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Gamma band plasticity in sensory cortex is a signature of the strongest memory rather than memory of the training stimulus

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A B S T R A C T

Gamma oscillations ($\sim$30–120 Hz) are considered to be a reflection of coordinated neuronal activity, linked to processes underlying synaptic integration and plasticity. Increases in gamma power within the cerebral cortex have been found during many cognitive processes such as attention, learning, memory and problem solving in both humans and animals. However, the specificity of gamma to the detailed contents of memory remains largely unknown. We investigated the relationship between learning-induced increased gamma power in the primary auditory cortex (A1) and the strength of memory for acoustic frequency. Adult male rats ($n = 16$) received three days (200 trials each) of pairing a tone (3.66 kHz) with stimulation of the nucleus basalis, which implanted a memory for acoustic frequency as assessed by associatively-induced disruption of ongoing behavior, viz., respiration. Post-training frequency generalization gradients (FGGs) revealed peaks at non-CS frequencies in 11/16 cases, likely reflecting normal variation in pre-training acoustic experiences. A stronger relationship was found between increased gamma power and the frequency with the strongest memory (peak of the difference between individual post- and pre-training FGGs) vs. behavioral responses to the CS training frequency. No such relationship was found for the theta/alpha band (4–15 Hz). These findings indicate that the strength of specific increased neuronal synchronization within primary sensory cortical fields can determine the specific contents of memory.

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1. Introduction

A major advance in broadening our understanding of the neural substrates of learning and memory has involved a shift in emphasis from linear stimulus–response circuits to neural networks. Following Hebb's insights (Hebb, 1949), it is now generally accepted that coordinated neuronal activity forms during learning to represent and store relevant information, serve cognition and ultimately behavioral action. Gamma frequency oscillations ($\sim$30–120 Hz) are thought to reflect the synchronous activity of neurons both within and across cortical fields (Buzsáki & Wang, 2012). The time-scale of gamma oscillations is appropriate for synaptic integration (Salinas & Sejnowski, 2000; Volgushev, Chistiakova, & Singer, 1998) and spike timing dependent plasticity (STDP) (Bi & Poo, 1998; Isaac, Buchanan, Muller, & Mellor, 2009; Wespatat, Tennigkeit, & Singer, 2004). Particularly relevant to the domain of learning and memory, increased gamma activity has been linked to processes such as attention (Börgers, Epstein, & Kopell, 2008) and

short-term memory (Lutzenberger, Ripper, Busse, Birbaumer, & Kaiser, 2002; Pesaran, Pezaris, Sahani, Mitra, & Andersen, 2002). Moreover, the level of gamma activity at the time of encoding can predict the degree of later recall (Fell & Axmacher, 2011; Osipova et al., 2006; Sederberg et al., 2006, 2007). An increase in cortical gamma power also develops during simple associative auditory classical conditioning in humans (Miltner, Braun, Arnold, Witte, & Taub, 1999), underscoring its ubiquity and the potential applicability of appropriate animal models to mechanisms of human learning.

Animal models of associative learning have identified candidate neural substrates for the representation and storage of signal stimuli in the cerebral cortex. For example when a tone is paired with a reinforcer, receptive fields (RF) in the primary auditory cortex (A1) shift to emphasize the frequency of the conditioned stimulus (CS) (Bakin & Weinberger, 1990; Edeline & Weinberger, 1993; Gao & Suga, 2000; Kisley & Gerstein, 2001). Such representational plasticity has the main attributes of associative memory: associativity, specificity, consolidation and long-term retention (reviewed in Weinberger, 2007). Gamma activity may play a critical role in the development of cortically based associative learning. For example, an increase in gamma power within A1 predicts both specific CS-directed cortical plasticity and also behaviorally validated

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learning 24 h later, but only during initial acquisition, not during maintenance of the memory (Headley & Weinberger, 2011; Weinberger, Miasnikov, & Chen, 2006).

Heretofore, it has been assumed tacitly that enhanced gamma activity induced by a signal stimulus during learning reflects the increased salience or behavioral relevance of that stimulus, e.g., the CS in simple associative learning. However, there is an alternative possibility. It is well known that even when perceptual, acquisition and storage processes are functioning optimally, the *content* of the resultant memory can *differ* from the actual experience. Subjects trained identically do not all acquire the exact same content (Bieszczad & Weinberger, 2010a, 2010b, 2012; Ohl, Scheich, & Freeman, 2001; Polley, Steinberg, & Merzenich, 2006). Therefore, the greatest increase in gamma activity may actually reflect the stimulus that has gained the greatest strength through learning, rather than the training stimulus. The relative strength of memory for different stimuli along a sensory dimension cannot be determined during training, but rather depends on obtaining post-training stimulus generalization gradients (Bouton, 2007; Mostofsky, 1965). Discrimination learning (i.e., reinforced CS+ with non-reinforced CS−) is a well-documented example. The peak of the post-training generalization gradient is generally not at the CS+ but is displaced to a stimulus value that is farther away from the CS− (Purtle, 1973). Such "peak shift" has been thought to reflect the summation of an excitatory neural gradient centered on the CS+ and an inhibitory neural gradient centered on the CS− (Spence, 1937).

