learned to respond to the CS+ upon reversal but had a difficult time extinguishing their response to the CS-. As stated above, one explanation of the rapid JM reversal might be that the jaw movement has already been partially conditioned to the CS that predicts airpuff, though evidence of independence between these two responses makes this explanation unlikely (Scavio, 1987). A second possible explanation for our results could be that the motivational state of the animal contributed to performance of the task, in that all animals were water-deprived, so they were more inclined to perform the JM. Additionally, it could be that appetitive learning is faster than aversive learning in general, as was found by Segal & Olds (1973), Steinmetz, Logue & Miller (1993) and Oliver et al (1993). However, not only were the two responses learned at similar rates in this study, but it was shown earlier that appetitive motivation like water deprivation can accelerate aversive learning as well (Berry & Swain, 1989). As expected, both JM and EB CS intrusion errors increased dramatically on the first day of reversal training and gradually decreased throughout the remainder of training.

The correspondence between the development of a simple conditioned behavioral response and a large increase in hippocampal unit activity has been noted for some time (e.g., Berger, Alger, & Thompson, 1976; McEchron, M. D., & Disterhoft, J. F., 1999). The present study replicated and extended those findings by evaluating changes in hippocampal activity during an appetitive-aversive discrimination task. As expected, hippocampal activity was significantly greater in the paired group than in the unpaired groups. Further, within the paired groups, the increase in neural activity developed across the ten days of conditioning. Taken together, these findings are consistent with a mnemonic interpretation of hippocampal function, with neural responses related to learned environmental contingencies. In addition to showing an associative increase in neural activity, the paired group also developed a different neural pattern in response to JM and EB trials, trials which differed in affective valence (incentive cuing) as well as behavioral responses. Thus, the unit responses were not a simple reaction to the stable (session-long) motivational state or states, but varied by trial type, consistent with the idea (Segal & Olds, 1973; Olds, 1975) that the hippocampus may play an interactive role in mediating mnemonic and motivational processes, much like incentive motivational theories would suggest (Tolman, 1951; Grossberg, 1975; Schmajuk & DiCarlo, 1991). The fact that responses in the unpaired group were small and did not develop over training demonstrates that the units do not code for unconditioned motor, motivational or sensory responses *per se*

Discriminative responding of hippocampal cells is not a new finding (Segal & Olds, 1973; Miller & Steinmetz, 1997; Timofeeva, Kotlyar, & Popovich, 1984), however, such past neural discriminations have often consisted of differences in magnitude, rather than differences in pattern. Specifically, the previously observed neural discriminations typically involved an increase of neural activity to one stimulus as opposed to no change or even a suppression of neural activity to another stimulus. While early (Day 1) differences between JM and EB trials here replicated this finding, later (Day 10) criterion behavioral performance was accompanied by excitation (but different temporal patterns) on both JM and EB correct trials. Although previous studies collectively provide support for distinctions in hippocampal neural activity on the basis of affective differences in stimuli within conditioning paradigms, each study examined only a single affective modality. In the present study, discrimination between EB and JM trials provided a direct comparison of hippocampal neural responses to stimuli that differed clearly and qualitatively in affective content and involved different motor response systems within a single conditioning task and session. The results indicate that the hippocampus is capable, not only of responding to the presence and type of a conditioned stimulus, but also of displaying a distinctive pattern related to its conditioned incentive value. This conclusion is supported by the finding of differential firing during the CS (before motor responses) in the paired groups, but not occurring during similar movements in the unpaired group.

An important question regarding the neural responses to the airpuff and water stimuli was whether the time-course, or topography, of the neural activity corresponded to the topography of the respective behavioral responses. The eyeblink response to the airpuff US is a monophasic response; whereas, the jaw movement response to the water US is rhythmic. Berger et al. (1976) and Oliver et al. (1993) found that the topography of the conditioned neural response on EB and JM trials corresponded to the time-course of the behavior in EB and JM conditioning, respectively. Perhaps some of the differences in these conditioned neural responses are related to differential involvement of hippocampal regions (dorsal vs ventral) in somatomotor and visceral behaviors (Weible et al., 2006). It should be noted for the JM responses that there is substantial rhythmicity of the local field potential in water deprived rabbits at or near the same periodicity as the movement and this may have been the basis of the rhythmicity reported by Oliver et al (1993). The autocorrelations in Figure 4 document that the rhythmicity of the JM conditioned neural response in the current study at least partially depends on trial sequence effects and does not occur during EB trials.

