Neuroscience 233 (2013) 146–156

BASOLATERAL AMYGDALA ACTIVITY DURING THE RETRIEVAL OF ASSOCIATIVE LEARNING UNDER ANESTHESIA

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

[a] *School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE15 5RD, UK*

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

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

Abstract—Associative learning can occur under anesthesia and its neural correlates have begun to be elucidated. During discrimination learning under anesthesia in rats, lateral amygdala excitability increases in response to a conditioned stimulus (CS+) previously paired with electrical stimulation of the paw but not to another stimulus presented alone (CS−). Similarly, medial prefrontal cortex activity increases selectively during CS+ presentation after discrimination learning but this occurs only in neurons receiving input from the basolateral amygdala (BLA), the main source of amygdaloid projections to this region. However, BLA activity during discrimination learning under anesthesia has not been investigated. Here we used *in vivo* electrophysiology to examine BLA activity before and after associative learning and during later memory retrieval in anesthetized rats. We examined extracellular unit and local field potential (LFP) activity using an auditory discrimination learning paradigm. Rats were repeatedly presented with two distinct sounds, one of which was paired with electrical stimulation of the paw. One hour later, the paired sound (CS+) was presented alone along with the sound not paired with electrical stimulation (CS−). We found increased unit firing late (1 h) but not early (5 min) after learning. LFP power was increased both early and late after learning. In control experiments we also found increased unit and LFP activity late after electrical stimulation alone. After discrimination learning, unit firing increased in response to CS+, but not CS−, presentation. LFP power also showed a modest increase during CS+, compared to CS−, presentation. These findings suggest that discrimination learning under anesthesia can occur at the neural level in BLA. The potential relevance of these results is discussed in relation to previous studies examining neural activity during fear learning and memory processing in conscious animals.

Key words: basolateral amygdala, consolidation, discrimination learning, memory, sensitization, fear conditioning.

INTRODUCTION

Associative learning has been shown to occur under general anesthesia under certain conditions. Animals subjected to auditory fear conditioning under anesthesia show conditioned fear responding during later retention testing while conscious if epinephrine is administered at the time of conditioning (Weinberger et al., 1984; Gold et al., 1985). More recent studies have examined the neural basis of associative learning in anesthetized animals. Neurons in the lateral nucleus of the amygdala (LA) exhibit synaptic plasticity during olfactory discrimination learning under anesthesia. Neuronal excitability in LA increases during repeated presentation of an odor (conditioned stimulus; CS+) paired with electrical stimulation of the paw and during CS+ alone presentation after conditioning. In contrast, repeatedly presenting another odor (CS−) without electrical stimulation decreases the excitability of LA neurons (Rosenkranz and Grace, 2002; Rosenkranz et al., 2003). Interestingly, this is broadly similar to what occurs during discriminative fear learning in conscious animals (Collins and Paré, 2000), in keeping with the known role of LA in mediating the association between conditioned and unconditioned stimuli during fear conditioning (Johansen et al., 2011).

Medial prefrontal cortex (mPFC) activity also increases in a subset of neurons during CS+, but not CS−, presentation after olfactory discrimination learning under anesthesia (Laviolette et al., 2005; Laviolette and Grace, 2006). Again, this is congruent with findings from studies in conscious animals showing a role for mPFC in mediating discriminative fear learning (Lauzon et al., 2009, 2012). Importantly, increased mPFC activity elicited by the CS+ only occurs in neurons that receive functional input from the basolateral nucleus of the amygdala (BLA), the primary source of amygdala afferents to mPFC (McDonald, 1991; McDonald et al., 1996). Moreover, the increase in activity in these mPFC neurons in response to the CS+ is inhibited by BLA inactivation, suggesting that BLA is involved in mediating discrimination learning under anesthesia.

*Corresponding author. Address: School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK. Tel: +44-115-95-16055; fax: +44-115-95-16302.
E-mail addresses: stxgf@exmail.nottingham.ac.uk (G. E. Fenton), clare.spicer@nottingham.ac.uk (C. H. Spicer), dh20@ohm.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+, conditioned stimulus; LA, lateral amygdala; LFP, local field potential; mPFC, medial prefrontal cortex; Post-E, early after learning; Post-L, late after learning; Stim-A, electrical stimulation alone.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2012.12.039

Although BLA activity during discriminative fear learning in conscious animals has been examined (Maren et al., 1991; Herry et al., 2008), comparable studies have not yet been conducted in anesthetized animals.

