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CS-specific *gamma*, *theta*, and *alpha* EEG activity detected in stimulus generalization following induction of behavioral memory by stimulation of the nucleus basalis

Dewey E. McLin III, Alexandre A. Miasnikov, and Norman M. Weinberger*

Center for the Neurobiology of Learning and Memory and Department of Neurobiology and Behavior, University of California, Irvine, CA 92697-3800, USA

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Abstract

Tone paired with stimulation of the nucleus basalis (NB) induces behavioral memory that is specific to the frequency of the conditioned stimulus (CS), assessed by cardiac and respiration behavior during post-training stimulus generalization testing. This paper focuses on CS-specific spectral and temporal features of conditioned EEG activation. Adult male Sprague–Dawley rats, chronically implanted with a stimulating electrode in the NB and a recording electrode in the ipsilateral auditory cortex, received either tone (6 kHz, 70 dB, 2 s) paired with co-terminating stimulation of the nucleus basalis (0.2 s, 100 Hz, 80–105 μA, ITI ~45 s) or unpaired presentation of the stimuli (~200 trials/day for ~14 days). CS-specificity was tested 24 h post-training by presenting test tones to obtain generalization gradients for the EEG, heart rate, and respiration. Behavioral memory was evident in cardiac and respiratory responses that were maximal to the CS frequency of 6 kHz. FFT analyses of tone-elicited changes of power in the *delta*, *theta*, *alpha*, *beta1*, *beta2*, and *gamma* bands in the paired group revealed that conditioned EEG activation (shift from lower to higher frequencies) was differentially spectrally and temporally specific: *theta*, and *alpha* to a lesser extent, decreased selectively to 6 kHz during and for several seconds following tone presentation while *gamma* power increased transiently during and after 6 kHz. *Delta* exhibited no CS-specificity and the *beta* bands showed transient specificity only after several seconds. The unpaired group exhibited neither CS-specific behavioral nor EEG effects. Thus, stimulus generalization tests reveal that conditioned EEG activation is not unitary but rather reflects CS-specificity, with band-selective markers for specific, associative neural processes in learning and memory.

Keywords: EEG; Classical conditioning; Learning; Association; Behavior; Cholinergic system

1. Introduction

The nucleus basalis (NB) has been implicated in state control of the cerebral cortex and in cognitive functions, such as memory and attention. Its cholinergic projections to the cortex (Lehmann, Nagy, Atmadja, & Fibiger, 1980; Mesulam, Mufson, Wainer, & Levey, 1983) are involved in activation of the electroencephalogram (EEG), i.e., a shift in spectral power from higher voltage, slower waves to lower voltage faster rhythms. For example, the discharge rate of cholinergic cortically projecting NB cells varies directly with the level of cortical activation, being highest during full waking and concomitant EEG activation and lowest during drowsiness and slow wave sleep (Buzsaki et al., 1988; Détári & Vanderwolf, 1987; Détári, 2000). Electrical stimulation of the NB elicits cortical activation, and release of cortical ACh (Buzsaki et al., 1988; Casamenti, Deffenu, Abbamondi, & Pepeu, 1986; Jiménez-Capdeville, Dykes, & Myasnikov, 1997; Kleiner & Bringmann, 1996; Kurosawa, Sato, & Sato, 1989; Metherate, Cox, & Ashe, 1992; Rasmusson, Clow, & Szerb, 1994). The muscarinic receptor antagonist atropine applied directly to the cortex blocks EEG activation induced by NB stimulation (Bakin & Weinberger, 1996). Lesions of the NB impair cortical activation (Buzsaki et al., 1988; Riekkinen, Sirviö, Hannila, Miettinen, & Riekkinen, 1990).

* Corresponding author. Fax: 1-949-824-4576.
E-mail address: nmweinbe@uci.edu (N.M. Weinberger).

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Involvement of the NB in learning is supported by neural correlates, such as its development of associative increased responses to signal stimuli during conditioning (Pirch, 1993; Richardson & DeLong, 1991; Whalen, Kapp, & Pascoe, 1994; Wilson & Rolls, 1990). Learning-induced plasticity in the NB can develop in only a few trials, slightly preceding the development of plasticity in the auditory cortex (Maho, Hars, Edeline, & Hennevin, 1995). These findings support the hypothesis that associative plasticity in the NB enables the development of cortical changes involved in the storage of specific sensory experiences.

Many lesion studies are consistent with an important role of the NB in learning and memory. For example, excitotoxic lesions of the NB produce deficits in memory (e.g., Connor, Langlais, & Thal, 1991; Santucci & Haroutunian, 1989; Turner, Hodges, Sinden, & Gray, 1992). However, such lesions are not selective for any particular neurotransmitter containing NB cells. Studies that have employed selective immunotoxic destruction of cholinergic neurons in the NB support the conclusion that these cells are involved in selective attention rather than in memory (reviewed in Everitt & Robbins, 1997; Wenk, 1997). Therefore, NB lesions affecting memory may involve non-cholinergic neurons. However, other studies report that a small percentage of cholinergic cells may be spared by immunotoxic lesions and these are sufficient to enable learning and memory in examined tasks (e.g., Leanza et al., 1996; Gutiérrez, 1999). Therefore, it is possible that cholinergic NB cells are involved in both attention and memory, but the issue is currently unresolved.

Studies of electrical stimulation of the NB are complementary to neural correlates and lesion approaches. Stimulation has the advantage of affecting its cholinergic and non-cholinergic neurons, both of which normally participate in NB responses to sensory stimuli (Nadal, Armario, Gill, & Janak, 2001), but also the potential problem of affecting non-NB cells given the somewhat diffuse nature of the NB (Mesulam et al., 1983). Nonetheless, several approaches using NB stimulation are consistent with its role in learning and memory. For example, post-training stimulation of the NB is reported to improve memory consolidation (Montero-Pastor et al., 2001). Additionally, the NB has been implicated in learning-related cortical plasticity. Receptive fields and tonotopic maps in the auditory cortex (ACx) are modified to emphasize a particular signal frequency when it gains behavioral significance during learning. This receptive field plasticity has major characteristics of behavioral memory, i.e., associativity, specificity, rapid induction, consolidation over hours and days, and very long term retention (reviewed in Weinberger, 1995, 1998, in press). Substitution of NB stimulation for a standard sensory reinforcer (e.g., food or shock) induces the same RF plasticity as that induced during such learning (Bakin & Weinberger, 1996; Bjordahl, Dimyan, & Weinberger, 1998; Dimyan & Weinberger, 1999; Kilgard & Merzenich, 1998; Kilgard et al., 2001) and NB-induced RF plasticity is blocked by application of atropine to the ACx (Miasnikov, McLin III, & Weinberger, 2001). These findings support the hypothesis that the NB enables such cortical reorganization by signaling the acquired behavioral significance of sensory stimuli. This is consistent with the fact that NB stimulation can produce long-lasting facilitation of auditory cortical responses to stimuli (e.g., Edeline, Maho, Hars, & Hennevin, 1994; Hars, Maho, Edeline, & Hennevin, 1993).

If the NB is involved in enabling the storage of information in the cortex, and perhaps elsewhere in the brain, then it might be possible to actually induce behavioral memory by substituting NB stimulation for a standard appetitive or aversive reinforcer. Recently, we obtained such a result (McLin, Miasnikov, & Weinberger, 2002a). Rats were conditioned for several days with a 6 kHz tone paired with weak stimulation of the NB. EEG changes and behavioral effects (heart rate and respiration) were assessed following training in the absence of NB stimulation. We selected changes in heart rate and respiration as behavioral indices of newly formed memory because they are highly sensitive, reliable, and robust indicators of behavioral conditioning (Lennartz & Weinberger, 1992).

Associative memory may be inferred from behavioral change if it meets the dual criteria of specificity and associativity, whether induced in standard learning situations (e.g., appetitive or aversive sensory unconditioned stimuli) or by the use of stimulation of the brain as the unconditioned stimulus (US). To assess conditioned stimulus (CS) specificity, we used the well-established metric of the stimulus generalization gradient, obtained when a subject is trained with one CS and later tested with many stimuli. We reasoned that if NB stimulation paired with a tone induces memory about that tone, then this CS frequency should later elicit the largest behavioral responses of all tones tested, i.e., occupy the peak of the frequency generalization function. An unpaired group controlled for possible non-associative sensitization effects of repeated presentation of tone and NB stimulation. The paired group did exhibit the greatest magnitude of cardiac and respiratory responses to the CS frequency, i.e., responses to the 6 kHz tone were at the peak of the gradients of stimulus generalization. The control group did not exhibit CS-specific behavioral responses. These findings indicate that behavioral memory can be induced by pairing a tone with NB stimulation.

This previous report also examined changes in the high frequency *gamma* band (32–59 Hz) of the electroencephalogram (EEG) during stimulus generalization, and found CS-specific increases in *gamma* power. This

band was analyzed because it is a reliable indicator of EEG activation, i.e., a coordinated shift from higher voltage slower waves to lower voltage faster rhythms (Maloney, Cape, Gotman, & Jones, 1997). Other EEG bands were not analyzed because we assumed that conditioned EEG activation during training was the same as conditioned EEG activation during testing for stimulus generalization. Therefore, we assumed that conditioned changes in other frequency bands had exhibited the same dynamics as the observed changes in the *gamma* band. However, subsequent analysis of all of the EEG bands revealed that this was not the case. Rather, individual bands of the EEG exhibit different changes in power to the CS vs. other acoustic frequencies, and different temporal dynamics within a test trial. This communication provides a detailed analysis of EEG activation during stimulus generalization. We also report additional findings about cardiac and respiration conditioned responses induced by tone paired with NB stimulation.

2. Methods

2.1. Surgery

Adult male Sprague–Dawley rats (350–690 g), housed individually with food and water ad lib. on a 12 h light/dark cycle, were anesthetized with sodium pentobarbital (35–40 mg/kg i.p., Abbott Laboratories, North Chicago, IL) and administered supplements as required to maintain an areflexic state. The animal was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) on a controlled heating pad (Model K-20; American Pharmaseal Company, Valencia, CA) and its eyes covered with opthalamic ointment to prevent drying of the cornea (Puralube Vet, Pharmaderm, Melville, NY). The central region of the scalp was excised, the temporal muscles were reflected, and the skull was leveled for subsequent stereotaxic NB electrode implantation. To permit removal of the ear bars while maintaining the subject's head in stereotaxic position, two 10 mm long threaded aluminum hexagonal standoffs and five to seven 1.5 mm diameter stainless steel screws inserted into the skull were attached to the calvaria using dental cement (methyl methacrylate, Co-Oral-Ite Dental MFG, Diamond Spring, CA). The aluminum standoffs were bolted to a support, thus permitting removal of the earbars for the duration of the surgery.

To determine the location of auditory cortex, a map was made of click evoked field potentials (100 μs, 1/10 s) recorded (1000×, 1–100 Hz, DAM-50H, World Precision Instruments, Sarasota, FL) from the temporal bone over the auditory cortex contralateral to the speaker, by a roving pointed stainless steel electrode. A 0.8 mm diameter stainless steel screw that served as the EEG recording electrode was inserted at site of the maximal click field potential evoked by the click (200–400 μV reproducible waveform, 8–15 ms latency positivity and longer latency negativity).

The subject was removed from the stereotaxic frame, and an EKG electrode was implanted subcutaneously (teflon-insulated 7-strand 140 μm stainless steel wire electrode, 0.0055 in. diameter, #7932, A-M Systems, Carlsborg, WA). Insulation was removed from a 5 mm segment of the wire, which was passed through the neck muscles via a guide probe and manually directed around the circumference of the rib cage, forming a loop around the chest with the uninsulated portion over the ventral thorax near the heart. To ensure sterility during implantation, wire was inserted into flexible polypropylene tubing coupled with a 50-ml syringe filled with 70% alcohol. The animal was then allowed to recover in an incubator and returned to its home cage.

The NB electrode was implanted in a second surgery. Because successful elicitation of cortical activation served as an important guide for proper positioning of the NB electrode, at least 3 days separated cortical electrode placement from implantation of the NB electrode to help ensure recovery of the cortex and stability of the cortical response. Rats were again anesthetized and a 13 mm long concentric bipolar stainless steel electrode (#SNEX-100 × 13 mm, David Kopf Instruments) was lowered through the contralateral cortex at a 45° angle in the frontal plan at AP −2.3, L 4.2 entering laterally and passing medially to the target NB. Beginning 8 mm from the cortical surface, bipolar (0.2 ms pulses of opposite polarity at 5 ms intervals) electrical stimuli (200–300 ms long trains, 100 Hz) were delivered via stimulus isolation units (PSIU6 and Grass S88 stimulator, Grass Instrument, Quincy, MA). The electrode was advanced in 0.2–0.3 mm steps with manually driven microdrive (Kopf 1460, David Kopf Instruments) until consistent EEG activation lasting 1–5 s was elicited from the epidural ACx electrode (DAM-50H amplifier, 1000×, 0.1–100 Hz, and Frequency Devices 9002 filter, Haverhill, MA, band pass 8-pole filter set at 1–10 Hz, digitized at 500 Hz with the Power-1401/Spike-3 interface/software, hereafter CED, Cambridge Electronic Design, Cambridge, UK and Pentium-based PC). The leads from all electrodes were crimped into individual pre-soldered gold-plated pins on a female miniature hexagonal connector (#719-4530, Allied Electronics, Irvine, CA) that was subsequently imbedded in dental cement on the subject's head.

