Neurobiology of Learning and Memory 92 (2009) 335–344

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Metabolic mapping of rat forebrain and midbrain during delay and trace eyeblink conditioning

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ARTICLE INFO

Article history:
Received 1 August 2008
Revised 3 April 2009
Accepted 7 April 2009
Available online 17 April 2009

Keywords:
Hippocampus
2-Deoxyglucose
Frontal cortex
MGN
Auditory cortex
Thalamus

ABSTRACT

While the essential neural circuitry for delay eyeblink conditioning has been largely identified, much of the neural circuitry for trace conditioning has yet to be determined. The major difference between delay and trace conditioning is a time gap between the presentation of the conditioned stimulus (CS) and the unconditioned stimulus (US) during trace conditioning. It is this time gap, which accounts for the additional memory component and may require extra neural structures, including hippocampus and prefrontal cortex. A metabolic marker of energy use, radioactively labeled glucose analog, was used to compare differences in glucose analog uptake between delay, trace, and unpaired experimental groups (rats, Long-Evans), to identify possible new areas of involvement within forebrain and midbrain. Here, we identify increased 2-DG uptake for the delay group compared to the unpaired group in various areas including: the medial geniculate nuclei (MGN), the amygdala, cingulate cortex, auditory cortex, medial dorsal thalamus, and frontal cortices. For the trace group, compared to the unpaired group, there was an increase in 2-DG uptake for the medial orbital frontal cortex and the medial MGN. The trace group also exhibited more increases lateralized to the right hemisphere, opposite to the side of US delivery, in various areas including: CA1, subiculum, presubiculum, perirhinal cortex, ventral and dorsal MGN, and the basolateral and central amygdala. While some of these areas have been identified as important for delay or trace conditioning, some new structures have been identified such as the orbital frontal cortex for both delay and trace groups.

1. Introduction

The delay eyeblink conditioning paradigm and its underlying neural circuitry have been studied extensively (Christian & Thompson, 2003; Thompson, 2005); whereas the neural circuitry for the trace eyeblink conditioning paradigm is less complete. During delay eyeblink conditioning, a conditioned stimulus (CS; e.g., 2 kHz tone) is paired with an unconditioned stimulus (US; e.g., periorbital shock), and the US elicits an unconditioned response (UR), which is eyelid closure. After several pairings of the tone and shock, the CS elicits a conditioned response (CR), where the eyelid closure occurs before the onset of the US.

For trace conditioning there is a time gap between the end of the CS and the beginning of the US, whereas no time gap exists between the CS and US in delay conditioning. This time gap or memory requirement during trace conditioning between the offset of the CS and the onset of the US is the primary difference between delay and trace conditioning paradigms. Here, we used metabolic mapping to examine forebrain and midbrain activity during delay and trace eyeblink conditioning. Results from the cerebellum were reported previously (Plakke, Freeman, & Poremba, 2007).

While the essential circuitry for delay eyeblink conditioning is mostly established and centered within the cerebellum and brainstem nuclei (for review see Christian & Thompson, 2003; Thompson, 2005), components of the auditory CS pathway have recently been added to the circuit including the medial geniculate nucleus and inferior colliculus (Freeman, Halverson, & Hubbard, 2007; Halverson & Freeman, 2006; Halverson, Poremba, & Freeman, 2008). In addition, other areas within the forebrain demonstrate modulation or illustrate parallel neurophysiological activity during delay conditioning including the hippocampus and amygdala (Berger, Alger, & Thompson, 1976; Blankenship, Huckfeldt, Steinmetz, & Steinmetz, 2005; Green & Arenos, 2007; Lee & Kim, 2004). While not crucial for simple delay conditioning, brain areas such as the hippocampus may play a role in more complex tasks such as during long delay conditioning (Beylin et al., 2001).

In trace conditioning, other structures may be necessary such as the hippocampus, medial prefrontal cortex, and anterior cingulate cortex (Kim, Clark, & Thompson, 1995; Kronforst-Collins & Disterhoft, 1998; McLaughlin, Skaggs, Churchwell, & Powell, 2002; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, &

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doi:10.1016/j.nlm.2009.04.001

Weisz, 1986; Takehara, Kawahara, & Kirino, 2003; Weible, McEchron, & Disterhoft, 2000; Weiss, Bouwmeester, Power, & Disterhoft, 1999). It is possible these other forebrain regions are needed due to the memory component that is embedded within trace conditioning, which is not inherent in delay conditioning. This memory trace may add another level of difficulty to the behavioral task and require additional processing by other neural structures (McLaughlin et al., 2002).

Here, we used metabolic mapping to compare neural circuitry for delay and trace eyeblink conditioning. A marker of metabolic activity [C^{14}] 2-deoxyglucose (2-DG), was used to compare glucose analog uptake across delay, trace, and unpaired groups of male rats. This glucose analog, is transported into cerebral tissue, including neurons and glial cells, and phosphorylated by hexokinase but, unlike glucose, is trapped within the cells (Sokoloff et al., 1977). Limitations of this method include not being able to distinguish between excitatory and inhibitory processes as both are energy consuming, and it does not allow differentiation between direct and indirect effects of a given stimulus. However, an entire pathway may be activated and this is an advantage when the goal is to identify the neural circuitry that mediates a specific behavioral response or in this experiment where direct comparisons are made in many brain regions between different types of behavioral training. In this experiment, the primary experimental parameter that differs between the delay and trace groups is whether or not there is a time gap between the offset of the CS and onset of the US. These groups are compared to an unpaired group given explicitly unpaired CS and US stimulus presentations. By using a metabolic marker throughout the forebrain and midbrain we may identify new areas of involvement for both delay and trace conditioning.