Recently, we found such shifted generalization peaks in simple associative conditioning, due to pre-training exposure to various tones that induced an inhibitory neural gradient in primary auditory cortex (A1) (Miasnikov & Weinberger, 2012). This disjunction between the training frequency and the peak of the generalization gradient provides a unique opportunity to determine whether enhanced gamma activity during learning is tied to the CS frequency or to the peak of the generalization gradient, i.e., to the tone that is most strongly represented in memory. If increased gamma activity reflects increased neural synchrony that is part of the substrate of auditory frequency memory, then the greatest increase in gamma should be tightly linked to the strongest memory, regardless of the training frequency. We report here the analysis of changes in gamma activity that had been recorded during the previous study. The same changes in gamma were analyzed two ways: based on the CS training frequency and based on the peak of the generalization gradient.

2. Materials and methods

As the present analysis concerns EEG activity obtained in our previous study (Miasnikov & Weinberger, 2012), the materials and methods are mainly the same and will be summarized briefly. All procedures were performed in accordance with the University of California, Irvine, Animal Research Committee and the NIH Animal Welfare guidelines. During training and testing, subjects were continuously monitored by video cameras.

2.1. Subjects and surgery

Sixteen adult male Sprague–Dawley rats (412 ± 28 g, mean ± sd) received an epidural recording electrode (stainless steel screw) into the calvaria over the right primary auditory cortex and screws over the frontal sinus to serve as references, while under general anesthesia. A concentric bipolar stainless steel stimulating electrode was implanted into the right (ipsilateral) caudal nucleus basalis (NB) (ventrolateral internal capsule, ventromedial lateral globus pallidus and nucleus basalis of Meynert), sites of cholinergic

projections to the auditory cortex (Bigl, Woolf, & Butcher, 1982; Moriizumi & Hattori, 1992). Stimulation of the nucleus basalis (NBstm) produced EEG activation: shift from lower frequency, higher voltage waves (e.g., theta and alpha) to higher frequency, lower voltage waves (especially gamma) in all animals (e.g., Celesia & Jasper, 1966; Détári, Rasmusson, & Semba, 1997, 1999; Duque, Balatoni, Détári, & Zaborszky, 2000). Implants were covered with a dental acrylic pedestal containing two aluminum hex threaded standoffs for mounting a thermistor assembly; all leads were connected to a miniature socket that could be led to a commutator. Subjects were allowed 1–2 weeks to recover from surgery.

2.2. Experimental design

To study stimulus-specific implanted memory, we first obtained behavioral baseline responses to many pure tone frequencies, then trained animals with one frequency and 24 h after the end of training, presented again many test frequencies. This protocol yielded pre- and post-training behavioral frequency generalization gradients (FGG). Pre-training gradients were obtained twice (Days 1–2), 200 trials/day total for the nine test frequencies (1.00–27.64 kHz), presented randomly (inter-stimulus intervals [ISI] = 53.8 ± 5.8 s, mean ± se). Day 2 behavioral data were used for the pre-training baseline because they were obtained within 24 h of the start of training. Training was conducted on Days 3–5. Each training trial consisted of a 2.0 s CS tone (3.66 kHz, 70 dB) followed after 1.8 s by NBstm overlapping the last 200 ms of CS presentation (CS−NBstm interval = 1.8 s), 200 trials/day (ISI = 54.5 ± 6.6 s, mean ± se). A post-training gradient was obtained on Day 6 (ISI = 51.3 ± 2.6 s, mean ± se) (Fig. 1A). The effect of pairing the CS with NBstm on memory was determined by subtracting the pre-training FGG on Day 2 from the post-training FGG on Day 6, yielding a difference frequency generalization gradient (ΔFGG) (see Section 2.4). A non-associative control group was not included because all previous studies of NB-induced memory implantation have shown that the effects of pairing tone with NBstm (tone−NBstm) are associative (McLin, Miasnikov, & Weinberger, 2002a; Miasnikov, Chen, Gross, Poytress, & Weinberger, 2008; Miasnikov, Chen, & Weinberger, 2006, 2008, 2011; Weinberger et al., 2006).

2.3. Stimuli

Training and testing took place while subjects rested quietly in an acoustically damped box (23 × 23 × 31 cm) contained in a double-walled acoustic chamber (Industrial Acoustics Co., Bronx, NY). Acoustic stimuli were 9 pure tones, 1.00–27.64 kHz, separated by ∼0.58 octaves (2.0 s duration, cosine 10 ms rise/fall time [10–90%], 70 dB SPL), produced by Tucker−Davis Technologies (TDT, Alachua, FL) System 3 components, delivered via calibrated speakers positioned ∼35 cm above the box floor. NBstm was a 0.2 s train of 100 Hz pulses, pairs of 0.2 ms opposite polarity, 100 μA (S88 stimulator and PSIU6 isolation units, Grass Instrument Co., Quincy, MA). NBstm was subthreshold to affect ongoing or initiate new behavior, as observed in video monitoring.

2.4. Respiration behavior: state control, recording and analysis

To assess the implantation of memory, we measured disruption of the ongoing pattern of regular respiration by all of the tones, before and after training. Respiration was detected as breathing-related thermal fluctuations by a glass-encapsulated thermistor attached to a lightweight pedestal-mounted assembly pre-adjusted in such way that a sensor is positioned in front of a naris. The amplified signal was fed to an ADC module, stored in a computer, and the autocorrelation function (AC) was calculated