A significant uncertainty accompanying the use of multiple unit recording is whether the same hippocampal neuron can display both conditioned patterns, or whether different cells condition to the two trial types. The signal-to-noise ratio required for inclusion in the current study suggests small numbers of neurons contributing to the standard scores and peristimulus histograms. Especially in the pyramidal cell layer of hippocampus, the likelihood would be strong that pyramidal cell firing corresponds to the overall shape of the multiple unit histogram (see Miller and Steinmetz, 1997). However, single unit recording during delay JM conditioning suggests that different cells may condition in the two paradigms. Specifically, EB conditioning seems to engage pyramidal cells, classified by low baseline firing rates and sometimes identified by antidromic activation by fornix stimulation (Berger, Rinaldi, Weisz, & Thompson, 1983). In contrast, Borgnis, Seager and Berry (1995; 1996) report in JM that slow firing neurons do not condition and that high baseline rate neurons (putative theta cells) develop conditioned responses during trials and also lose their preferred phase relation to the local field potential during the high firing rate of the conditioned neural response. Unfortunately, the multiple days of training required for the current task was not compatible with isolation and holding of single units during acquisition and reversal. New recording methods may, in the future, resolve this issue by allowing classification and sorting of single units during stable asymptotic performance of EB/JM discrimination.

While our data have provided empirical support thus far for an incentive motivation (both affective and mnemonic) interpretation of hippocampal function, results from ablation studies suggest involvement in response modulation. That is, a hypothesized role of the limbic system is either to facilitate or to inhibit ongoing behavior. Berger and Orr (1983) reported that rabbits with bilateral hippocampectomies were capable of exhibiting normal acquisition of a discrimination task (go/no-go), but failed to differentiate between the stimuli during subsequent discrimination reversal. Thus the hippocampus is essential for learning a discrimination reversal but not for learning the initial discrimination. It is noteworthy that the reversal deficit was a failure to suppress the previously correct response. Our reversal findings surprisingly indicate no significant difference in neural responsiveness between day 10 of discrimination and day 1 of reversal for JM trials but a slight decrease for the late CS3 response on EB trials. In a go/no-go reversal task (Miller and Steinmetz, 1997), associative hippocampal neural activity changed (decreased or absent) on the first day of reversal training, and did not return until after behavioral reversal was accomplished. In our study, the CS period 3 neural response for JM trials, but not EB trials, was well-developed on Day R1 but both trial types eventually recovered the same temporal pattern by R10 as they had on D10. Our neural responses to the US changed immediately upon reversal and did not increase over the course of reversal training. Thus, the behavioral and neural reversal was very rapid for JM trials but took longer for EB trials. The immediate behavioral reversal of the JM response in the current study may have facilitated or permitted the rapid development of hippocampal responses as the contingencies were reversed. In any case, ours, Disterhoft and Segal's (1978) and Miller and Steinmetz' (1997) studies support the idea that the roles of hippocampal neural activity may differ importantly between acquisition and reversal phases of a complex discrimination task.

Behavioral intrusion errors were not obviously accompanied by hippocampal CRs of the wrong pattern (See Fig. 3A; i.e. if both behaviors occurred on the same trial, hippocampal CRs matched the appropriate response.). This suggests that hippocampal CRs are more related to the signal value or incentive of the CS than to the motor pattern *per se*. In a similar fashion, in a go/no-go discrimination reversal task (Miller & Steinmetz, 1997), hippocampal neural responses were observed only on trials where a CR was given to the appropriate tone. When CRs were given to the inappropriate tone, no conditioned neural responses were observed. Thus, we suggest that the hippocampal unit CR in our study reflects the incentive value of the stimulus rather than movement itself.