2.2. Training

All experimental manipulations took place in a sound attenuation chamber (I.A.C., Bronx, New York). Subjects were placed in a training box (25 × 22 × 26 cm; Sterilite, Townsend, MA) that contained bedding

identical to that in their home cage. Prior to the first day of training, the EEG was monitored until low voltage high frequency activity was replaced by relatively synchronized activity. NB stimulation (0.2 ms pulses, 100 Hz, 200 ms, biphasic) was then presented at varying current levels in order to determine the minimal current required to consistently elicit 3–4 s of visually identifiable EEG activation while not provoking movement or change in ongoing behavior. This minimal current was subsequently used during training. Tones were delivered via a speaker mounted over the training box. The speaker had been calibrated (0–80 dB SPL, Bruel and Kjaer condenser microphone type 4134, sound level preamplifier type 2204, Hewlett–Packard 3581A wave analyzer) at locations approximating those of the rat's head. Tones were generated using a sine wave generator (Hewlett–Packard, 202CR) led through a shaped rise/fall gate (Coulbourn Instruments, Allentown, PA) controlled by a triggering output from the DAC output device of the CED.

Training began the next day. For the pairing group ($n = 4$) a tone (6 kHz, 70 dB, 2000 ms, 20 ms rise/fall) was paired with a co-terminating 200 ms train of NB stimulation, i.e., the CS-US interval (onset-to-onset) was 1.8 s. Subjects were given approximately 200 trials daily, with random inter-trial intervals averaging 45 s (range 30–60 s) for 14 (±2) days. The EEG signal was recorded starting 2 s before tone onset. To control for non-associative effects, a second group ($n = 5$) received explicitly unpaired trials, consisting of ~200 presentations of both the tone and NB stimulation, randomly presented with neither occurring more than three times consecutively for 14 (±2) days. There was no significant difference between groups for the number of training days (paired = 13.0, unpaired = 12.6; $t(7) = 0.69$, $p > .05$).

2.3. Testing: Determination of frequency generalization gradients

After completion of training, generalization data were obtained for the EEG, heart rate, and respiration in responses to a series of 7 tones (15, 1, 12, 2, 10, 3, 6 kHz; 70 dB; 2000 ms; 10 ms rise/fall). This testing was performed in an experimental room different from that used during training (see below). Recordings were obtained before, during tone presentation and for 9 s thereafter. Tones were not presented until at least 2 s of visually judged stable respiration were present following the minimal inter-tone intervals of 30 s (range 30–300). To further ensure stability, tones were not presented when paroxysmal EEG activity, characterized by spike waves of 8–14 Hz that are several fold larger in amplitude than background activity, was present (Vergnes, Marescaux, Depaulis, Micheletti, & Warter, 1987). After testing, electrolytic marking lesions were made via the NB stimulation electrode with the subjects under general (barbiturate) anesthesia, for histological verification of electrode placement (Paxinos, 1998).

2.4. Recording and analysis of the EEG

To determine the time course of tone-elicited effects on the EEG, the records for both training and testing were analyzed in 1 s intervals: two intervals before the tone, one interval beginning 200 ms after tone onset, and nine intervals beginning 200 ms after tone offset. While this entire temporal analysis could be achieved for testing data, only the pre-trial and during tone intervals could be analyzed for training data due to NB stimulation co-terminating with the tone in paired subjects, rendering further changes unattributable to either stimulus exclusively. The changes following NB stimulation in unpaired subjects were used to evaluate the effectiveness of NB stimulation on activation of the EEG in the training situation.

Fast Fourier transformations (FFTs) with a 0.98 Hz resolution were performed off-line on each 1 s epoch to determine the power in the following EEG bands: *delta*, 1.09–3.26 Hz; *theta*, 3.27–8.68 Hz; *alpha*, 8.69–15.19 Hz; *beta1*, 15.20–19.53 Hz; *beta2*, 19.54–32.55 Hz; *gamma*, 32.56–59.68 Hz. It should be noted that the frequencies contained within the *alpha* band include those associated with spindle (*sigma*) activity. With this in mind, we use the term "*alpha*" rather than "*sigma*," in accordance with prior studies of the cholinergic system and the EEG of the rat (e.g., Dringenberg & Diavolitsis, 2002; Holschneider, Leuchter, Scremin, Treiman, & Walton, 1998). To permit pooling of effects across trials and subjects, the data were normalized; the tone effect on each EEG band for each trial was quantified on a second-by-second basis as the ratio of power during and following the tone to the average power during the 2 s immediately preceding the tone (post-/pre-average). The resulting ratios were used to analyze the effects of tone presentation during training and testing. A ratio of 1.0 indicates no change, smaller values show a decrease and larger numbers show an increase in power.

One-factor (tone frequency) ANOVAs were performed for stimulus generalization data in both groups, for each 1 s period ("time slice") during the 10 s of recording on each trial (1 s during the tone, 9 s post-tone). The purpose of this analysis was to determine if changes in power within each of the EEG bands were differentially affected by acoustic frequency. For all cases of statistical significant ANOVAs, an a priori orthogonal contrast was performed to determine if response change to the CS frequency of 6 kHz was significantly smaller or larger than to the other frequencies combined (Kirk, 1995). The rationale for a priori selection of the CS frequency of 6 kHz for contrast analysis is that we previously found EEG (*gamma* band) and behavioral specificity only for this frequency (McLin et al., 2002a).

However, those findings do not preclude frequency-specific effects for other test frequencies. Therefore, for all cases of a significant ANOVA, contrasts were performed for each of the other frequencies (i.e., 1, 2, 3, 10, 12, 15 kHz). A total of five of these data points were significant: 2 kHz unpaired (*beta1*); 10 kHz unpaired (*delta*); 12 kHz paired (*beta2*); 15 kHz paired (*gamma*); 15 kHz unpaired (*gamma*). These are included in Section 3. There were also two non-6 kHz frequencies with significant contrasts in the paired group: 12 kHz (*beta2*); 15 kHz (*gamma*). In the unpaired group, three non-6 kHz frequencies had significant contrasts: 2 kHz (*beta1*); 10 kHz (*delta*); 15 kHz (*gamma*).

2.5. Recording and analysis of EKG

The EKG signal was passed through the differential input of a DAM 50H amplifier (1000×, 1.0–100 Hz) digitized at 1000 samples/s with the analog-to-digital converter of a Power-1401 interface operated under the Spike-3 (CED) data acquisition/analysis software and stored on a Pentium-based PC. Heart rate was calculated off-line based on inter-beat intervals. Responses to tones were characterized by a short latency bradycardia (~1 s after tone onset) followed by a longer latency tachycardia (~5 s after tone onset). Statistical analysis used the same methods as described for EEG analysis. In the case of heart rate, we analyzed each of the two temporally discrete phases of this response, bradycardia and tachycardia. A priori orthogonal contrasts, based on the prediction that the paired frequency of 6 kHz would elicit the largest response, were also performed on frequency generalization gradients constructed for the time intervals containing the maximal bradycardia and the interval containing the maximal tachycardia.

2.6. Recording and analysis of respiration

Respiration was recorded via a lightweight head-mounted assembly of our own design that permits prolonged recording from awake freely moving animals. The assembly consists of three adjustable arms holding a 3-s time constant glass encapsulated 250 Ω (at 25 °C) style-1 thermistor (#837-5170, Allied Electronics, Fort Worth, TX). The thermistor was placed as close as possible to the nostril without touching or blocking it in order to maximize sensitivity. The thermistor served as one of the arms in a resistor bridge circuit (pre-balanced with a 1 kΩ potentiometer) making it sensitive to the local temperature fluctuations caused by the animal's respiration. The output signal from the bridge was fed to the differential input of a DAM 50H amplifier (1000×, 1–100 Hz) digitized at 2000 samples/s (CED) and stored on a Pentium-based PC.

As with the EEG, FFTs were performed on 1 s epochs of digitized respiratory recordings (two pre-stimulus, one during the stimulus and nine post-stimulus), resulting in power spectra for each interval. The maximum possible frequency resolution with the 2000 Hz sampling rate was extracted, yielding 1.63 Hz bins. To eliminate artifacts that might result from increased power due to DC offset, the 0–1.63 Hz bin was discarded. Analysis of spectrograms derived from epochs of spontaneous respiration in the absence of tones or NB stimulation revealed that, after discarding the 0–1.63 Hz bin, 99% of the power was below 4.88 Hz. These results are consistent with the respiration rates we observed, generally ranging from approximately 1.5 to 2.5 Hz. Consequently, the respiration analysis was confined to the power spectrum between 1.63 and 4.88 Hz.

Quantification of respiration was achieved with an index (respiration change index, RCI) that is sensitive to changes in rate and magnitude of the signal across the frequency spectrum, independent of the direction of those changes.

$$\mathrm{RCI} = \overline{X}(|\mathrm{Post}\ a_i - \mathrm{Pre}\ a_i|/(\mathrm{Pre}\ a_i + \mathrm{Post}\ a_i))$$

for i = frequency bands 1.63–3.25 and 3.26–4.88 Hz where Pre is the average power in the 2 s preceding the tone. To obtain temporal specificity, RCIs were calculated on a second-by-second basis. As for the EEG and heart rate, ANOVAs were performed on the RCIs to determine the effects of acoustic frequency. A priori orthogonal contrasts between responses to 6 kHz vs. all other tone frequencies were performed for the time intervals corresponding to the periods of tone presentation, 2 s after tone presentation and maximal response at 5 s after the tone.

3. Results

3.1. Effectiveness of NB stimulation during training

Stimulation sites are shown in Fig. 1. They lie within the area of the NB that projects to the auditory cortex (Mesulam et al., 1983; Moriizumi & Hattori, 1992) and are intermixed between groups. Current levels did not differ between paired and unpaired groups (83.3 ± 2.36 and 103.3 ± 18.68 μA, respectively; $t_7 = 0.48$, $p > .05$). Assessment of the effectiveness of NB stimulation to produce EEG activation during training was limited to the unpaired group because NB stimulation was preceded by the tone in the paired group, precluding determination of an NB effect per se (Section 2). Stimulation elicited cortical activation in unpaired subjects. Fig. 2A provides an example and Fig. 2B gives group data for the relative change in power for each EEG band. To determine if stimulation produced significant changes, the ratios for each of the 6 s following each NB stimulation across training were compared to a value of 1.0 (no change). These analyses showed that NB

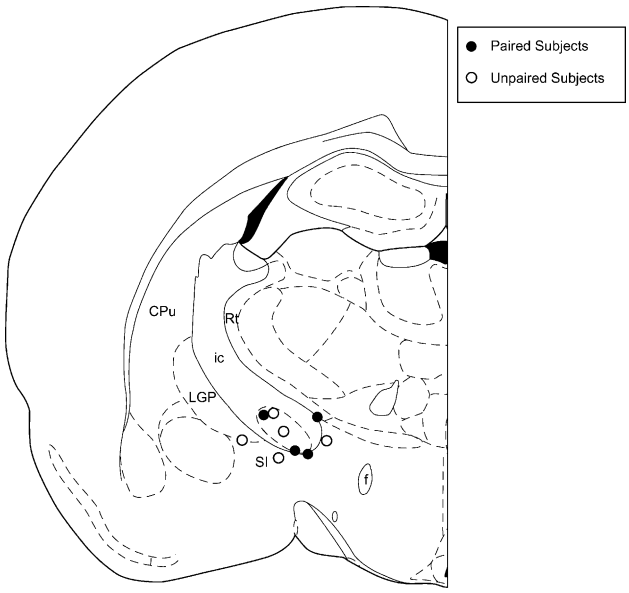

Fig. 1. The location of NB stimulation in paired and unpaired subjects. Electrode placements are ±0.25 mm in the anterior/posterior plane, projected to frontal section AP = −2.1 (Paxinos, 1998).

stimulation significantly reduced power in the *delta*, *theta*, *alpha*, and *beta1* bands for each 1 s time period following stimulation (one sample *t* tests, all $p < .05$). In contrast, *beta2* and *gamma* power were significantly increased (one sample *t* tests, both $p < .05$). These findings show that NB stimulation was effective in producing EEG activation in the unpaired group during training. Although comparable data could not be obtained for the paired group, an independent study of NB stimulation alone, using the same range of current values employed in this experiment, revealed consistent elicitation of EEG activation (McLin, Miasnikov, & Weinberger, 2002b).

3.2. EEG activation during training

Pairing tone and NB stimulation resulted in EEG conditioned cortical activation in the paired group but not in the unpaired group. Conditioned activation developed rapidly and was maintained; it was evident during the first day of training and still present during the last day of training. Examples are presented in Fig. 3A. Statistical analysis of ratios between the tone period and the pre-tone activity pooled across training days revealed that all EEG bands exhibited a decrease in power in the paired group relative to the unpaired group, except for the high frequency *gamma* band, which showed an increase in power. The differences (Fig. 3B) were statistically significant (one-sampled *t* tests against a mean difference of zero), except for the *alpha* band (*alpha*: $t_{13} = 1.36$, $p = .20$; *delta*: $t_{13} = 10.65$, $p < .0001$; *theta*: $t_{13} = 3.22$, $p = .0067$; *beta1*: $t_{13} = 5.98$, $p < .0001$; *beta2*: $t_{13} = 4.64$, $p = .0005$; *gamma*: $t_{13} = 5.79$, $p < .0001$).