2. Methods

2.1. Subjects

Twenty male Long Evans rats weighing 200–250 g were used for this study. All rats were housed in Spence Laboratories at the University of Iowa and kept on a 12-h light/dark cycle. The rats were fed standard rat chow ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Iowa. The rats were divided into three conditioning groups: delay, trace, and unpaired.

2.2. Surgery

For surgery, each rat was anesthetized and fitted with differential electromyogram (EMG) electrodes implanted in the left upper eyelid muscle (orbicularis oculi). A ground electrode was attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins held in a plastic connector, which was secured to the skull with dental acrylic. A bipolar stimulating electrode (for delivering the shock US) was implanted subdermally, immediately caudal to the left eye. The bipolar electrode terminated in a plastic connector that was secured to the skull with dental acrylic. Both sets of connectors for the EMG and bipolar electrode were connected to a lightweight cable that allowed the rats to move freely during conditioning. The rats were allowed to recover for 7–10 days before training began.

2.3. Conditioning apparatus

Rats from each group were trained in one of four sound attenuating chambers (BRS/LVE, Laurel, MD). These small animal sound attenuating chambers surrounded a small-animal operant chamber where the rats were kept during conditioning (BRS/LVE, Laurel, MD). One wall of the operant chamber was fitted with two speakers. The back wall of the sound attenuating chamber was equipped with a small house light and exhaust fan. The electrode leads from the rat's headstage were connected to peripheral equipment and a desktop computer. Computer software controlled the delivery of stimuli and the recording of eyelid EMG activity (JSA Designs, Raleigh, NC). The shock stimulus (1–2 mA, DC constant current) was delivered through a stimulus isolator (Model number 365A, World Precision Instruments, Sarasota FL). EMG activity was recorded differentially, filtered (500–5000 Hz), amplified (2000×), and integrated by equipment (JSA Designs, Raleigh, NC) described in other reports (Halverson & Freeman, 2006).

2.4. Behavioral paradigm

2.4.1. Training groups

Rats were given delay, trace, or unpaired conditioning. The conditioned stimulus (CS) was a tone (2 kHz, 85 dB, 250 ms) paired with a unilateral, left side, periorbital shock unconditioned stimulus (US, 25 ms). For the delay group ($n = 7$), the CS terminated with the onset of the US, yielding an interstimulus interval (ISI) of 250 ms. For the trace group ($n = 6$), the CS offset was separated by 250 ms from the US onset, resulting in a 500 ms ISI. For both groups given paired conditioning there was a variable intertrial interval with an average length of 30 s. The unpaired group ($n = 7$) received the CS and US in an explicitly unpaired fashion so that the CS and US never overlapped in a predictive manner, and the variable intertrial interval had a length of 15 s equating the total length of each unpaired training session to the training sessions for the delay and trace groups.

2.4.2. Training procedure

For delay and trace conditions, training consisted of 10 blocks of 10 trials for a total of 100 trials per session per day. Each block consisted of nine pairings of the CS and US and one CS alone presented as the 10th test trial in each block. CRs for all groups were defined as responses that crossed a threshold of 0.4 V above baseline during the CS period after 80 ms.

Rats in all groups were trained for 4 days. Before training each day, the rats were habituated to a small containment box, where the tail was restrained. This procedure readied the rats for the injection that occurred on the last day. On the 5th and final day, rats were weighed and then given an i.v. tail injection of (20 μCi/100 g) body weight, glucose analog, 2-deoxyglucose (2-DG) radiolabeled with ^{14}C (American Radiolabeled Chemicals, St. Louis) in 0.3 ml sterile (0.9%) saline (Breier, Crane, Kennedy, & Sokoloff, 1993). Experimental rats were then run in their final training session. The computer program was altered to allow the final session to run continuously for 2 h (rather than the typical 1 h session) so that each rat could be run separately. The rats were only allowed to experience 80 trials, or about 40 min of training compared to their normal 100 daily training trials to optimize uptake of the 2-DG, which remained trapped in the cells, for the 45 min post injection period after which the rats were decapitated. The brains were extracted in less than 6 min and frozen in hexane cooled to −37 °C and then stored at −80 °C until sectioned.

2.4.3. Histology

Before sectioning, brains were removed from the freezer and allowed to equilibrate for 10 min. The brain was cut just anterior to the cerebellum so that the cerebellum could be sliced horizontally. Results from the cerebellum were previously reported (Plakke et al., 2007). The tissue anterior to the cerebellum was sectioned in the coronal plane at 40 μ in a series of seven with four sections saved for 2-DG analyses, one saved for Nissl staining and two saved for other staining. Tissue mounted for 2-DG was exposed to ^{14}C