The role of incentive motivation (the prediction of a biologically significant event by a learned cue) has been of interest to scientists since Tolman's model of learning (for review see Tolman, 1951). Tolman emphasized the influence of three variables on learning: the motivational state of the animal, the animal's representation of the value attributed to stimuli in the environment, and the animal's attraction to, and expectations of, significant objects (i.e., Where they are in the environment, or when they occur). Grossberg's attentional network model (1975) was one extension of these ideas, proposing that the pairing of the CS with the US brought about a long-term association of the sensory representation of the CS with the drive-association of the US ("incentive-motivation" learning). The attentional network model emphasized the important role of the hippocampus, especially so when it was later extended by Schmajuk & DiCarlo (1991). The results presented here indicate that the role of the hippocampus in incentive learning may need to be further investigated if we are to better understand the neural processes underlying complex associative learning.

In conclusion, this experiment employed a novel discrimination task that required the animals to differentiate between stimuli predicting consequences with opposite affective values. Our results suggest that the hippocampus can respond with different firing patterns in the same animal depending on the incentive cue and the required response. We suggest that this provides evidence that the hippocampus may play a key role in integrating motivation with learning. Such findings support computational models that posit a role for the hippocampus in incentive motivation or related processes (Grossberg, 1975; Schmajuk & DiCarlo, 1991; Wyble, Hyman, Rossi & Hasselmo, 2004). From this perspective, the effects of hippocampal damage in other studies might be related to an inability to energize and flexibly guide adaptive behavior using incentive cues that occur prior to, or at a distance from, primary consummatory or unconditioned stimuli. Tasks such as that used here, which examined the relationships between conditioned neural and behavioral responses with explicit manipulation of motivational and incentive processes, may lead to new testable hypotheses on the exact role of the hippocampus in learning and memory.

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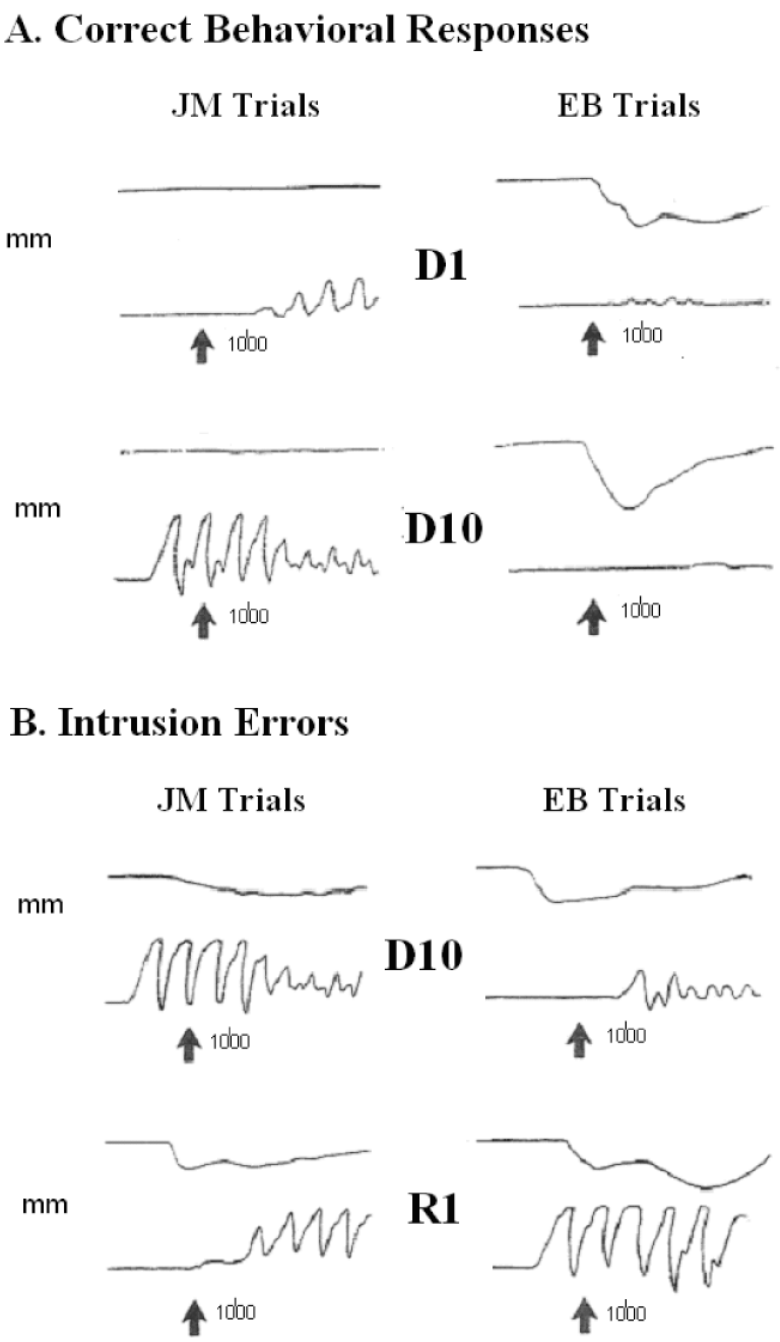