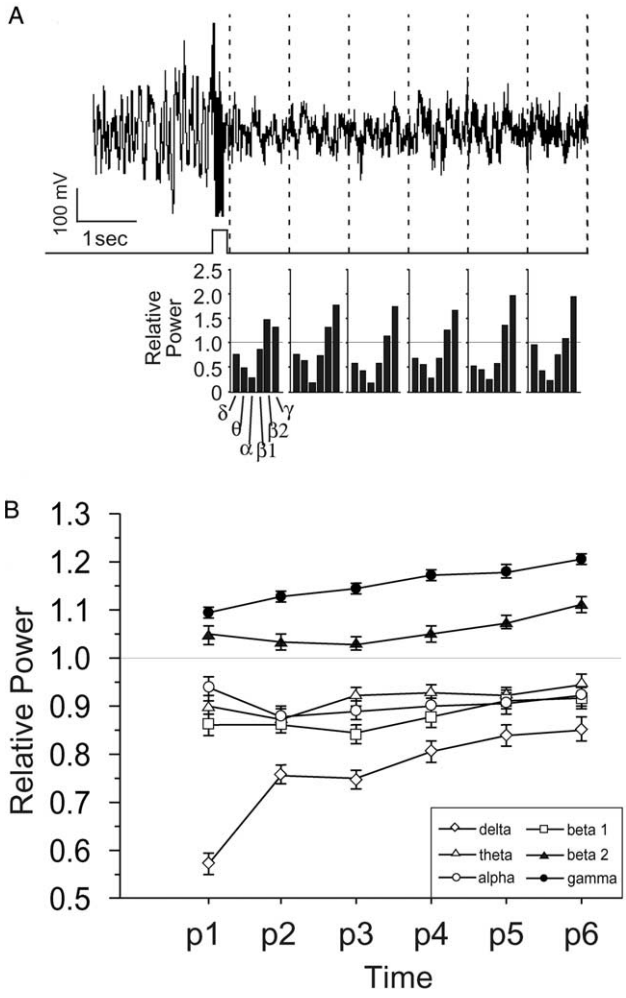

Fig. 2. (A) An example of EEG waveform that reflects activation elicited by NB stimulation. Below the trace are spectra histograms reflecting the relative change in power on a second-by-second basis for each EEG band. The histogram bars are arranged from lowest frequency band to highest frequency band within each second (*delta*, *theta*, *alpha*, *beta1*, *beta2*, and *gamma*). Note that the stimulation causes a reduction in power for the lower frequency bands and an increase in power for the two highest frequency bands. (B) Group data showing the change in power elicited by NB stimulation in each EEG band across time (mean ± SE). The power at lower frequency *delta*, *theta*, *alpha*, and *beta1* bands is significantly reduced across the 6 s (post-stimulus seconds 1–6: P1–P6) whereas the power at the highest frequency *beta2* and *gamma* bands is significantly increased. This indicates that NB stimulation successfully activated auditory cortex.

3.3. Temporal and frequency-specific changes in the electroencephalogram

As noted in Section 2, the specificity and associativity of conditioned EEG activation were determined during the testing phase that followed training. Fig. 4 presents examples of EEG responses to all test tones for a paired and an unpaired subject. Note that 6 kHz elicited visually identifiable EEG activation in the paired subject (left column) but not in the unpaired subject (right

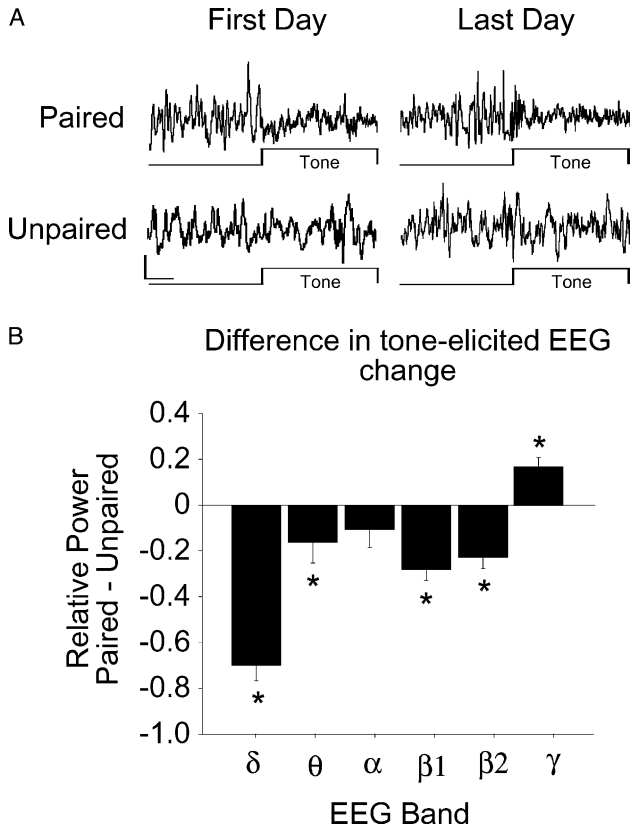

Fig. 3. (A) Examples of EEG responses to 6 kHz during training for a paired and unpaired subject on the first and last day of training. The tone elicited EEG activation on both the first and last day of training for the paired, but not the unpaired, subject. Calibration bars: 100 μV, 0.5 s. (B) Average difference (±SE) of change elicited in each EEG band between the paired and unpaired subjects. Tone-elicited changes in each of the EEG bands of the unpaired group were subtracted from those of the paired group; a value of zero indicates no difference, positive values indicate more power in paired subjects and negative values indicate less power in paired subjects. The power within the *delta*, *theta*, *beta1*, and *beta2* bands was significantly reduced in the paired group relative to the unpaired group. The power of *gamma* was significantly increased during pairing.

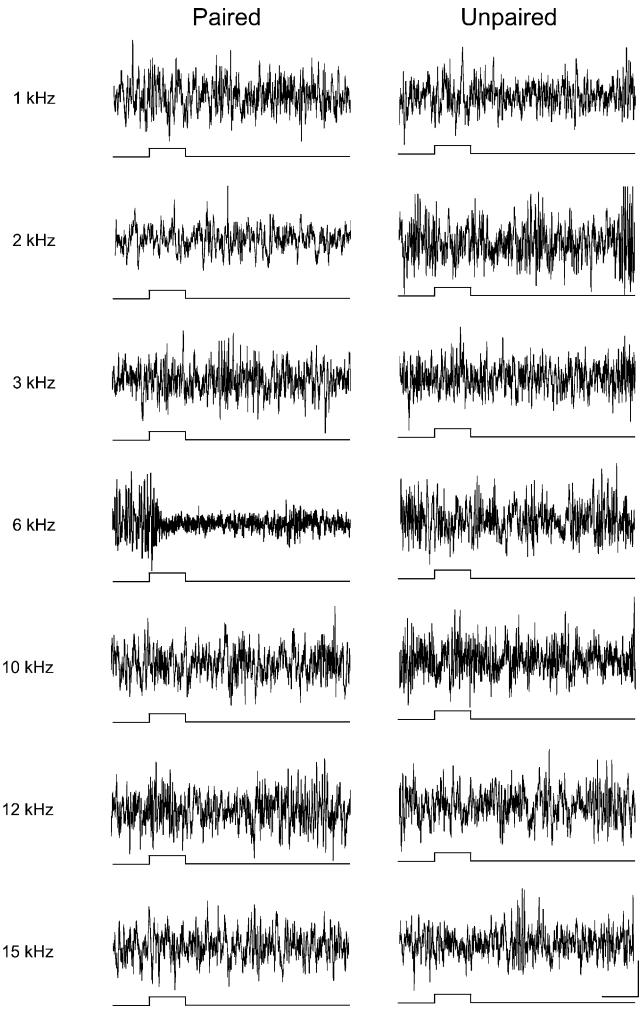

Fig. 4. Examples of EEG records to each test frequency for a paired and unpaired subject. The 6 kHz tone elicited greater EEG activation in the paired than in the unpaired subject; in the paired subject, 6 kHz elicited greater activation than did other test frequencies. Calibration bars: 2 s, 100 μV.

column). Other frequencies elicited smaller or no discernable changes in the EEG for both subjects.

The analysis of each EEG band revealed somewhat complex findings that cannot be discerned from visual inspection of individual records. The spectral and temporal changes are best understood by considering them in several complementary figures (Figs. 5–9). The mean group results are shown in Fig. 5 which presents a global color-coded depiction of the magnitude of EEG changes to all test frequencies across the entire time of recording for both groups. The extent of specificity of EEG changes can be seen as patterns of color extending horizontally, i.e., across time within trials. The paired group exhibited evidence of specificity to the CS frequency of 6 kHz for some EEG bands, compared to the control group in which there is little, if any, evidence of specificity to any frequency. In the paired group, *delta* exhibited decreased responses that were not specific. In contrast, *theta* exhibited a decrease in power for up to ~8 s, mainly at the CS frequency of 6 kHz. Similar specificity was evident for *alpha* while *beta1* showed a longer latency, somewhat weaker decrease in power. *Beta2* exhibited only a weak effect, largely confined to ~3 s after the 6 kHz tone. In contrast to all other EEG bands, *gamma* power showed a marked increase in power, but this is largely restricted to 6 kHz and to the period of actual tone presentation. This phasic effect (previously reported: McLin et al., 2002a) is distinctive in its brief duration compared to the longer lasting changes in other frequency bands. Thus, overall, the degree of CS frequency specificity, magnitude, and temporal dynamics differed across EEG bands. This heterogeneity may be contrasted with the uniform effects over time of NB stimulation alone, i.e., relatively sustained decreased or increased power over time (Fig. 2).

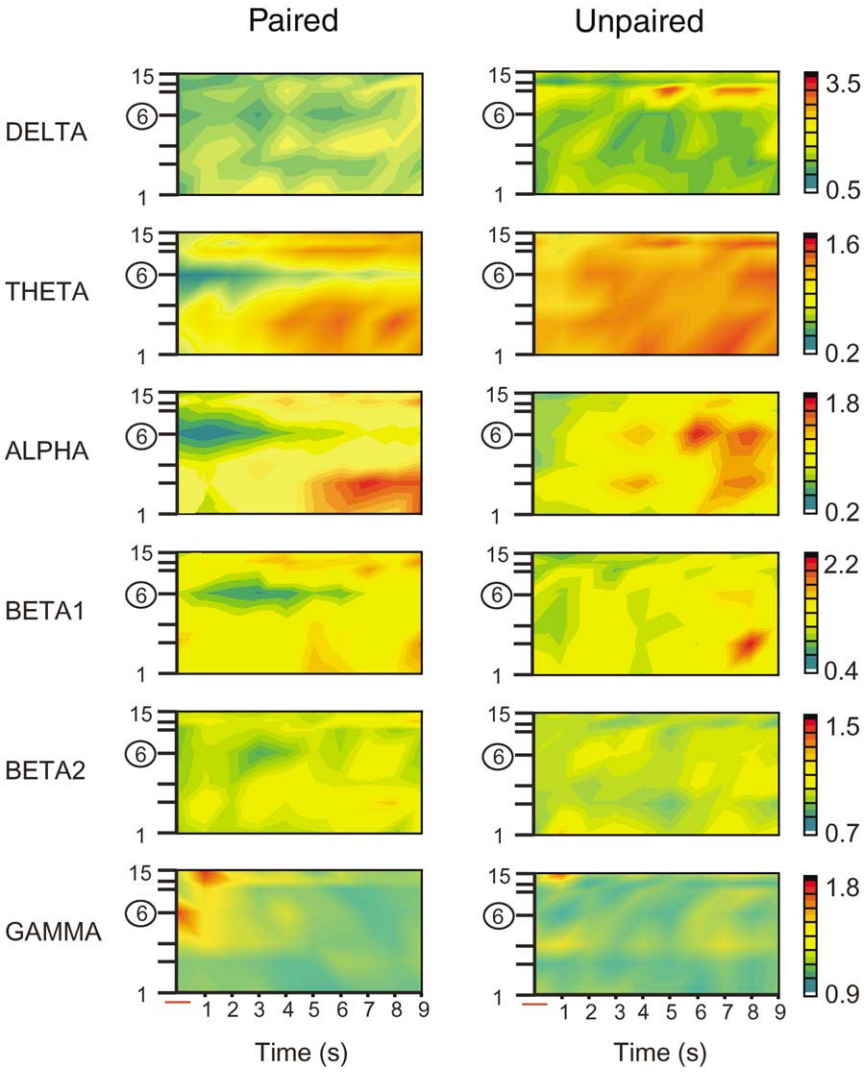

Fig. 5. Color representation of the change in EEG power for paired and unpaired groups across both time and frequency. The *X*-axis represents time in seconds after tone offset. The *Y*-axis represents tone frequency on a logarithmic scale. The color represents the relative change in power; red indicating the greatest increase and blue indicating the greatest decrease. In the paired group, frequency specificity can be seen as horizontal bands of color extending across time at and around the CS frequency of 6 kHz. Note the difference in the time course of the response between bands. The unpaired subjects do not exhibit any specificity to the 6 kHz tone. Horizontal red bars show the duration of tone presentation.

Figs. 6 and 7 supplement the overall representation of the data by providing the means, standard errors, and denotations of statistically significant effects. To assess frequency specificity, one-factor ANOVAs were performed for the data from each 1-s time slice for each EEG band. In those cases where the effect of tone frequency was significant, an orthogonal contrast was performed to test whether the response elicited by 6 kHz was significantly different from that elicited by the other frequencies. There were a total of 37 significant frequency effects (ANOVAs) in the paired group, 22/37 orthogonal contrasts for 6 kHz were significant. In the unpaired group, there were 6 significant frequency effects of which 1/6 contrasts for 6 kHz were significant. Significant 6 kHz temporal contrasts are indicated by an asterisk in Figs. 6 and 7.