In this study we examined BLA activity using a discrimination learning paradigm conducted under anesthesia that was adapted from the olfactory conditioning procedure described above. We modified this procedure in an attempt to make certain parameters more directly relevant to those typically used during fear learning experiments in conscious animals. We used auditory stimuli given that most cued fear conditioning studies are conducted using a sound as the CS+. A duration of 1 h was used between discrimination learning and later CS+ and CS− presentation to examine if any lasting increase in BLA activity is observed after learning. This occurs after fear learning in conscious animals and is thought to be involved in memory consolidation (Pelletier et al., 2005; Popa et al., 2010). To further address this issue we also conducted separate control experiments examining the effects of electrical stimulation alone on later BLA activity. Finally, we examined local field potential (LFP) oscillations and action potential firing pattern and synchrony given the important roles of neural population rhythms and temporal coding in mediating short-term plasticity (Ainsworth et al., 2012). For example, theta and gamma oscillations and synchronized action potential firing in BLA may facilitate memory consolidation by promoting synaptic plasticity locally and in downstream projection areas (Pelletier et al., 2005; Bauer et al., 2007; Popescu et al., 2009; Popa et al., 2010). Similarly, burst firing may also enhance local synaptic transmission by mediating selective communication between BLA neurons (Paré et al., 1995; Krahe and Gabbiani, 2004).

EXPERIMENTAL PROCEDURES

Animals

Experiments were conducted in male Lister hooded rats (Charles River, UK) weighing 250–400 g. Animals were group housed on a 12-h light/dark cycle (lights on at 0700 h) with free access to food and water. Experimental procedures were conducted with internal ethical approval and in accordance with the Animals (Scientific Procedures) Act 1986, UK. Every effort was made to minimize the number of animals used and the suffering of the animals.

Surgery

Anesthesia was induced with 3.5% isoflurane (IVAX Pharmaceuticals, UK) in medical air and maintained ~2.0% throughout surgery to ensure complete inhibition of the hindpaw withdrawal reflex. Body temperature was maintained at ~37°C using a homeothermic heating pad (Harvard Apparatus Ltd., UK). The animal was placed in a stereotaxic frame using custom-made hollow ear bars connected to earphones. A scalp incision was made and the skull over the right amygdala was removed. The dura mater was excised and an eight-wire microelectrode array (Teflon-coated stainless steel wires, 50 µm diameter/wire; NB Labs, TX, USA) was lowered into the amygdala, with four or eight microwires aimed at BLA (3.0 mm posterior and 4.7 mm lateral to bregma, 7.6 mm ventral to the surface of the brain Paxinos and Watson, 1997). Two 25-gauge needles connected to an electrical stimulator (Neurolog System, Digitimer Ltd., UK) were also inserted into the footpad of the left hindpaw (i.e. contralateral to the recording site).

Recording procedure

The recording procedure used has been described in detail elsewhere (Stevenson et al., 2007, 2008a,b). The electrode array was connected via a headstage to a preamplifier. Extracellular action potential unit spikes and LFPs (units: gain 1000×, band-pass filtered at 250 Hz–8 kHz; LFPs: band-pass filtered at 0.7–170 Hz, digitized at 1 kHz) were linked to a Plexon multichannel acquisition processor (Plexon Inc., TX, USA) connected to a PC. This provided simultaneous 40 kHz A/D conversion on each channel at 12-bit resolution. Unit activity was displayed on a 507 analog–digital oscilloscope (Hameg Instruments, Germany) and monitored aurally through a speaker.

Auditory discrimination learning paradigm

We adapted a previously described olfactory conditioning procedure used in anesthetized rats (Rosenkranz and Grace, 2002; Rosenkranz et al., 2003; Laviolette et al., 2005; Laviolette and Grace, 2006) for use with auditory stimuli (Fig 1A). Two sounds (3 kHz tone and white noise; 90 dB each) were each presented four times. One sound was chosen at random to be paired with electrical stimulation of the hindpaw (counter-balanced between animals). Pairing consisted of presenting the sound (CS+) for 10 s together with electrical stimulation for 5 s (5 mA, 20 Hz, 0.5 ms pulse duration), such that the CS+ and stimulation co-terminated. This was followed 60 s later by presenting the non-paired sound (CS−) for 10 s. Sound presentation and electrical stimulation were computer controlled (Cool Edit 96, Syntrillium Software Co, AZ, USA). After 60 min, the animals were presented with the CS+ and CS− (one each) as above except that electrical stimulation was omitted. In separate control experiments some animals were subjected to electrical stimulation in the absence of auditory stimuli and neuronal activity was recorded afterward for 60 min. All recording sessions lasted ~75 min.

Histology

At the end of each experiment the animal was deeply anesthetized (5% isoflurane) and current (0.1 mA) was passed for 5–7 s through a pair of microwires to deposit ferric ions at the electrode array tip. The animal was then culled and its brain was removed and placed in a solution of 4% paraformaldehyde/4% potassium ferrocyanide. This allowed for the marking of the recording sites using the Prussian blue reaction. BLA sections were later obtained and stained for acetylcholinesterase (Fig. 1C) as previously described (Stevenson et al., 2007, 2008a).

Unit sorting

The parameters used have been described in detail elsewhere (Stevenson et al., 2007, 2008a,b). Unit discrimination was achieved with Off-Line Sorter (Plexon Inc.) using automatic and manual sorting techniques (Fig. 1D). Principal component analysis was used to display the waveforms recorded from each electrode in three-dimensional space (Fig. 1E). Each electrode was checked for noise artefacts which were removed