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MEDIAL PREFRONTAL CORTEX CIRCUIT FUNCTION DURING RETRIEVAL AND EXTINCTION OF ASSOCIATIVE LEARNING UNDER ANESTHESIA

G. E. FENTON,[a] D. M. HALLIDAY,[b] R. MASON[c] AND C. W. STEVENSON[a]*

[a] *School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK*

[b] *Department of Electronics, University of York, Heslington, York YO10 5DD, UK*

[c] *School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK*

Abstract—Associative learning is encoded under anesthesia and involves the medial prefrontal cortex (mPFC). Neuronal activity in mPFC increases in response to a conditioned stimulus (CS+) previously paired with an unconditioned stimulus (US) but not during presentation of an unpaired stimulus (CS−) in anesthetized animals. Studies in conscious animals have shown dissociable roles for different mPFC subregions in mediating various memory processes, with the prelimbic (PL) and infralimbic (IL) cortex involved in the retrieval and extinction of conditioned responding, respectively. Therefore PL and IL may also play different roles in mediating the retrieval and extinction of discrimination learning under anesthesia. Here we used *in vivo* electrophysiology to examine unit and local field potential (LFP) activity in PL and IL before and after auditory discrimination learning and during later retrieval and extinction testing in anesthetized rats. Animals received repeated presentations of two distinct sounds, one of which was paired with footshock (US). In separate control experiments animals received footshocks without sounds. After discrimination learning the paired (CS+) and unpaired (CS−) sounds were repeatedly presented alone. We found increased unit firing and LFP power in PL and, to a lesser extent, IL after discrimination learning but not after footshocks alone. After discrimination learning, unit firing and LFP power increased in PL and IL in response to presentation of the first CS+, compared to the first CS−. However, PL and IL activity increased during the last CS− presentation, such that activity during presentation of the last CS+ and CS− did not differ. These results confirm previous findings and extend them by showing that increased PL and IL activity result from encoding of the CS+/US association rather than US presentation. They also suggest that extinction may occur under anesthesia and might be represented at the neural level in PL and IL.

Key words: prelimbic, infralimbic, discrimination learning, extinction, retrieval, *in vivo* electrophysiology.

INTRODUCTION

In certain circumstances associative learning occurs under general anesthesia. Undergoing fear learning while anesthetized can result in learned fear expression after recovery from anesthesia if epinephrine is given during learning (Weinberger et al., 1984; Gold et al., 1985). The neural mechanisms that mediate associative learning under anesthesia have begun to be elucidated. During olfactory discrimination learning in anesthetized rats, the lateral amygdala shows increased neuronal excitability in response to an odor (conditioned stimulus; CS+) previously paired with footshock (unconditioned stimulus; US), but not to another odor (CS−) presented without the US (Rosenkranz and Grace, 2002; Rosenkranz et al., 2003). We have recently shown similar results in the basolateral amygdala (BLA) during auditory discrimination learning under anesthesia, where BLA activity increases in response to CS+, but not CS−, presentation after learning (Fenton et al., 2013). These findings are comparable to changes in LA and BLA activity during discriminative fear learning (Maren et al., 1991; Collins and Paré, 2000; Herry et al., 2008).

Activity in the medial prefrontal cortex (mPFC) also increases selectively during CS+ presentation after olfactory discrimination learning under anesthesia (Laviolette et al., 2005; Laviolette and Grace, 2006). This agrees with findings from similar studies showing a role for mPFC in discriminative fear learning. Neural activity in mPFC is increased during CS+, compared to CS−, presentation after successful discriminative fear learning (Likhtik et al., 2014). Temporary mPFC inactivation before testing the retention of discriminative fear learning impairs CS+/CS− discrimination (Lee and Choi, 2012). The mPFC is a heterogeneous area comprising the prelimbic (PL) and infralimbic (IL) cortex. Fear learning studies in conscious animals have shown

*Corresponding author. Tel: +44-115-95-16055; fax: +44-115-95-16302.

E-mail addresses: stxgf@exmail.nottingham.ac.uk (G. E. Fenton), david.halliday@york.ac.uk (D. M. Halliday), rob.mason@nottingham.ac.uk (R. Mason), carl.stevenson@nottingham.ac.uk (C. W. Stevenson).

Abbreviations: ANOVA, analysis of variance; BLA, basolateral amygdala; CS−, unpaired stimulus; CS+, conditioned stimulus; HSD, Honestly Significant Difference; IL, infralimbic; LFP, local field potential; mPFC, medial prefrontal cortex; PL, prelimbic; US, unconditioned stimulus.