The *delta* band exhibited few selective decreases of power to 6 kHz presentation, at post 1 and post 4 s. In contrast, the *theta* band showed the longest duration of decrease to 6 kHz; this effect was significant from the period during the tone presentation through post 9 s, except for post 8 s. The *alpha* band also exhibited CS-specific decreased power parallel to that of *theta*, although to a lesser degree: significant contrasts were found during the 6 kHz tone and at post 1, 2, 3, 4, and 6 s. *Beta1* power also decreased in response to the CS frequency of 6 kHz, but the effect was smaller and only significant at post 3 s. *Beta2* power exhibited little consistent specificity to 6 kHz; a significant reduction occurred at post 3 that seemed to be part of a phasic specific decrease in power (see also 4 s point) and there was also an effect at 9 s; however, inspection of these

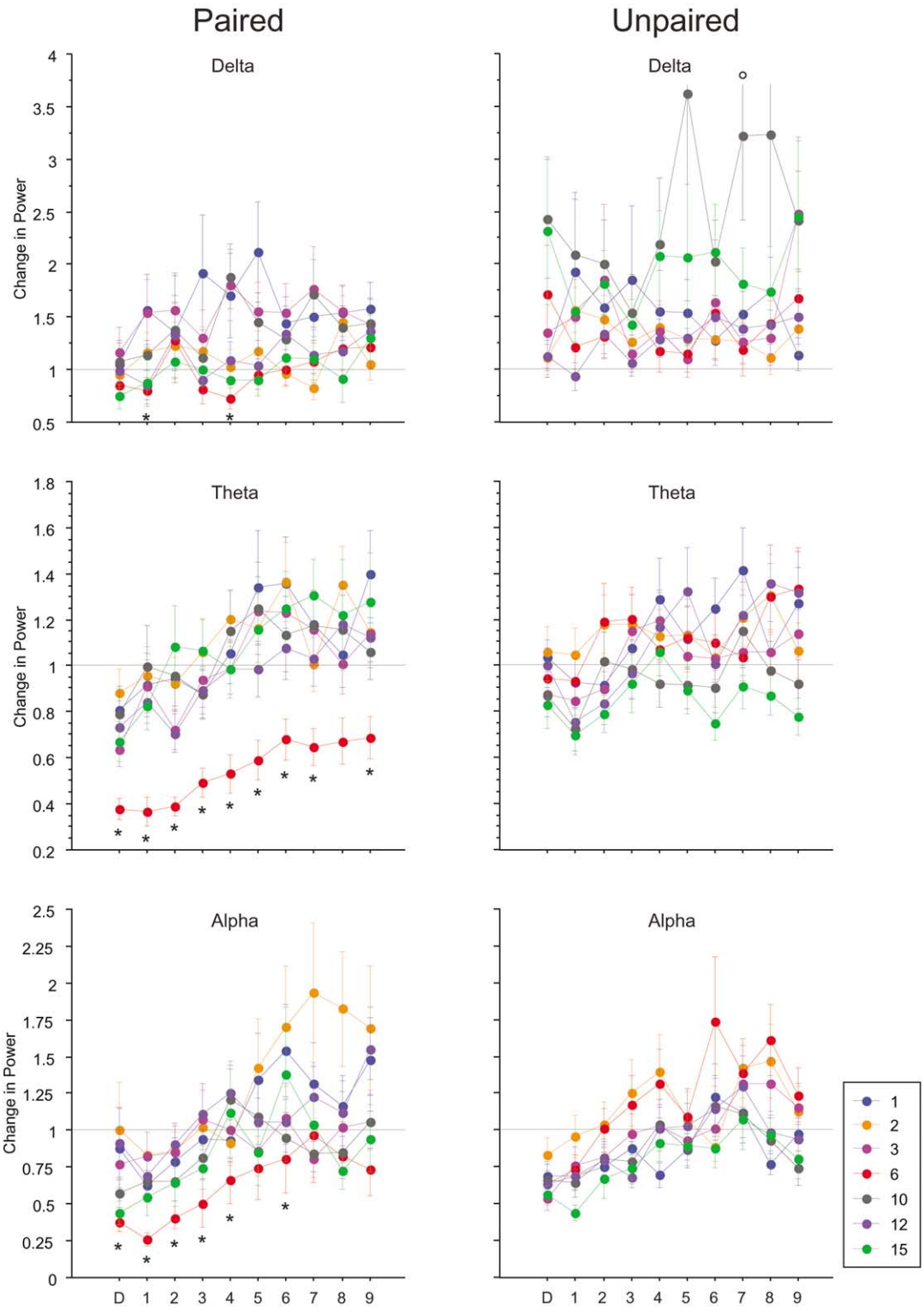

Fig. 6. Mean (±SE) change elicited in the power of the *delta*, *theta*, and *alpha* EEG bands by test tones for the paired (left column) and unpaired (right column) groups. Asterisks indicate a significant effect (ANOVA) of tone frequency where the response to 6 kHz differs significantly (orthogonal contrast) from that to other tones at each particular point in time. Open circles indicate significant changes in response to test frequencies other than 6 kHz. Markers at the *X*-axis indicate the time points at which the power spectra were determined: *D* = during the tone; 1–9 = seconds following the tone offset. Numbers in the insert denote the test frequencies in kHz.

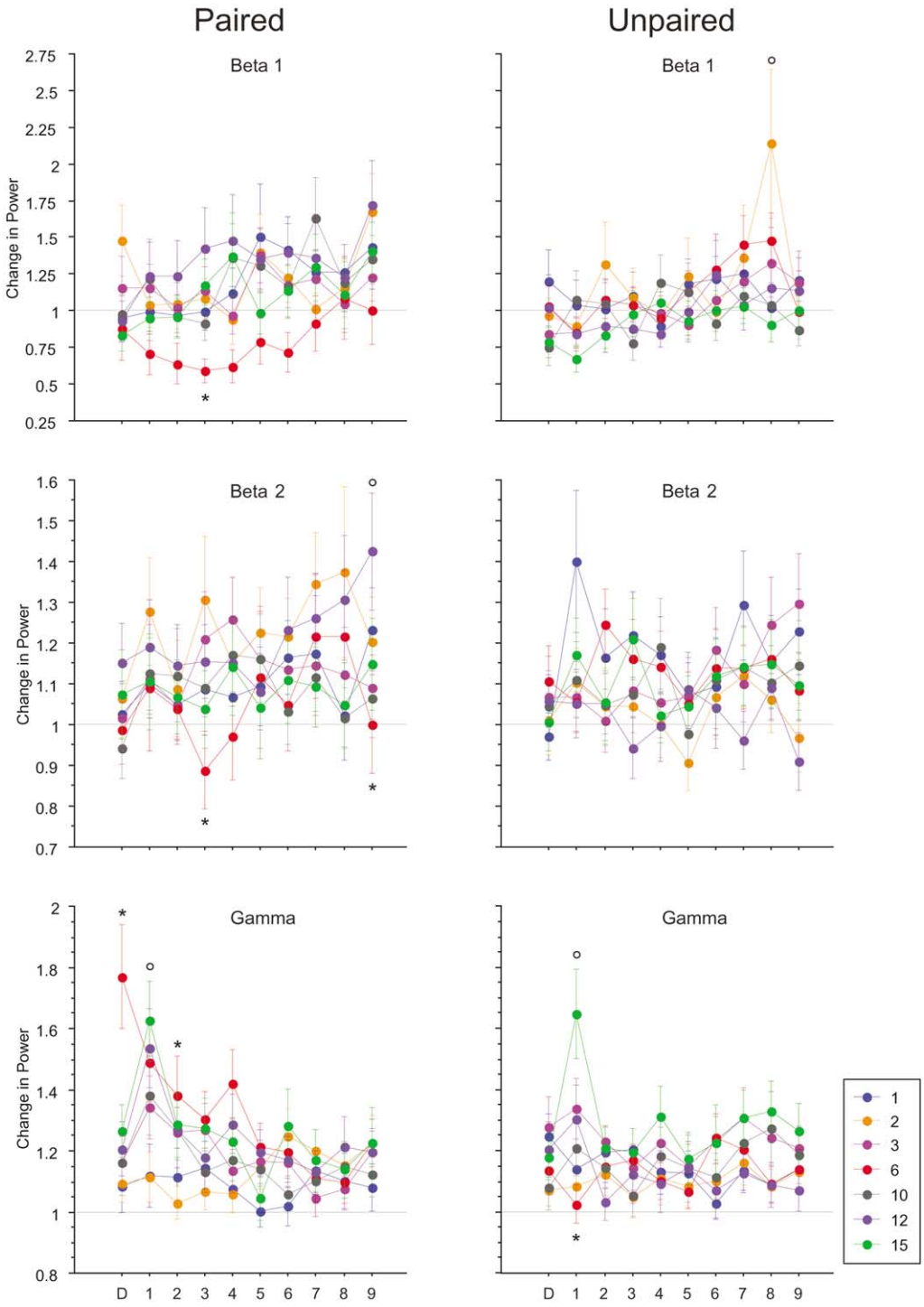

Fig. 7. Mean (±SE) change elicited in power within the *beta1*, *beta2*, and *gamma* EEG bands by test tones for the paired (left column) and unpaired (right column) groups. All the markers are the same as in Fig. 6.

data in Fig. 7 suggests that the latter point may have been spurious. The *gamma* band changes were distinct from other bands. *Gamma* showed a marked increase in power during presentation of the 6 kHz tone. However, this frequency-specific increase quickly faded, was not significant at post 1, was significant at post 2, and then not significant at any other time point. The reason *gamma* was not significant at post 1 was not the lack of a 6 kHz response, which was still apparent, but because of the presence of a transient response to 15 kHz seen in both the paired and unpaired group. The response to 15 kHz is so great that the 6 kHz-elicited *gamma* activity in the unpaired group was significantly *smaller* than that of the other frequencies at post 1 s.

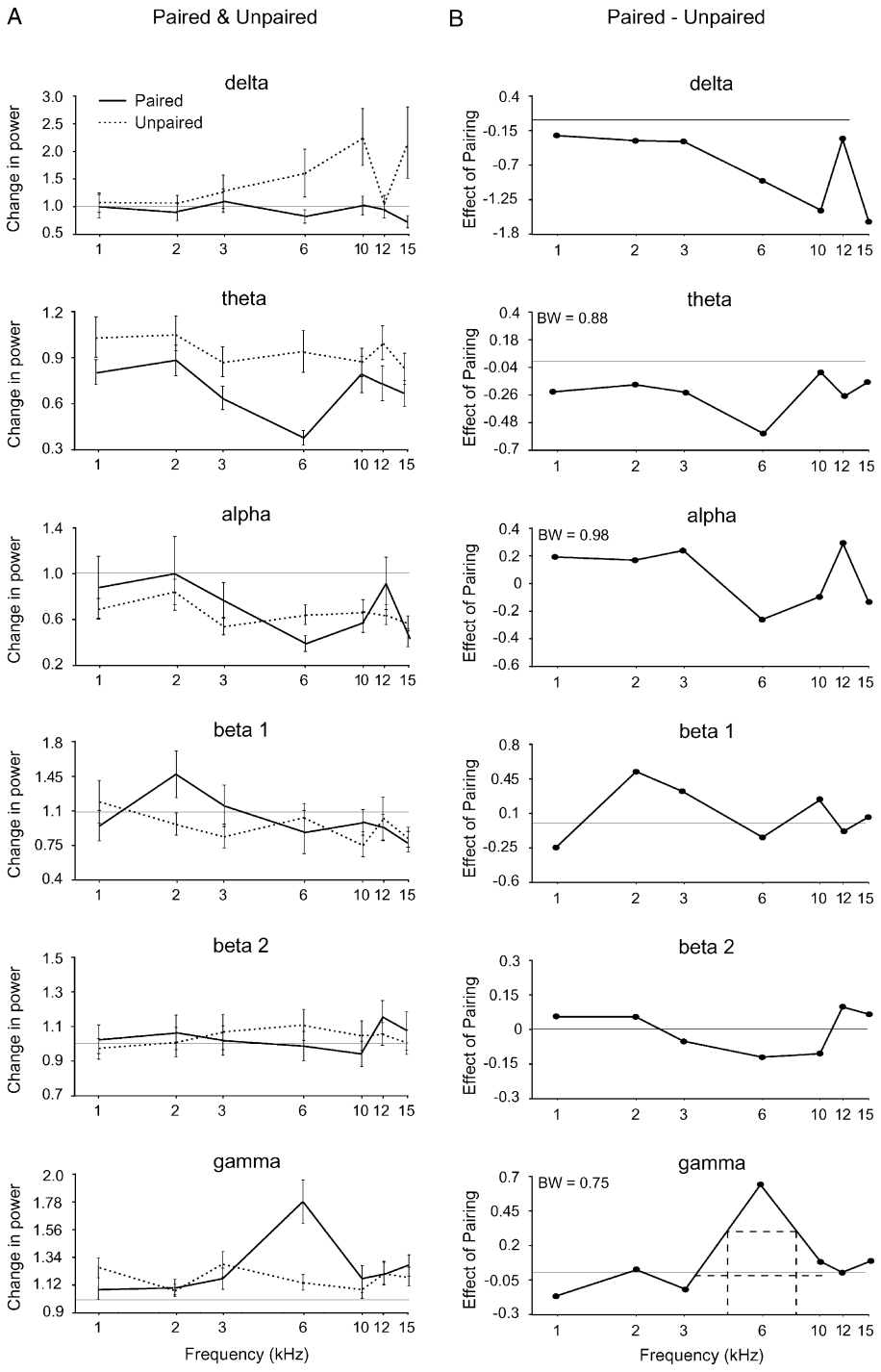

Fig. 8. (A) Frequency generalization gradients showing the mean (±SE) change found within each EEG frequency band elicited by each test tone frequency for the paired (solid lines) and unpaired (dotted lines) groups *during* the tone. (B) Group differences (subtraction of function representing the unpaired group from the function representing the paired group) showing the specificity of EEG changes during the tone due to the difference in training. The bandwidth (BW) of the difference function is indicated in the upper left corner of the graphs for the *theta*, *alpha*, and *gamma* bands. The dashed lines in the difference function for *gamma* illustrate the method used to calculated the BW at 50% of the response to 6 kHz. The lower horizontal dashed line indicates the average of the responses to 3 and 10 kHz. The upper horizontal line lies halfway (50%) between the average response to 3 and 10 kHz and the magnitude of response to 6 kHz. The two vertical lines indicate the BW at 50%, crossing the frequency axis at 4.65 and 7.80 kHz. The BW was 0.75 octaves, calculated as follows: difference (octaves) $= \log_2(7.80/4.65)$.