Figure 1.
Examples of (A) correct behavioral responses and (B) intrusion errors. In each of the eight pairs of polygraph records the top tracing is the EB response and the bottom tracing is the JM response measured in millimeters. Arrows indicate US onset. The time duration of each polygraph record is approximately 2000 msec, with UCS onset at 700 msec. D1, D10—days 1 and 10 of discrimination training; R1—day 1 of reversal training.

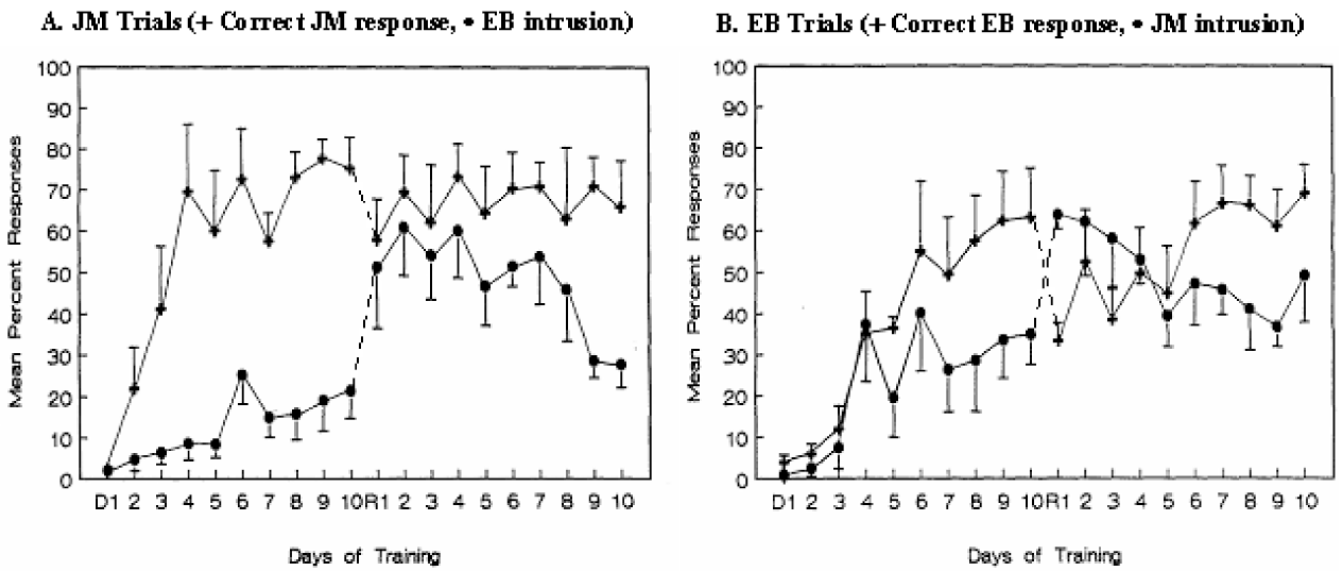