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dissociable roles for PL and IL in mediating different memory processes. While PL is involved in the retrieval or expression of conditioned responses, the suppression and extinction of conditioned responding involve IL (Vidal-Gonzalez et al., 2006; Sierra-Mercado et al., 2011; Fenton et al., 2014). Thus PL and IL may play different roles in memory processing related to discrimination learning. Moreover, these mPFC subregions share reciprocal connections that are functionally relevant, raising the possibility that PL–IL synchrony is also involved in discrimination learning (Jones et al., 2005; Hoover and Vertes, 2007; van Aerde et al., 2008; Ji and Neugebauer, 2012; Zelikowsky et al., 2013).

Here we examined PL and IL activity using a modified version of the auditory discrimination learning paradigm conducted under anesthesia that we have recently described (Fenton et al., 2013). We examined activity before and after learning given that increased mPFC activity during and after fear learning in awake animals may play a role in memory consolidation (Popa et al., 2010; Tan et al., 2011). In separate control experiments we examined activity before and after US presentations alone to further address this issue. Given the recent finding that fear extinction occurs during altered states of consciousness (Hauner et al., 2013), we also repeatedly presented the CS+ and CS− alone after learning in an attempt to examine activity during both retrieval and extinction in this discrimination learning paradigm. Assessing activity in PL and IL concurrently also allowed for the examination of functional connectivity within mPFC circuitry during these memory processes while under anesthesia.

EXPERIMENTAL PROCEDURES

Animals

All experimental protocols were performed in accordance with the Animals (Scientific Procedures) Act 1986, UK, and internal ethical approval. Male Lister hooded rats (250–350 g; Charles River, UK) were group housed on a 12-h light/dark cycle (lights on at 0700) and had free access to food and water. Every effort was made to minimize the number, and suffering, of the animals used.

Surgery

Anesthesia was induced under 3.5% isoflurane (IVAX Pharmaceuticals, UK) in medical air. Anesthesia was gradually reduced to and maintained at ~2.0% throughout the experimental protocol, ensuring complete lack of the hindpaw withdrawal reflex. Body temperature was maintained at ~37 °C using a homeothermic heating blanket (Harvard Apparatus Ltd., UK). Rats were placed in a stereotaxic frame with customized hollowed ear bars connected to earphones. An incision was made in the scalp, and the skull and dura over mPFC were removed. An eight-wire micro-electrode bundle (Teflon-coated stainless steel wire, 50-μm diameter/wire; NB Labs, TX) was lowered into right mPFC. The electrodes were 'staggered' such that four wires were 1 mm longer than the other four, allowing for simultaneous recordings from PL and IL (2.7 mm anterior, 0.5 mm lateral to bregma; 3.3 (PL) and 4.3 (IL) mm ventral to the brain surface; (Paxinos and Watson, 1997)). The electrode was allowed to settle for 1 h before recordings began. Two 25-gauge needles connected to an electrical stimulator (Neurolog system, Digitimer Ltd., UK) were also inserted into the ventral surface of the left hindpaw, contralateral to the recording site.

Recording procedure

The recording protocol has been described in detail previously (Stevenson et al., 2007, 2008). The electrode was connected to a preamplifier via a headstage. Units and local field potentials (LFPs) were linked to a PC via a Plexon multichannel acquisition processor (Plexon Inc., TX) and filtered (units: gain 1000x, bandpass filtered at 0.25–8 kHz; LFPs: bandpass filtered at 0.7–170 Hz, digitized at 1 kHz). This provided simultaneous 40-kHz A/D conversion on each channel at 12-bit resolution. Unit activity was monitored visually and aurally using a 507 analog–digital oscilloscope (Hameg Instruments, Germany) and a speaker, respectively.

Auditory discrimination learning paradigm

The paradigm used was adapted from our previously described auditory discriminative learning protocol (Fenton et al., 2013). Basal activity was recorded for 3 min. During learning, rats were presented with a sound (CS+) for 10 s paired with a footshock (US; 5 mA, 20 Hz, 0.5-ms pulse duration) of 5 s duration that co-terminated with the CS+. A second sound (CS−) was presented 60 s later for 10 s in the absence of footshock. The CS+/US pairings and CS− presentations were repeated four times. The two sounds (3-kHz tone or white noise, 90 dB each) were counterbalanced between the CS+ and CS− between animals. Presentations of sound and footshock were automatically controlled (Cool Edit 96, Syntrillium Software Co., AZ). After 3 min, rats were presented with 12 CS+ and 12 CS− presentations as above except that footshocks were not given (Fig. 1A). In separate control experiments, rats received four footshocks alone and activity was recorded for 3 min afterward.

Histology

At the end of each experiment rats were culled by isoflurane overdose. A current (0.1 mA) was briefly passed through a pair of electrodes in PL and IL, depositing ferric ions at the electrode tips. Brains were removed and stored in a solution of 4% paraformaldehyde/4% potassium hexacyanoferrate (Sigma, UK), marking the recording sites by the Prussian blue reaction. Electrode placements were later confirmed by obtaining mPFC sections of 200-μm thickness (Fig. 1B, C).