Fig. 8 presents the frequency generalization gradients for all EEG bands *during* tone presentation. Fig. 8A presents the gradients for both groups and Fig. 8B depicts the differences between the paired and unpaired groups. Note that CS-specific plasticity would be indicated if the greatest increase or decrease in the gener-

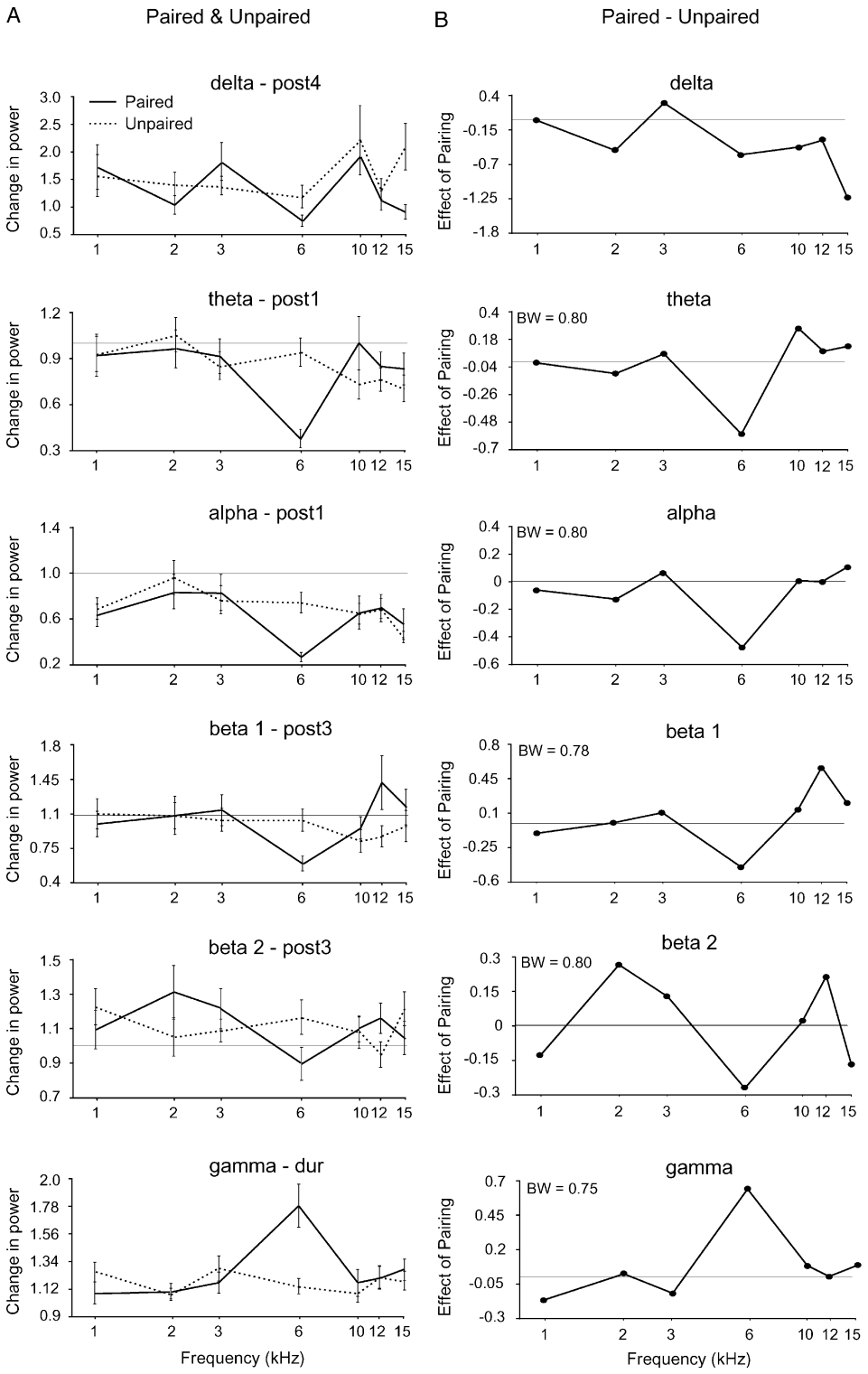

Fig. 9. (A) Frequency generalization gradients showing the mean (±SE) change found within each EEG frequency band elicited by each test tone frequency for the paired and unpaired groups, during the time periods in which the 6 kHz test tone elicited the maximal response in each EEG frequency band. ANOVAs and orthogonal contrasts revealed that 6 kHz tone elicited a significantly greater response than the other test frequencies for the *theta*, *alpha*, *beta1*, and *gamma* bands in paired subjects. The unpaired group did not show significant changes in response to 6 kHz in any of the EEG frequency bands. (B) Group difference functions, showing the specificity of EEG changes during the tone due to the difference in training. The bandwidth of the difference function is indicated in the upper left corner of the graphs for the *theta*, *alpha*, *beta1*, and *gamma* EEG frequency bands.

alization function occurs at the CS frequency of 6 kHz. This criterion was met for the *theta*, *alpha*, and *gamma* bands, but not for the *delta*, *beta1*, and *beta2* bands in the paired group. The unpaired group shows variable functions, all lacking 6 kHz specificity. The difference functions (Fig. 8B) provide an estimate of the degree of associative specificity. Although these group difference functions do not permit statistical evaluation, it was possible to obtain estimates of the degree of CS-specificity by calculating the bandwidth (BW) centered on 6 kHz. BW was calculated as 50% of the response magnitude for 6 kHz compared to the average response magnitude of the two adjacent frequencies of 3 and 10 kHz; (see Fig. 8 legend). The BW values for EEG bands that exhibited CS-specificity were 0.88 octaves for *theta*, 0.98 octaves for *alpha*, and 0.75 octaves for the *gamma* band. These values suggest that changes in the *gamma* band may be more specific than *theta* which in turn may be more specific than *alpha*. However, in the absence of statistical tests, no firm conclusions should be drawn.

As noted previously (Figs. 5–7), EEG bands displayed different temporal patterns of change. Maximal changes occurred as follows: *delta*, post 4; *theta*, post 1; *alpha*, post 1; *beta1*, post 3; *beta2*, post 3; *gamma*, during tone. Therefore, we also determined frequency generalization functions for the time period of maximal change to 6 kHz for each EEG band (Fig. 9A). *Delta* shows the greatest decrease for 6 kHz but the function is irregular and provides little confidence of genuine CS-specificity. This conclusion is supported by the difference function (Fig. 9B), in which the greatest difference between paired and unpaired groups is at 15 kHz. However all other bands do exhibit specificity to 6 kHz, *theta*, *alpha*, *beta1*, and *beta2* exhibiting decreased power. (As the maximal change for *gamma* was during the tone, this presentation of maximal data provides no new information for *gamma*.) BW values for these EEG bands are the same (0.80 octaves) or nearly so (*beta1* = 0.78 octaves). An overall summary of the findings is presented in Table 1.

3.4. Behavioral indices of memory

3.4.1. Heart rate

Examples of heart rate responses to a 6 kHz tone during testing for a paired and an unpaired subject are shown in Fig. 10A. The paired subject's response consists of a biphasic change, composed of a brief bradycardia followed by a longer tachycardia. Neither of the components of this response are present in the unpaired subject's heart rate record. To examine the dynamics of change, heart rate was analyzed in 200 ms bins. Fig. 10B shows the change from baseline heart rate across acoustic frequency and across time for the paired and unpaired groups. This change was calculated by subtracting the heart rate in each 200 ms bin after tone onset from the average heart rate 2 s before the tone for 9 s of recording. The extent of specificity of heart rate changes can be seen as patterns of color extending across time. Note that the paired group shows a specific response to 6 kHz, consisting of a decrease in heart rate about 1 s after tone onset followed by an increase that peaks about 6 s after onset. Other frequencies also elicited heart rate responses, but not as great as those to 6 kHz. The unpaired group did not show this 6 kHz specificity.

The main temporal features of the specific heart rate response to 6 kHz are the short latency bradycardia and longer latency tachycardia. To evaluate each of these components, the average response for the time period of maximal bradycardia and the average response for the time period of maximal tachycardia were analyzed separately. The responses for each trial were averaged in order to construct frequency generalization gradients for both time periods. The resulting gradients are shown in Fig. 11A. An ANOVA for tone frequency was performed for each group at each time point. Frequency was significant for the paired group for both the bradycardia ($F_{6,398} = 2.541$, $p = .02$) and tachycardia ($F_{6,398} = 6.84$, $p < .0001$). Orthogonal contrasts confirm that the response to 6 kHz is of greater magnitude than that of the other frequencies for both the bradycardia

Table 1
Summary of differential conditioned changes of power in EEG bands

	Delta	*Theta*	*Alpha*	*Beta1*	*Beta2*	*Gamma*
CS-specificity during tone?[a]	No	Yes	Yes	No	No	Yes
CS-specificity at maximum?[b]	No	Yes	Yes	Yes	Yes	Yes[g]
Direction of significant change[c]	↓	↓	↓	↓	↓	↑
Periods of significant change[d]	P 1,4	D, P 1–9[e]	D, P 1–6[f]	P 3	P 3,9	D, P 2
Periods of max. significant change	P 4	P 1	P 1	P 3	P 3	D

[a] CS frequency (6 kHz) at maximum or minimum of generalization gradient during tone.
[b] CS frequency at maximum or minimum of generalization gradient at period of largest magnitude of change.
[c] Decreases (down arrow) or increases (up arrow) of significant change.
[d] Significant ANOVA (tone frequency) and orthogonal polynomial. D, during; P, post-tone periods.
[e] *Theta*—All periods had significant change except post 8.
[f] *Alpha*—Period 5 was not significant.
[g] *Gamma*—The CS-specificity at maximum was also during the period of tone presentation.

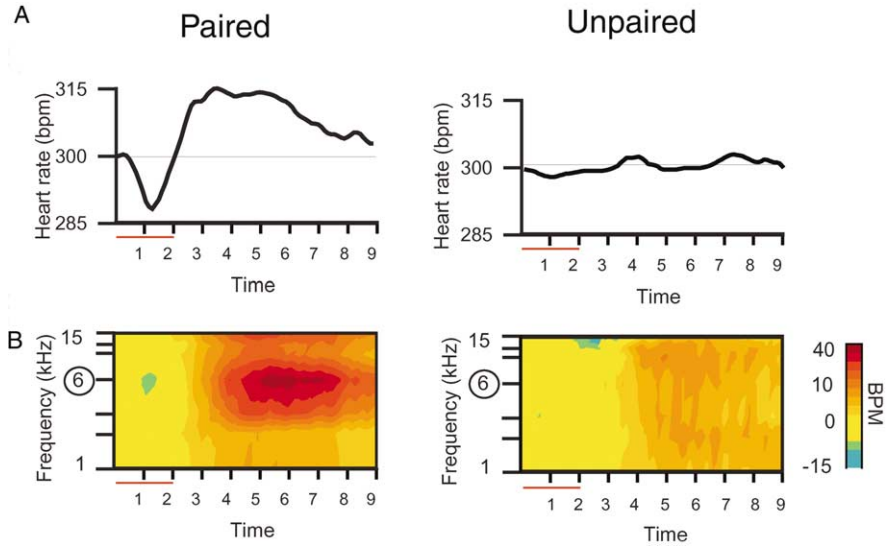

Fig. 10. (A) Examples of heart rate responses (beats per minute, BPM) to the 6 kHz tone during testing for a paired and an unpaired animal. Deflections below the baseline (thin horizontal line) indicate bradycardia while those above the baseline show tachycardia. Horizontal bar indicates period of test tone presentation (2 s). The paired subject's response consists of a brief bradycardia followed by a longer latency and longer duration tachycardia. No such response is seen in the unpaired subject. (B) Group data showing the mean change in heart rate for paired and unpaired subjects across both time (*X*-axis) and frequency (*Y*-axis). The time marks on the *X*-axis represent time (s) after tone presentation. The color represents the relative change in heart rate from baseline, red indicating the greatest increase and blue indicating the greatest decrease in response to the test tones. In the paired group, pairing-induced frequency specificity can be seen as horizontal band of colors corresponding to the 6 kHz tone extending across time. Note the decrease (bradycardia) specific to 6 kHz about 1 s after tone onset. The largest long-latency increase in heart rate is also centered on 6 kHz, peaking at about 6 s after tone onset, but with greater spread to adjacent frequencies. In contrast, the unpaired group does not show 6 kHz specificity. Unpaired subjects do show a frequency-specific decrease; however, it is to 15 kHz with a latency of about 2.5 s. The most striking feature in the response of the unpaired group is a tachycardia, peaking at about 6 s with no frequency specificity. Horizontal red bars indicate the duration of tone presentation.