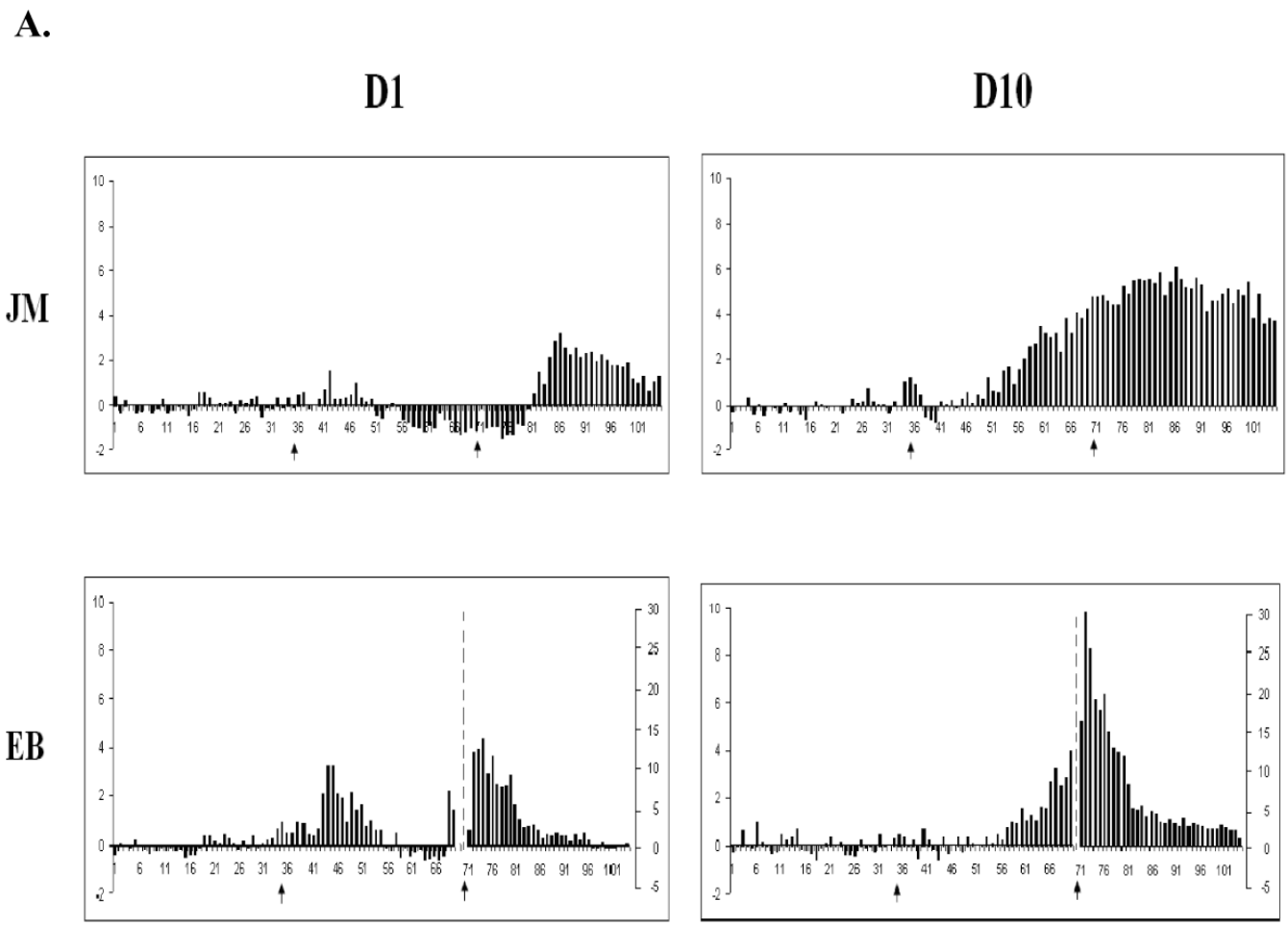


Figure 2.

Average Percent of CS Period CRs and Intrusion Errors. JM responses on EB trials are more intrusive during the CS than EB responses on JM trials. (A) Correct JM responses and CS EB intrusion errors on JM trials during paired discrimination training (D1-10) and reversal (R1-10). (B) Correct EB responses and CS JM intrusion errors on EB trials during discrimination (D1-10) and reversal (R1-10). Error bars = S.E.M. (n = 10).



