Neurobiology of Learning and Memory 95 (2011) 286–295

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Consolidation and long-term retention of an implanted behavioral memory

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ARTICLE INFO

Article history:
Received 22 October 2010
Revised 6 December 2010
Accepted 7 December 2010
Available online 13 December 2010

Keywords:
Acetylcholine
Association
Behavioral state
Nucleus basalis

ABSTRACT

Hypothesized circuitry enabling information storage can be tested by attempting to implant memory directly in the brain in the absence of normal experience. Previously, we found that tone paired with activation of the cholinergic nucleus basalis (NB) does induce behavioral memory that shares cardinal features with natural memory; it is associative, highly specific, rapidly formed, consolidates and shows intermediate retention. Here we determine if implanted memory also exhibits long-term consolidation and retention. Adult male rats were first tested for behavioral responses (disruption of ongoing respiration) to tones (1–15 kHz), yielding pre-training behavioral frequency generalization gradients. They next received 3 days of training with a conditioned stimulus (CS) tone (8.0 kHz, 70 dB, 2 s) either paired ($n = 7$) or unpaired ($n = 6$) with moderate electrical stimulation of the nucleus basalis (~65 μA, 100 Hz, 0.2 s, co-terminating with CS offset). Testing for long-term retention was performed by obtaining post-training behavioral frequency generalization gradients 24 h and 2 weeks after training. At 24 h post-training, the Paired group exhibited specific associative behavioral memory, manifested by larger responses to the CS frequency band than the Unpaired group. This memory was retained 2 weeks post-training. Moreover, 2 weeks later, the specificity and magnitude of memory had become greater, indicating that the implanted memory had undergone consolidation. Overall, the results demonstrate the validity of NB-implanted memory for understanding natural memory and that activation of the cholinergic nucleus basalis is sufficient to form natural associative memory.

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1. Introduction

Several standard approaches are used to investigate neural circuitry that is hypothesized to enable memory storage. These include recording changes in the activity of a putative involved structure consequent to learning, pre or post-training inactivation (permanent or reversible), direct stimulation (electrical or chemical) to facilitate or impair memory and targeting the neural structure by molecular genetics methods. However, if the hypothesized mechanism is sufficient to normally form behavioral memory, then it should be possible to *implant* memory directly into the brain by appropriate activation of that circuitry.

We have used this approach in a series of experiments to test the hypothesis that experience-based activation of the nucleus basalis (NB) (which is the major source of cortical acetylcholine (ACh); Mesulam, Mufson, Levey, & Wainer, 1983), is engaged in the formation of at least some types of behavioral memory. The model under test postulates that activation of the cholinergic nucleus basalis serves as a "final common path" that is sufficient to promote or induce long-term memory based on the formation of information storage in the cerebral cortex, and perhaps elsewhere. In this schema, the nucleus basalis is "downstream" of motivational and emotional systems (Weinberger, 1998; Weinberger et al., 1990, chap. 3). It is derived, in part, from long-standing evidence that the cholinergic system is involved in the formation of memory (Deutsch, 1971; Flood, Landry, & Jarvik, 1981) and memory-related cortical plasticity (Edeline, 2003).

We explicitly distinguish behavioral memory from associative neural plasticity, which was previously known to be induced by tone paired with electrical stimulation of the nucleus basalis (NBstm) (Bakin & Weinberger, 1996; Bjordahl, Dimyan, & Weinberger, 1998; Dimyan & Weinberger, 1999; Kilgard et al., 2001). Unfortunately, such neural plasticity is often erroneously assumed to constitute "memory" rather than a memory substrate. Insofar as memory is validated at the behavioral level, assuming that a neural process is memory constitutes a "category error", i.e., equating a part with the whole (Ryle, 1963).

We emphasize that putative implanted memory must pass the test of having the same attributes as natural memory. Indeed, it should be difficult to determine whether memory-dependent changes in behavior reflect natural or implanted memory based on standard behavioral measures. Another important criterion is that memory-enabling stimulation of the brain not be mnemonically effective simply because it replaces either rewarding or punishing effects of standard reinforcers. Such motivational substitution

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1074-7427/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.nlm.2010.12.004

effects have long been known (e.g., reward, Olds, 1962; nociception, Guilbaud, 1985).

The initial study in this program of research revealed that pairing a tone with NBstm produced associative memory. Furthermore, this implanted memory was specific to the frequency of the conditioned stimulus (CS) (McLin, Miasnikov, & Weinberger, 2002). Subsequent studies revealed that this putative implanted memory shares other cardinal features of natural associative memory, including rapid development, consolidation and retention and that NBstm was motivationally neutral (Weinberger, 2007).

To provide for a comprehensive characterization of implanted memory, the goal of this experiment was to determine if it exhibits a critical feature of natural associative memory, *viz*., long-term retention, herein defined as maintenance of specific memory for 2 weeks. In so doing, it was possible to also determine the consolidation dynamics of NBstm-implanted memory, i.e., the extent to which it changes in specificity and strength without any further training, attributes previously found for auditory cortical specific associative plasticity (Galván & Weinberger, 2002).