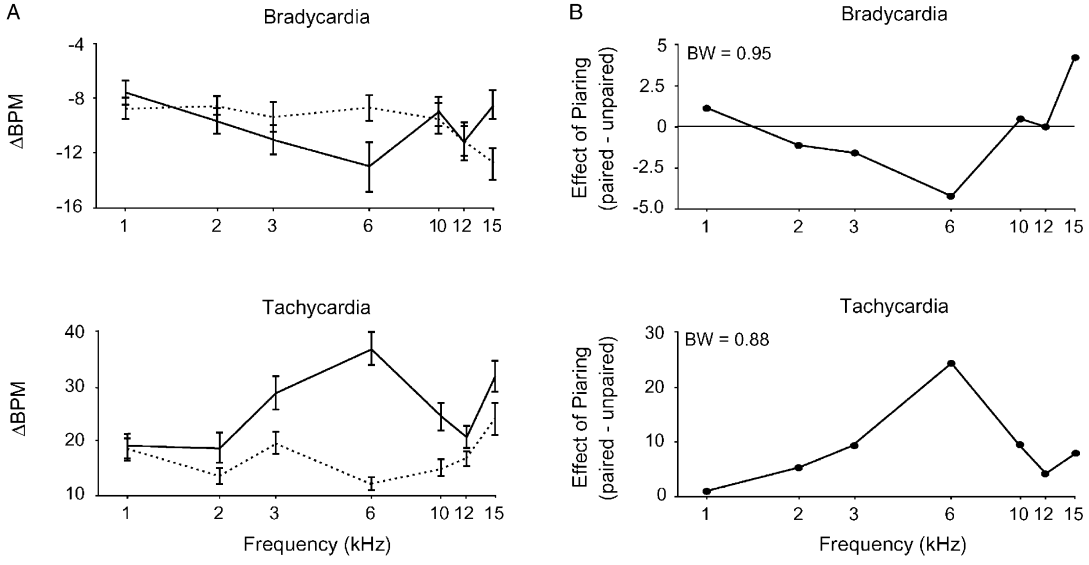

Fig. 11. (A) Frequency generalization gradients showing the mean (±SE) change in heart rate elicited by each test tone frequency during the period of maximal bradycardia (upper panel) and maximal tachycardia (lower panel). The responses in the paired group are represented by the solid line and those in the unpaired group are indicated by the dotted line. ANOVAs and orthogonal contrasts revealed that 6 kHz elicited a significantly greater bradycardia and tachycardia than the other frequencies in paired subjects. The unpaired group did not show a significantly greater response to 6 kHz for either change in heart rate. (B) Group differences (subtraction of unpaired from paired group functions) showing the specificity of heart rate changes during the time period of maximal cardiac response due to the difference in training. The bandwidth of each difference function is shown in the upper left corner of the graphs.

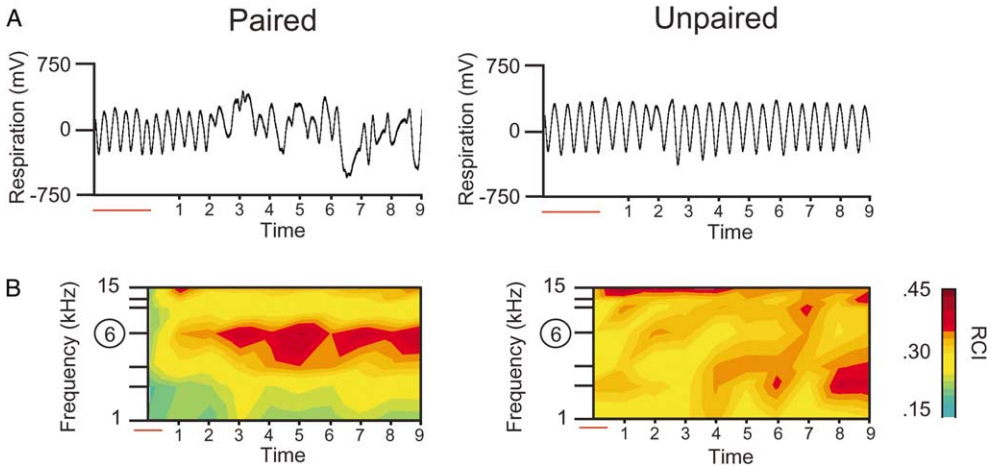

Fig. 12. (A) Examples of individual respiration responses to the 6 kHz tone during testing for one animal each in the paired and unpaired groups. The red horizontal bars indicate tone presentation. The paired subject's respiration is greatly disrupted following the tone whereas the unpaired subject's respiration remains relatively stable. (B) Group data showing the change in respiration (respiration change index, RCI) for paired and unpaired subjects across both time and frequency. The *X*-axis represents time in seconds following tone presentation. The *Y*-axis represents tone frequency. The color represents the RCI value, with red indicated the greatest values and blue indicating the smallest (the absence of change would correspond to a value of zero). In the paired group, pairing-induced frequency of response specificity can be seen as red horizontal band around 6 kHz extending across time. The response increases gradually over several seconds, reaching asymptote at about 5 s after the tone. Responses in the unpaired group do not show specificity to 6 kHz, the greatest response being found around the 15 kHz test frequency.

($t_{413} = -2.42$, $p < .05$) and the tachycardia ($t_{413} = 6.41$, $p < .01$). Unpaired subjects also had a significant effect of frequency for both the bradycardia ($F_{6,308} = 2.263$, $p = .04$) and tachycardia ($F_{6,308} = 4.70$, $p < .0001$). However, orthogonal contrasts indicate that the response to 6 kHz is not larger than that to other tones for bradycardia ($t_{308} = 0.34$, $p > .05$) or tachycardia ($t_{308} = -0.77$, $p > .05$). The results for the unpaired group indicate that presentation of tone alters heart rate for some frequencies more than for others, but such effects are not specific to 6 kHz. To indicate the specificity of the response due to pairing, the difference in generalization functions between the groups was calculated (Fig. 11B). The bandwidth at 50% maximum response of the bradycardia component was 0.95 octaves; the bandwidth of the tachycardia component was 0.88 octaves. Although these bandwidth values cannot be compared statistically (see Section 4), they suggest that the different components of the conditioned cardiac response might be differentially selective, with the tachycardia being more selective.

3.4.2. Respiration

Examples of respiration responses to the 6 kHz tone during testing are shown in Fig. 12A. Respiration was altered to a marked extent in the paired, but not unpaired, record. To quantify the changes in respiration, Fourier analysis was used to generate a RCI (see Section 2). Previously we reported that the largest average change across time within a trial occurs to 6 kHz in the paired group (McLin et al., 2002a). To understand the dynamics of the conditioned respiration response, RCI values were calculated on a second-by-second basis. Fig. 12B shows the average change in respiration, as RCI values, across time and tone frequency for the paired and unpaired groups. Orange-red colors indicate greater amounts of change and yellow-blue colors indicate lesser amounts of change. As with the heart rate changes, the frequency specificity of change can be seen as patterns of colors extending across time. The paired group shows specificity to 6 kHz which begins to emerge about 2 s after tone offset and reaches a peak at about 5 s. No such indication of specificity is present in the unpaired group. The pattern observed in Fig. 12 suggests that respiration, like EEG and heart rate, also develops associative and specific responses to the 6 kHz paired tone.

Visual inspection of the data indicated several time points of interest with regard to the specificity of the response. To more fully assess response dynamics, frequency generalization gradients were constructed for the time period during the tone and the time period with the maximal 6 kHz response. Additionally, a generalization gradient was also constructed for the time period corresponding to the emergence of the response, 2 s after the tone (Fig. 12B). The resulting gradients are shown in Fig. 13A. ANOVAs for test frequency were performed for both groups for each of these time periods. Frequency was not significant for paired subjects during the tone ($F_{6,1245} = 0.835$, $p = .54$), but was significant at post 2 ($F_{6,1245} = 7.22$, $p < .0001$) and post 5 ($F_{6,1245} = 10.32$, $p < .0001$). Orthogonal contrasts indicate that the 6 kHz response was significantly greater for paired subjects at both 2 and 5 s after the tone ($t_{413} = 2.58$, 3.36, respectively, $p < .05$). Frequency was not a significant factor for unpaired subjects during the tone ($F_{6,1431} = 1.81$, $p = .09$) (Fig. 13, top panel) or at post 2 s

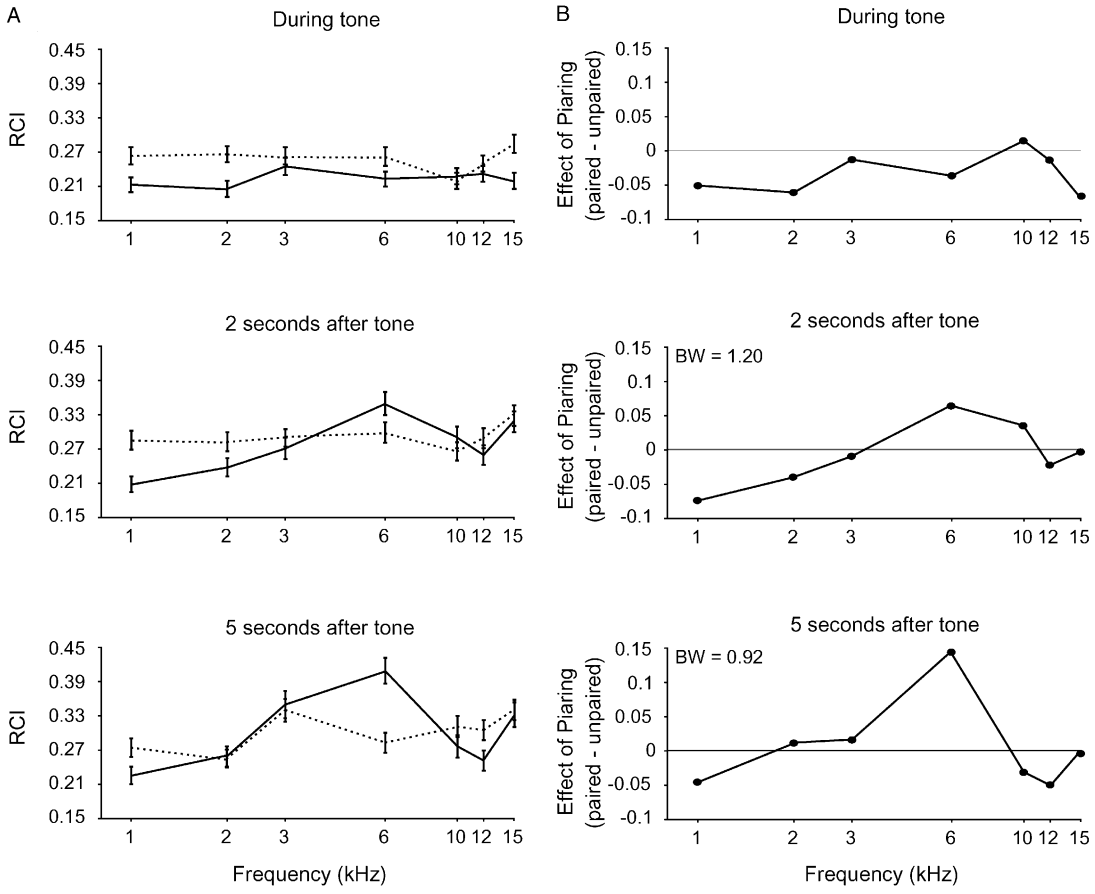

Fig. 13. (A) Frequency generalization gradients showing the mean (±SE) change in respiration elicited by each tone frequency during the tone (upper panel) 2 s after the tone (middle panel) and at the peak of the response 5 s after the tone (lower panel); paired group, solid line and unpaired group, dotted line. ANOVAs and orthogonal contrasts revealed that the 6 kHz test tone elicited a significantly greater change in respiration at both 2 and 5 s after the tone in the paired group. This was neither the case for any time period in the unpaired group, nor during the tone for the paired group. (B) Group difference functions (subtraction of unpaired from paired group functions) showing the specificity of respiration changes due to the difference in training. The bandwidth of the difference function is shown in the upper left corner of the graphs for the post 2 and post 5 s time periods. Note the lack of specificity in respiratory response during the tone, although the specificity is apparent by 2 s after the tone and is even more specific 5 s after the tone.

($F_{6,1431} = 1.31$, $p = .25$) but it was significant at post 5 s ($F_{6,1431} = 3.39$, $p = .0025$). However, the orthogonal contrast for 6 kHz evoking a larger response than other frequencies at post 5 s for unpaired subjects was not significant ($t_{518} = -0.61$, $p > .05$). To indicate the specificity of the response due to pairing, the difference in response between the groups was calculated (Fig. 13B). The bandwidth at 50% maximum response could not be calculated for the time period during the tone because 6 kHz did not elicit the maximum response. The bandwidth at post 2 s was 1.20 octaves and at post 5 s was 0.92 octaves, suggesting that specificity increases as the response develops.

4. Discussion

4.1. Overview of the findings

This report largely constitutes a detailed analysis of the spectral and temporal characteristics of conditioned EEG activation that is induced by the pairing of a tone with NB stimulation. We have previously reported that this treatment produces selective conditioned EEG activation, as indexed by increased power in the *gamma* band during presentation of the 6 kHz frequency of the conditioned stimulus. Of greatest importance in the previous report, this pairing also induces CS-specific behavioral memory, as indexed by post-training assessment of tone frequency generalization gradients for heart rate and respiration. The effects are associative as they failed to develop in the control group receiving unpaired tone and NB stimulation (McLin et al., 2002a).

The lack of either conditioned CS-specific EEG effects or behavioral memory in the unpaired group cannot be attributed to ineffective NB stimulation (Bjordahl et al., 1998) because it produced unconditioned activation in these animals (Fig. 2). Explicitly unpaired, rather than random, presentation of 6 kHz and NB stimulation was chosen to prevent accidental pairing. This precaution was used because it is not known whether or not

accidental forward pairings would be equally offset by accidental backward pairings, given the novel use of NB stimulation as an unconditioned stimulus. As NB stimulation produces several seconds of unconditioned EEG activation, a random design probably would have produced many trials in which the tone overlapped or shortly preceded a state of NB-induced activation. The unpaired protocol did not produce inhibitory EEG conditioning although the CS during training signaled the imminent absence of NB stimulation.