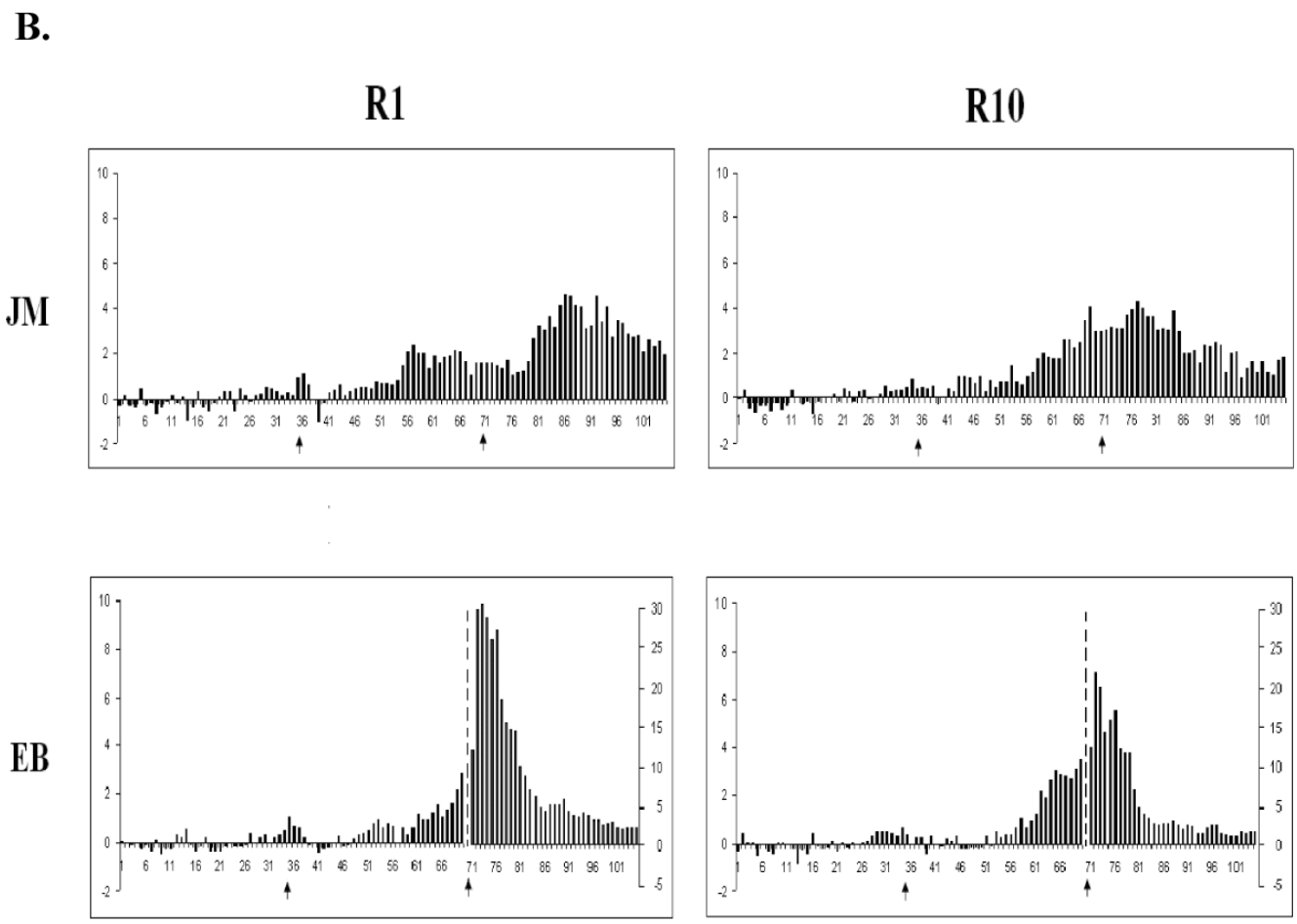


Figure 3.

Comparison of hippocampal responses during discrimination and reversal in the paired group. (A) Group average histograms showing hippocampal unit activity during JM (top) and EB (bottom) trials on the first and last days of discrimination training (D1 and D10) and (B) the first and last days of reversal training (R1 and R10). Markers indicate stimulus onset; ISI = 700 ms. Note different y-axis scale for the UCS period of airpuff trials due to the extremely large responses observed.