2. Materials and methods

The materials and methods were mainly identical to those previously reported (Weinberger, Miasnikov, & Chen, 2009), and will be described only 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

The subjects were 13 adult male Sprague–Dawley rats (396 ± 34 g, mean ± sd), housed individually with *ad libitum* food and water, on a 12/12 h light–dark cycle (lights on at 6:15 am). Following several days of adaptation to the vivarium, they were handled and learned to sit calmly during attachment of a thermistor assembly and a cable to their skull pedestal. Under general anesthesia (sodium pentobarbital, 40 mg/kg, i.p.), a stainless steel epidural screw electrode was inserted over the right primary auditory cortex at the locus showing the largest amplitude evoked potential to a contralateral noise burst. Two screws over the frontal sinus served as reference electrodes. The EEG from the auditory cortex and respiration recordings were used to assess arousal state during training and testing. The EEG was also used to ensure the NBstm-elicited cortical activation, which is an index of the cortical release of acetylcholine during natural behavior and by NBstm (e.g., Celesia & Jasper, 1966; Détári, Rasmusson, & Semba, 1997, 1999; Duque, Balatoni, Détári, & Zaborszky, 2000). A concentric bipolar stainless steel stimulating electrode was implanted either through the contralateral (left) hemisphere or vertically into the right nucleus basalis, aimed at the caudal nucleus basalis (ventrolateral internal capsule, ventromedial lateral globus pallidus and nucleus basalis of Meynert), which are sites of cholinergic projections to the auditory cortex (Bigl, Woolf, & Butcher, 1982; Moriizumi & Hattori, 1992). The effective locus was confirmed by obtaining at least a few seconds of auditory cortical EEG activation to NBstm (pairs of 0.2 ms opposite polarity pulses, 100 Hz, 200 ms trains; S88 stimulator, PSIU6 isolation units, Grass Instrument Co., Quincy, MA). A dental acrylic pedestal was built with two aluminum hex threaded standoffs embedded therein, and all leads connected to a miniature socket that could be led to a commutator via a multi-conductor cable. Subjects were allowed 1–2 weeks to recover from surgery.

2.2. Stimuli, recording and data analyses

Training and testing took place while each subject was in an acoustic-damping box (23 × 23 × 31 cm) supplied with fresh bedding, contained in a double-walled acoustic chamber. Acoustic stimuli were pure tones (1.0–15.0 kHz, 2 s duration, cosine 10 ms rise/fall time [10–90%], 70 dB SPL) produced by Tucker–Davis Technologies (TDT, Alachua, FL) System 3 components and delivered to a calibrated loudspeaker positioned about 35 cm above the floor of the box. NBstm current used during training was ~65 μA, a moderate level that elicited no muscular or behavioral responses but was known to be sufficient to induce specific associative memory (e.g., Weinberger, Miasnikov, & Chen, 2006).

To assess the implantation of memory, we measured disruption of the ongoing pattern of regular respiration to various tones, before and after training. Respiration is a sensitive measure of state and associative learning (see Section 4.2). Respiration was detected as breathing-related thermal fluctuations with a glass-encapsulated thermistor attached to a lightweight pedestal-mounted assembly positioned in front of a naris, as described previously (McLin et al., 2002). Usage of such noninvasive method to measure ventilatory variables without restraining the animals is important because restriction is a potent stressor that strongly affects breathing (Dauger, Nsegbe, Vardon, Gaultier, & Gallego, 1998). The amplified output signal was fed to an ADC module, and the autocorrelation function (AC) was calculated on-line. The AC was used to present tones only when the subject was in a quiescent behavioral state (Miasnikov, Chen, & Weinberger, 2008), thus excluding states such as exploration/grooming and paradoxical (REM) sleep (Fig. 1A, B and D). The pattern of respiration can serve as a reliable marker for each state (Weinberger et al., 2006). Trials meeting the criterion of regular baseline ($0.700 < AC < 0.975$) for over 4 s (Fig. 1C) were presented if the scheduled inter-trial interval period had passed. This state control was employed to avoid giving stimuli when very high levels of ACh were being released in the cortex, as during exploration or REM sleep, or very low, as during slow-wave sleep (Giovannini et al., 2001; Jasper & Tessier, 1971) to prevent ceiling or floor effects, thus promoting a physiologically-effective release of ACh by NBstm.

Major evoked changes in respiration occurred within the first 13 s after tone onset (although certain changes in respiration can be detected at up to 20 s). The collected data were used to calculate a "Respiration Change Index" (RCI), on a second-by-second basis. The index was sensitive to increases and decreases of both frequency and amplitude of respiration response. RCIs were calculated as: $RCI_i = (|Post_i – Pre|)/(Post_i + Pre)$ where Post and Pre were the values of power of respiration signal (McLin et al., 2002). An RCI value of zero would indicate no change and a value of 1.0 would indicate complete cessation of respiration. An example of a record of the tone-elicited disruption of respiration is provided in Fig. 1C2 and its RCI quantification in Fig. 1C3. Statistical analyses used SPSS v. 17 software (SPSS, Chicago, IL).

2.3. Experimental design

The subjects were assigned to two groups, Paired ($n = 7$) and Unpaired ($n = 6$). There was no difference in age ($t_{(11)} = 1.308$, $p > 0.20$, two-tailed t-test; 92 ± 7 days of age, mean ± sd for the population) or weight ($t_{(11)} = 0.027$, $p > 0.95$; 396 ± 34 g) between the Paired and Unpaired groups. After recovery from surgery, NBstm thresholds were determined while subjects were in the state of slow-wave sleep. NBstm was delivered every few minutes at increasing levels starting at ~30 μA (100 Hz bipolar, 200 ms train) until stimulation reliably elicited 3–5 s epoch of cortical activation (decrease in low frequency activity often accompanied by