Conditioned EEG activation (also termed "desynchronization") is the oldest neurophysiological finding in the neurobiology of learning and memory. First discovered in the 1930s as conditioned *alpha* blocking in the human (e.g., Jasper & Cruikshank, 1936), it was subsequently extended in numerous studies of humans (e.g., Jasper & Shagass, 1941) and animals (reviewed in Morrell, 1961). As noted in Section 1, we had assumed that our detection of conditioned increased *gamma* power during CS presentation merely indexed the establishment of conditioned activation, which we used as an assay to demonstrate that tone-NB pairing had induced cortical plasticity (McLin et al., 2002a). Subsequent, detailed analysis of all EEG bands and time periods has now revealed that conditioned EEG activation is more complex. These complexities were revealed in data obtained during post-training determination of behavioral and electrographic tone frequency generalization functions. They revealed a "fractionation" of conditioned EEG activation, specifically that associatively induced changes in power for various EEG bands consist of differential CS-specificity and temporal dynamics. The EEG findings are summarized in Table 1 and will be discussed from low to high frequency bands. The present analyses also provided additional information on CS-specific temporal changes in heart rate and respiration and these will be addressed later.

4.2. Differential spectral and temporal characteristics of conditioned EEG activation

4.2.1. Statistical analyses and frequency generalization functions

Before discussing the detailed findings, it will be helpful to consider the bases for interpretation of the findings. Data analyses consist both of standard statistical testing and also quantitative findings that could not be subjected to statistical evaluation. The former consist of *t* tests, ANOVAs and their related orthogonal contrasts to determine if responses to the CS frequency of 6 kHz differed significantly from responses to other frequencies. The latter consist of differences between acoustic frequency generalization functions, specifically functions for the unpaired group subtracted from functions for the corresponding EEG band for the paired group (Figs. 8B and 9B). These difference functions were determined because they provide an estimation of the *associative* effects of the experiment. Generalization functions for the unpaired group are likely to represent non-associative effects while those for the paired group may contain some non-associative effects as well as associative effects. For example, some acoustic frequencies had unconditioned effects in both groups, as indicated by the data for 15 kHz in the *gamma* band (Figs. 5 and 7). Such effects apparently also occurred in the *delta* band for the period of its maximum 6 kHz decrease in power (Fig. 9A). However, the unpaired group exhibited this same effect but the difference between the generalization functions for these groups failed to reveal 6 kHz specificity (Fig. 9B). We used the generalization difference functions to determine if responses to the CS frequency were at the maximum decrease ("valley" of the function) or maximum increase ("peak" of the function). However, these difference functions are not amenable to statistical analysis because they include no estimate of variance. Generalization gradients for individual subjects comprised the mean group gradients but could not individually be compared to gradients for unpaired subjects because there were no parallel (e.g., "yoked") subjects. (Individual subject generalization gradients that yield associative effects might be achieved in the future by use of a two-tone discrimination paradigm in which responses to the CS− tone might be subtracted from responses to the CS+ tone.) The bandwidth measures from the generalization difference functions serve only a descriptive function and may have a heuristic value for the future, but we do not draw inferences from the bandwidth values.

4.2.2. Delta band

Delta waves are a prominent feature of the EEG, particularly during periods of lower levels of arousal, such as relaxed waking, especially during slow wave sleep. Conditioned EEG activation in animals has been well established (reviewed in Morrell, 1961), but spectral analyses of changes in *delta* power to tone conditioned stimuli appear to have been rare. Recently, Whalen et al. (1994), found a CS-elicited reduction of *delta* power during classical conditioning in the rabbit, using a two-tone discrimination protocol with shock as the US. This conditioned decrease developed rapidly, being present within the first block of six trials. The decrease in *delta* power was maintained throughout training to the CS+ but it dissipated to the CS− starting with the third block of six trials. We also found a significant change in *delta* power during training: *delta* power was significantly reduced in the paired group compared to the unpaired group (Fig. 3B). Moreover, we also observed that conditioned activation was present during the first training day and was maintained across days. However, we cannot determine how quickly conditioned *delta* effects

developed within the first session. As the focus of the present experiment concerned post-training generalization data, for which we could find no precedents in the literature, we did not perform trial-by-trial analyses, but rather combined the data across ~200 trials per day.

Analysis of the tone frequency generalization data revealed that changes in *delta* power exhibited little, if any, CS-specificity. Two time periods, 1 and 4 s after tone presentation, did exhibit a significantly greater decrease for 6 kHz than for the other frequencies. However, the *delta* function for change at each trial time period showed no evidence of a consistently lower power in response to 6 kHz (Fig. 6A), particularly compared to the *theta* band (below). Also, the CS frequency was not at the peak of *delta* decrease (i.e., the "valley" of the function) for generalization gradient difference functions either during tone presentation (Fig. 8B) or at it point of maximum change at post 4 s (Fig. 9B). In the absence of such specificity, bandwidths were not computed for the *delta* band.

4.2.3. Theta band

During training, *theta* power was significantly reduced in the paired group compared to the unpaired group (Fig. 3B). Of greater importance, *theta* power exhibited the most pronounced CS-specific change of any EEG band. It had a significant 6 kHz contrast during tone presentation and for every post-tone period except at 8 s (Fig. 6). Generalization gradients showed that the decrease in *theta* power was greatest to the 6 kHz tone both during tone presentation (Fig. 8B) and at its period of maximum change, 1 s after the tone (Fig. 9B). The bandwidths of specificity were 0.88 and 0.80 octaves, respectively. This suggests that specificity may become greater after, rather than during, tone presentation, a possibility that requires future investigation with designs yielding bandwidth data amenable to statistical evaluation. To permit a finer grain analysis of bandwidth, further studies of stimulus generalization should use a larger number of acoustic frequencies at smaller frequency distances.

4.2.4. Alpha band

Power in this band was reduced during training, but the difference between the paired and unpaired groups did not attain statistical significance (Fig. 3B). During generalization testing, *alpha* power paralleled the decrease observed for the *theta* band but was not as pronounced: contrasts were significant during 6 kHz and post 1–4 and post 6 s (Fig. 6). CS-specificity was also evident in the generalization gradients where *alpha* showed greatest decrease in power during the tone (Fig. 8B) and at its maximum effect 1 s after tone (Fig. 9B). The bandwidths of 0.98 and 0.80 octaves also suggest that specificity increases after tone offset, as indicated for the *theta* band.

4.2.5. Beta1 band

This band had a significant reduction in power during training (Fig. 3B) but little specificity during generalization testing. Its generalization gradient was not specific to 6 kHz during tone presentation. However, it did develop a decrease in power that was evident from post 1 to post 7 s but the contrast attained statistical significance only at post 3 s (Fig. 7A). Its generalization gradient exhibited 6 kHz specificity at this time point (Fig. 9B). The *beta1* bandwidth at this single period of specificity was 0.78 octaves, comparable to that of the *theta* and *alpha* bands. As *beta1* exhibited CS-specificity after but not during the tone, it seems that CS-specificity can develop over trial time.

4.2.6. Beta2 band

This EEG band traditionally has been considered to represent lower voltage, high frequency activity that increases during cortical activation. However, Maloney et al. (1997) found that this is not the case for changes in sleep–waking states and spontaneous behavior in the freely moving rat. Rather, they found that EEG activation was characterized by an increase of power in the *gamma* band (see also below) while power in the *beta2* band was decreased. In contrast, we found that *beta2* power was significantly increased during EEG activation caused by stimulation of the NB (Fig. 2B). This difference in findings is consistent with the view that, while the cholinergic NB is a major factor in EEG activation (e.g., Buzsaki et al., 1988; Casamenti et al., 1986; Détári & Vanderwolf, 1987), other neuromodulatory systems contribute to this cortical state (Berridge & Foote, 1991; Peck & Vanderwolf, 1991). However, *beta2* power did decrease significantly during training (Fig. 3B). This was the only EEG band in which the direction of change in power was different during training vs. testing for stimulus generalization. We do not have an explanation for this paradox, but it does indicate that the nature and processes underlying EEG activation appear to differ, depending on the circumstances within which it occurs. This differential role for the *beta2* band is consistent with the overall theme of the current findings, that EEG activation is a complex rather than unitary process.

Similar to the *beta1* band, changes in the power of *beta2* were not specific during tone presentation (Fig. 8) but became specific 3 s after the 6 kHz tone (Fig. 9). Its bandwidth of specificity at this time was 0.80 octaves, the same as *theta* and *alpha* at their respective periods of maximum change. In contrast to *beta1* there was no systematic development of a decrease in power to the CS frequency over time; rather, the effect at post 3 s was limited to that time point (Fig. 7A).

Beta activity has been linked to centrifugal control of information processing during expectancy for a specific sensory stimulus. Thus Kay and Freeman (1998) have reported that the *beta* band (12–35 Hz) is involved in

projections from the entorhinal cortex to the olfactory bulb, to facilitate processing of an expected odor during learning. Wrobel (2000) has reported similar centrifugal (cortical–geniculate) beta activity in the visual system that may selectively lower the threshold for attended visual stimuli. The present study of classical conditioning revealed only minimal CS-specific changes in the beta band (15.20–32.55 Hz), perhaps because our protocol did not permit expectancy of the presentation of the CS frequency. That is, because the protocol involved testing for stimulus generalization, the subjects received seven different frequencies, not merely a single, predicted CS tone. Neuper and Pfurtscheller (2001) reported that *beta* activity is reduced focally when a relevant cortical area is active, e.g., during motor imagery.Thus, beta activity might be reduced in the auditory cortex during processing of the CS frequency. We observed a tendency for a reduction of *beta1* (15.20–19.53 Hz) activity in the paired group during presentation of the CS frequency, but this failed to reach statistical significance except for a single 1 s time period (Fig. 7). Stronger findings during classical conditioning might be revealed by discrimination training, with sharper behavioral generalization gradients that would indicate a higher degree of selective processing of the CS frequency.

4.2.7. Gamma band

The *gamma* band is distinctive as the *only* component of the EEG for which power was increased by NB stimulation (Fig. 2), increased during training (Fig. 3B) and exhibited a CS-specific increase in stimulus generalization (Fig. 8). *Gamma* also differs from all other bands in exhibiting maximal specificity *during* presentation of 6 kHz in frequency generalization gradients, and then rapidly losing this effect (significant contrast thereafter only at post 2 s). Some other bands also exhibited a small number of time points with significant contrasts (e.g., decreased power for *delta*, post 1 and 4; *beta1*, post 3; *beta2*, post 3 and 9), but none exhibited the *gamma* pattern of change. The increase in *gamma* power observed in this study is consistent with the increase in this band during spontaneous EEG activation (Franken, Dijk, Tobler, & Borbély, 1994; Maloney et al., 1997), now extended to the specificity of activation by conditioned sensory stimuli.

Cortical *gamma* rhythms appear to be generated endogenously (reviewed in Jeffreys, Traub, & Whittington, 1996 see also Freeman & Barrie, 2000) but can be enhanced by electrical stimulation of the thalamus (e.g., posterior intralaminar nucleus, Brett & Barth, 1997; thalamic reticular nucleus, MacDonald, Fifkova, Jones, & Barth, 1998). Stimulation of the NB can increase *gamma* activity both by electrical (McLin et al., 2002a; Metherate et al., 1992) and chemical (Cape & Jones, 1998; Cape, Manns, Alonso, Beaudet, & Jones, 2000) stimulation. However, Brett and Barth (1997) failed to increase *gamma* power with NB stimulation but they suggested that the low current level of 15 μA was responsible. Currents of ~50–100 μA produce increased *gamma* both in anesthetized (Metherate et al., 1992) and unanesthetized animals (McLin et al., 2002a). Similar levels also have been used to elicit cortical release of acetylcholine (Kurosawa et al., 1989). The present study elicited unconditioned increases in *gamma* power (Fig. 2) using ~83–103 μA of current in unanesthetized animals, consistent with prior successful demonstrations of electrical stimulation of the NB.

Gamma activity has been of increasing interest because it has been linked to several important physiological and psychological processes. For example, *gamma* oscillations appear to be involved in increased synchronization of discharges among neurons (Bressler, 1990; Gray & Singer, 1989; Murthy & Fetz, 1992), which may provide neuronal "binding" of stimulus components into perceptual objects (Joliot, Ribary, & Llinás, 1994; Jones & Barth, 1997; Keil, Gruber, & Müller, 2001a). *Gamma* waves are prominently induced in all sensory cortices by modality-specific sensory stimulation (reviewed in Sannita, 2000). This is consistent with the view that *gamma* waves are involved in the perception of sensory stimuli (Gray, König, Engel, & Singer, 1989), and in selective attention (Bouyer, Montaron, & Rougeul, 1981), including visual search (Tallon-Baudry, Bertrand, Delpuech, & Pernier, 1997). Transient synchrony as manifested in phasic bursts of *gamma* rhythms (30–60 Hz) also has been hypothesized to signal the "recognition" of space–time patterns across an array of neurons (Hopfield & Brody, 2001).

Prior studies of learning using standard appetitive and aversive reinforcers, report enhanced *gamma* activity in the cerebral cortex of animals (Amzica, Neckelmann, & Steriade, 1997; Barrie, Freeman, & Lenhart, 1996; Dumenko, 1995) and humans (Keil, Müller, Gruber, Wienbruch, & Elbert, 2001b; Miltner, Braun, Arnold, Witte, & Teub, 1999). The present approach, of obtaining post-training stimulus generalization gradients, permits determination of the degree of stimulus specificity in associative processes. As noted above, the generalization findings revealed that conditioned enhancement of *gamma* power is highly specific to the frequency of the conditioned stimulus and that it is confined largely to the period of presentation of the CS frequency. These findings support the hypothesis that *gamma* activity has a special role in the processing of sensory stimuli that acquire behavioral importance. In the present experiment, such behavioral significance was independently verified by the associative induction of behavioral memory by assaying cardiac and respiratory behavior.