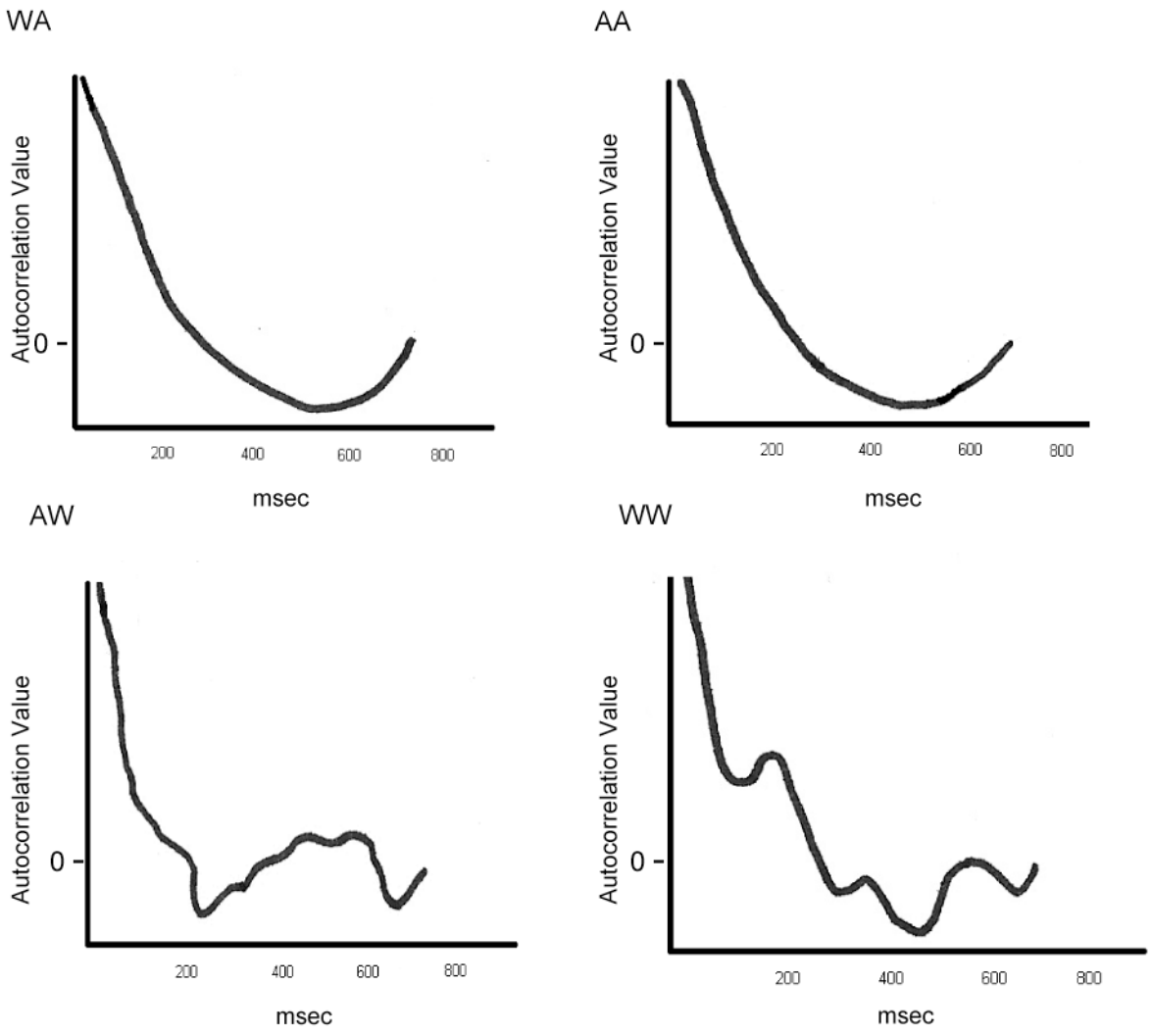


Figure 4.

Comparison of the pattern of hippocampal activity between EB and JM trials. Autocorrelations reflecting degree of rhythmicity of group averaged hippocampal unit responses to (WA) EB trials preceded by a JM trial, (AA) EB trials preceded by another EB trial, (AW) JM trials preceded by an EB trials, and (WW) JM trials preceded by another JM trial. Note the absence of rhythmicity in the EB trials as compared to the JM trials. Autocorrelation values on the Y axis are arbitrary units corresponding to the cross products of A/D interface values. The x-axis represents time in milliseconds.