4.2.8. Future studies of conditioned EEG activation and stimulus generalization

The present use of NB stimulation as an unconditioned stimulus is novel. Therefore the present findings

raise the issue of generality to conditioning with orthodox appetitive or aversive reinforcers. To what extent are the current results indicative of the normal role of the nucleus basalis in learning? NB cells respond to standard reinforcers and develop discharge plasticity to conditioned stimuli during such conditioning (Maho et al., 1995; Pirch, 1993; Richardson & DeLong, 1991; Whalen et al., 1994; Wilson & Rolls, 1990). Therefore, assuming that NB stimulation mimics some of this NB activity, it is likely that similar specificity of EEG bands will be found in future studies of EEG stimulus generalization during standard conditioning. Such studies will help place the present findings of selective EEG spectral and temporal specificity within the broader context of learning and memory. They will also break new ground in providing information on the degree of stimulus specificity of conditioned EEG activation.

The current findings of differential spectral and temporal CS-specificity of conditioned EEG activation raise issues of both function and mechanism. We have discussed some potential functional aspects for the *gamma* band, based on prior and extensive studies of *gamma* activity. The present results suggest that *delta* activity is highly sensitive to all acoustic stimuli, to the extent that it exhibits a lack of CS-specificity. When *delta* activity is particularly high, however, it tends to spill over into *theta* range (Maloney et al., 1997). It is possible that *delta* does show frequency specificity, but primarily in the upper *delta* ranges that spill over into the *theta* band, obscuring tone frequency specificity in the *delta* band or contributing to tone frequency specificity in the *theta* band.

Unlike *delta*, *alpha*, which is generally considered to be highly sensitive to sensory stimulation, does exhibit a significant degree of CS-specificity. Because the *alpha* band, as defined here, would include spindle (*sigma*) activity, the frequency-specific conditioned decreases observed could be due to spindling decreases, which were visually observable and are strongly associated with arousal (Klemm, 1969). The *alpha* band also includes paroxysmal EEG patterns which several investigators have observed to occur spontaneously in rats (e.g., Vergnes et al., 1987). Such activity is readily identifiable visually, characterized by spike waves several fold greater in amplitude than background activity. Although we have noted such activity in our subjects as well, we specifically did not present trials during such activity to minimize variability in background activity. Further, no tone elicited such activity in any animal.

A wealth of literature describes functional and behavioral correlates of EEG activity. Interpretation of the current results within the theoretical frameworks that are evolving regarding such functional and behavioral correlates is complicated by the fact that the EEG changes we induced (a) were conditioned responses and (b) were likely generated by direct activation of the NB cholinergic system which modulates EEG. Nonetheless, the current findings suggest that functional relationships do exist for conditioned EEG effects induced with stimulation of the NB. Because the primary objective of this study was to further elucidate the NBs role in the mediation of cortical plasticity, more than a cursory consideration of the functional aspects of various EEG bands is beyond the scope of this paper.

The present findings do provide a novel means of investigating neural processes in learning and memory. While association per se, indicated by conditioned EEG activation and other measures, has been studied extensively, the processes underlying stimulus specificity in conditioning have received much less attention. The current delineation of EEG frequency bands that either reflect CS-specificity (*theta*, *alpha*, *gamma*) or are relatively insensitive to conditioned tone specificity (*delta*, *beta1*, *beta2*), provide markers for seeking sources of specific, associative neural processes. Future research needs to compare and contrast the neural bases of the two groups of EEG frequency bands because this line of inquiry has the potential to identify neural mechanisms affiliated with associative specificity, that is, the differential representation of acquired stimulus attributes in conditioning, and perhaps by extension, other forms of learning and memory.

4.3. Behavior

Cardiac behavior was analyzed separately for the short latency bradycardia and the longer latency tachycardia. Previously, we noted that the overall magnitude of the cardiac response exhibited CS-specificity (McLin et al., 2002a). Separate analyses in this report show that each of the components exhibited CS-specificity. The unpaired subjects exhibited changes in heart rate for both components, but there was no specificity to the CS frequency of 6 kHz, indicating that cardiac behavior in the paired group was both associative and specific to the CS frequency. Interestingly, the bandwidth values for the differences between groups suggest that the bradycardia (BW = 0.95) is less specific than the longer latency conditioned tachycardia (0.88). As noted above, the design used in this experiment does not permit statistical comparisons of these descriptors. As for analysis of EEG bandwidths of group differences, future studies may directly address this issue by using a within-subject two-tone discrimination design.

Temporal analysis of respiration behavior extended our previous report of behavioral specificity (McLin et al., 2002a). In the present case, it became evident that there was no significant response for either the paired or unpaired group during tone presentation. Specificity became evident 2 s after tone offset and became maximal at 5 s in the paired group. Although the unpaired group exhibited differential response to frequency at post 5 s,

this was not specific to the CS frequency (Fig. 12).The BWs for the differences between the groups at post 2 and post 5 s are 1.20 and 0.92 octaves, respectively, suggestive of increasing specificity to the CS frequency for time within a trial.

The temporal dynamics for cardiac and respiratory CS-specific conditioned responses are thus different. Notably, specific bradycardia was evident during tone presentation but comparable significant changes in respiration were not present during the tone (compare Figs. 10 and 12). Also, the highest level of CS-specificity was attained with a longer latency for respiration (post 5 s) than heart rate, as even the bradycardia during tone presentation attained a clear degree of specificity during the tone; even the respiration response at 2 s appears less specific than the bradycardia during the tone (compare Figs. 11 and 13). Therefore, heart rate is a more sensitive measure of NB-induced memory than respiration, particularly during tone presentation. These results suggest that while both measures adequately reveal NB-induced memory regarding 6 kHz, if a single measure is used, heart rate may be preferred.

4.4. Stimulation of the nucleus basalis as a reinforcer

Standard appetitive and aversive reinforcers have hedonic value and elicit unconditioned responses. Does NB stimulation act in the same manner although it was applied to the brain? For example, stimulation of the ventral tegmental area (VTA) is positively reinforcing and when paired with a tone, produces auditory cortical plasticity (Bao, Chan, & Merzenich, 2001; Kisley & Gerstein, 2001) that in some ways resembles receptive field plasticity and tonotopic re-organization induced by pairing a tone with stimulation of the nucleus basalis (Bakin & Weinberger, 1996; Bjordahl et al., 1998; Dimyan & Weinberger, 1999; Kilgard & Merzenich, 1998). Furthermore, stimulation of the NB elicits unconditioned EEG activation, cardiac, and respiratory responses (McLin et al., 2002a; McLin et al., 2002b). However, these similarities do not force the conclusion that NB stimulation acts as a standard (i.e., motivational) reinforcer for conditioned EEG activation and the induction of behavioral memory, for the following reasons. First, in contrast to the VTA, the nucleus basalis is not part of any known hedonic or motivational system (reviewed in Pennartz, 1995). Second, motivationally neutral sites can elicit unconditioned responses that produce EEG activation and behavioral arousal. For example, Olds and Peretz (1960) reported separable positive, negative, and behaviorally motivationally neutral regions of the midbrain of the rat, whose stimulation elicited EEG activation. Moreover, Wester (1971, 1972) found that stimulating sites in the midline and intralaminar nuclei of the thalamus, which he found to be without hedonic value in behavioral tests, elicited behavioral arousal as well as EEG activation. Notably, the midbrain sites were later identified as including ascending projections of the midbrain cholinergic system, some of which innervate the medial and intralaminar thalamus while others innervate the nucleus basalis (Steriade & Buzsaki, 1990).

Stimulation of the NB also increases cerebral blood flow (CBF) (e.g., Biesold, Inanami, Sato, & Sato, 1989; Lacombe et al., 1989). It might be argued that increases in CBF might be in some way aversive, hence imparting negative hedonic value to the NB. However, the stimulus durations used to elicit CBF effects have been minimally 10 s (reviewed in Sato, Sato, & Uchida, 2001). We used a 200 ms train of stimulation, which is 1/50th the duration of the briefest stimulation employed in CBF studies. Therefore, this brief stimulus probably did not produce notable CBF effects, but even if so, the possible hedonic effects are by no means certain. Thus, resolution of this issue remains to be determined.

It should be remembered that an unconditioned stimulus need not have motivational impact in order to establish associations. Sensory preconditioning is such an instance (Macintosh, 1974). The NB may act in yet a different manner. Ordinarily, the NB may operate "downstream" of systems that evaluate and determine the hedonic value of stimuli. As these systems have efferents to other brain systems, it is possible that the NB is ordinarily engaged to modulate the cerebral cortex, and perhaps other structures, by the release of acetylcholine, having received excitatory input from evaluative systems. In such a location in the processing stream, the NB could exert widespread cortical effects in response to a multitude of inputs without itself constituting part of any motivational system. The issue can be further clarified by additional direct investigation of possible hedonic NB effects.

4.5. Limitations of the present study

There are two major limitations of the current findings: specificity of effects to the nucleus basalis and, related, the circuitry underlying the EEG and behavioral effects.

The present findings, in conjunction with previous reports, are consistent with the view that the conditioned EEG (but not necessarily the behavioral) effects are mediated by projections of the NB to the auditory cortex, including its cholinergic projections. The study of NB-induced EEG and behavioral memory was motivated by prior findings that have implicated the NB, and its cholinergic projections to the cerebral cortex, in learning and memory. These include the findings that tone paired with NB stimulation induces the same auditory cortical plasticity that develops during standard conditioning when tone is paired with an appetitive or aversive unconditioned stimulus (Bakin & Weinberger,

1996; Bjordahl et al., 1998; Dimyan & Weinberger, 1999; Kilgard & Merzenich, 1998). Necessarily, investigation of such NB effects requires the use of similar methods, including electrical stimulation of the NB. The advantages and limitations of stimulating the NB have been provided by Rasmusson (2000).

In the present experiment, NB stimulating electrodes were placed under physiological control, the final sites were determined as those producing EEG activation at the lowest stimulus levels; the threshold current levels were 50–100 μA (see Section 2). Also, our stimulating electrodes were located within regions recently shown to have relatively high concentration of cortically projecting cholinergic cells (Zaborszky, Pang, Somogyi, Nadasdy, & Kallo, 1999). However, the cells that comprise the NB are not densely compacted and some reside within the internal capsule as well as the globus pallidus and substantia innominata (see Fig. 1) (Mesulam et al., 1983). Therefore, stimulation may have engaged pallidothalamic projections involving the ventral anterior and ventral lateral thalamic nuclei that project to motor cortex (although no movement was detected in any subject). Stimulation might also have engaged thalamocortical fibers passing through the posterior limb of the internal capsule.

Regarding conditioned EEG effects, NB activation of the cortical EEG is known to be mediated by its cholinergic projections (Casamenti et al., 1986) and also by its GABAergic projections to the cortex (the latter producing inhibition of cortical inhibitory interneurons (Freund & Meskenaite, 1992; Jiménez-Capdeville et al., 1997)). It seems likely that cortical activation in this study was also so mediated.

Moreover, there is prior evidence that NB-produced plasticity requires engagement of muscarinic receptors in the auditory cortex. Miasnikov et al. (2001) induced CS-specific associative receptive field plasticity in the auditory cortex of the rat by pairing a tone with NB stimulation. They found that the induction of plasticity was blocked by the administration of atropine sulfate directly to the auditory cortex. Their findings also indicate that NB induced cortical plasticity does not depend upon peripheral autonomic responses to the NB stimulus. The conditioning protocol and sites of stimulation used in the present study were the same as those employed by Miasnikov et al. (2001). Therefore it seems likely that the current findings also involved muscarinic receptors in the auditory cortex. However, in the absence of combined pharmacological investigation in the present study, the specificity of conditioned EEG effects cannot be attributed definitely to cholinergic NB projections to the auditory cortex.

With reference to the second point, circuitry underlying the conditioned autonomic cardiac and respiratory responses is more problematic. The NB projects to the amygdala as well as to the cerebral cortex. Even if the behavioral results are mediated in part via the NB, as is likely for the EEG effects,the circuitry underlying the behavioral effects need not be limited to one or the other pathway. In fact, the conditioned EEG activation might be mediated via effects on the cortex while the behavioral indices of memory might be mediated via the amygdala. Therefore we make no claims about the neurotransmitters or circuitry that are involved in the cardiac and respiratory responses (McLin et al., 2002b). The current study should be regarded as a preliminary step to more directly understand the role of the nucleus basalis in cortical plasticity and in behavioral memory.

4.6. Nature of the induced memory

Viewing the behavioral stimulus generalization gradients (Figs. 11 and 13), one could not determine if the animals had been trained with tone paired with food or shock vs. tone paired with stimulation of the nucleus basalis. These data meet the two essential criteria for inferring memory from conditioned behavior: associativity and specificity. Therefore, it is reasonable to conclude that behavioral memory had been induced.

What aspects of memory might have been induced by tone paired with NB stimulation? Normally, memories are thought to include both the "*sensory content*" of an experience and the *level of behavioral importance* of the experience. However, part of the sensory content would appear to be missing, i.e., that of a standard unconditioned stimulus. The NB is neither itself part of any sensory system, nor does it appear to be a hedonic substrate. Thus, the NB may have had a singular effect. It would have induced only the *increased importance* of 6 kHz, without storage of the normal CS–US sensory–sensory or sensory–motivational relationship, as these were absent. In short, the paired subjects came to regard 6 kHz as important, in the objective sense that this frequency gained the power to control their heart rate and respiration. The present approach appears to provide a method to investigate memory for stimulus importance, relatively isolated from memory of normal sensory–motivational events, thereby allowing the "dissection" of memory components for reductionistic analyses.

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