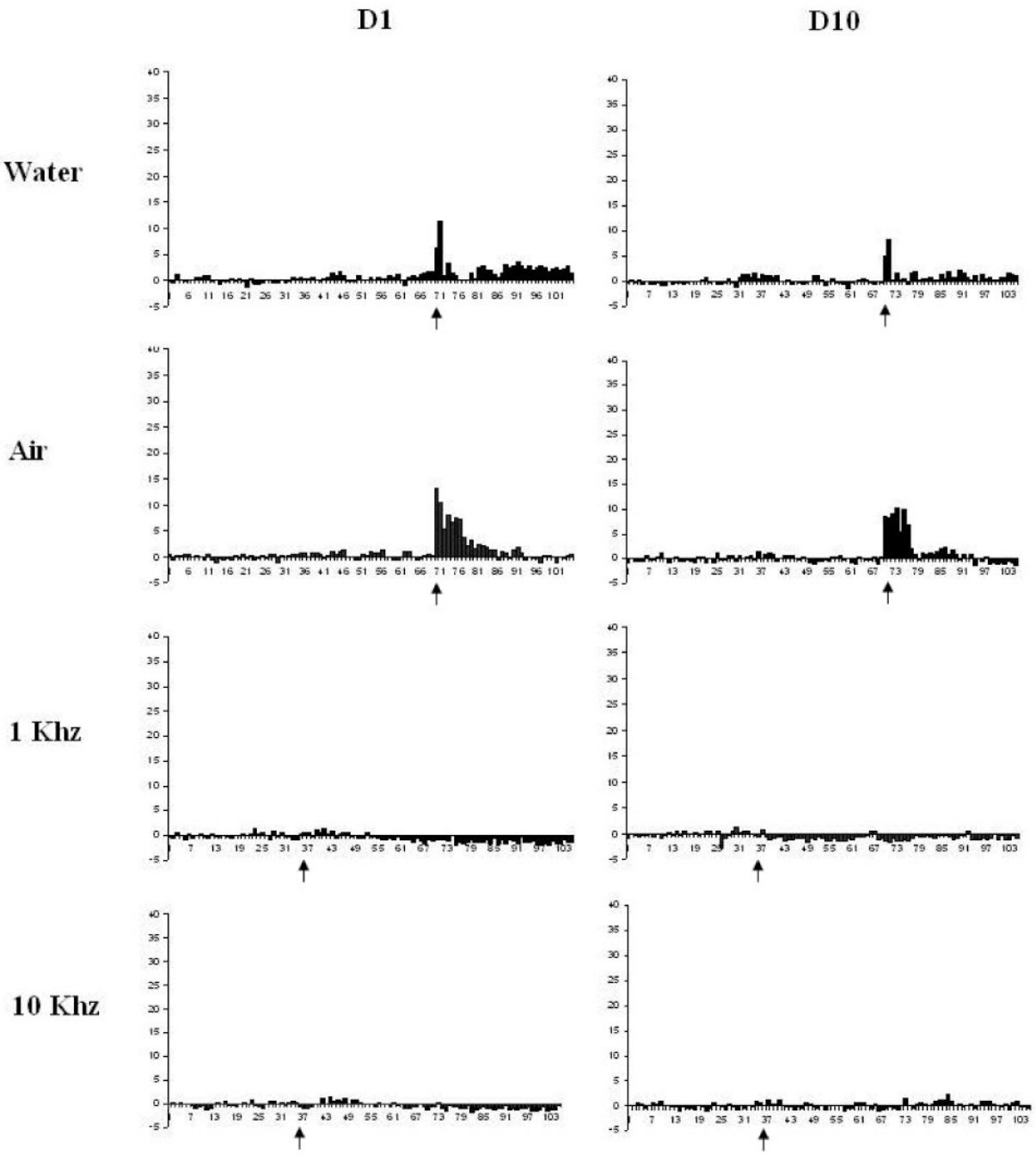


Figure 5.
Unpaired mean hippocampal responses to training stimuli across training sessions. Group average hippocampal peristimulus histograms of neural activity during the baseline period and the period following stimulus onset during the first (D1) and last (D10) days of training for the unpaired (n = 4) group. Each histogram bin is 20 msec and the total duration is 2100 msec. Arrow indicates stimulus onset.