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Method of euthanasia affects amygdala plasticity in horizontal brain slices from mice

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ABSTRACT

An important consideration in any terminal experiment is the method used for euthanizing animals. Although the prime consideration is that the method is humane, some methods can have a dramatic impact on experimental outcomes. The standard inhalant anesthetic for experiments in brain slices is isoflurane, which replaced the flammable ethers used in the pioneer days of surgery. To our knowledge, there are no data available evaluating the effects of the method of euthanasia on plasticity changes in brain slices. Here, we compare the magnitude of long-term potentiation (LTP) and long-term depression (LTD) in the lateral nucleus of the amygdala (LA) after euthanasia following either ether or isoflurane anesthesia, as well as in mice decapitated without anesthesia. We found no differences in input–output curves using different methods of euthanasia. The LTP magnitude did not differ between ether and normal isoflurane anesthesia. After deep isoflurane anesthesia LTP induced by high frequency stimulation of cortical or intranuclear afferents was significantly reduced compared to ether anesthesia. In contrast to ether anesthesia and decapitation without anesthesia, the low frequency stimulation of cortical afferents induced a reliable LA-LTD after deep isoflurane anesthesia. Low frequency stimulation of intranuclear afferents only caused LTD after pretreatment with ether anesthesia. The results demonstrate that the method of euthanasia can influence brain plasticity for hours at least in the interface chamber. Therefore, the method of euthanasia is an important consideration when brain plasticity will be evaluated.

1. Introduction

Anesthetic and sedative agents exert their effects by altering brain function. Rapidly acting anesthetics are usually characterized by low water and high lipid solubility; as a result, following cessation of circulation, washout of a given anesthetic is slow. Recently we found that capsaicin, through activation of the transient receptor potential vanilloid type 1 (TRPV1) receptor, induces suppression of long-term potentiation (LTP) in the lateral nucleus of the amygdala (LA) when using ether anesthesia before decapitation (Zschenderlein et al., 2011). Ether was used as it was recently shown that isoflurane causes a sensitization of TRPV1 (Cornett et al., 2008; Harrison and Nau, 2008; Matta et al., 2008). In accordance with these results, capsaicin caused an enhancement of LTP when deep isoflurane anesthesia (8%) was given before decapitation (Zschenderlein et al., 2011), whereas, 4% isoflurane did not provoke a sensitization (unpublished results).

Ether can form an explosive mixture with oxygen; therefore, its use in an open system can be dangerous. Isoflurane and diethyl ether are irritants to mucous membranes and cause copious secretions, which tend to obstruct the airway. The behavior studies on rats and mice, together with safety aspects and time to collapse, confirm that isoflurane is the preferred anesthetic for euthanasia to obtain brain slices. One reason decapitation without anesthesia is chosen over other methods is that anesthetics act on different molecular receptors and alter brain metabolism parameters. It is currently being debated whether decapitation is a humane method of euthanasia in awake animals (van Rijn et al., 2011).

Long-lasting changes in the synaptic efficacy of signaling between neurons in the central nervous system are thought to be involved in memory consolidation and recall. General anesthetics are thought to affect LTP, a model of learning and memory, since clinically relevant concentrations of volatile anesthetics seem to modify ligand-gated ion channels such as glutamate receptors and gamma-aminobutyric acid type A ($GABA_A$) receptors. $GABA_A$ receptors, the major inhibitory receptors in the brain, are important targets of many drugs, including diethyl ether (Suzuki and Smith, 1988; Lin et al., 1992; Raines et al., 2003) and isoflurane (Simon

Abbreviations: ACSF, artificial cerebral spinal fluid; EC, external capsule; EPSPs, excitatory postsynaptic potentials; $GABA_A$, gamma-aminobutyric acid type A; HFS, high-frequency stimulation; IN, intranuclear; I/O, input/output; LA, lateral nucleus of the amygdala; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; TRPV1, transient receptor potential vanilloid type 1.

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et al., 2001; Grasshoff et al., 2005). However, it is important to consider that the stress of handling during the decapitation procedure may also modulate metabolite levels. Many publications describe the effects of clinically relevant concentrations of isoflurane on different ligand-gated ion channels; yet, to our knowledge, our results offer the first indication that molecular events induced by short anesthesia before decapitation provoke long-lasting changes in the plasticity of neurons and that isoflurane is maintained for a prolonged period in slices. To compare different methods of euthanasia, extracellular recordings were made from each animal group after euthanasia by decapitation with or without ether or isoflurane anesthesia.

2. Methods

2.1. Animals

Eight to 12-week-old male C57BL/6J mice were used. Four mice per cage were kept under standardized conditions with an artificial 12-h dark–light cycle and a room temperature of 22 °C. The mice had free access to food and water. All of the experimental protocols were approved by government authorities (Landesamt für Gesundheit und Soziales Berlin ID: T0344/05), performed according to the German Animal Welfare Act of May 25th, 1998, and conformed to the European Communities Council Directive of November 24th, 1986 (86/609/EEC). All efforts were made to minimize suffering.

2.2. Preparation and recording

Detailed methods for slice preparation and positioning of the electrodes have been previously described (Drephal et al., 2006; Muller et al., 2009) and are provided here in brief. The mice were decapitated without anesthesia or anesthetized with diethyl ether or isoflurane and decapitated. The brains were removed quickly and placed in ice-cold artificial cerebral spinal fluid (ACSF) (NaCl: 129 mM; KCl: 3 mM; $NaHCO_3$: 21 mM; Na_2HPO_4: 1.25 mM; $MgSO_4$: 1.8 mM; $CaCl_2$: 1.6 mM; glucose: 10 mM). Hemisected horizontal slices (400 μm) were prepared with a vibroslicer (Campden Instruments, Silbey, UK). The appropriate slices were placed in an interface chamber and allowed to equilibrate for 120 min at 35 °C. They were superfused continuously with ACSF (1.5 mL/min). The pH was maintained at 7.4 by oxygenating and carbogenating the solution with 95% O_2 and 5% CO_2.

Glass microelectrodes (Science Products, Hofheim, Germany) were filled with ACSF (tip resistances 1 MΩ) and placed in the caudoventral part of the LA to record field potentials. Bipolar stimulation electrodes were used to stimulate afferents running through the external capsule (EC) or located within the LA (intranuclear stimulation, IN). Single stimuli (duration 100 μs) were presented every 10 s. Signals of the evoked responses were amplified and filtered (bandpass: 0.1 Hz–3 kHz) by a pre-amplifier (World Precision Instruments, Sarasota, FL, USA), displayed on a storage oscilloscope, and fed via a CED laboratory interface (Cambridge Electronic Design, UK) to a computer disk.

2.3. Stimulation parameters

An input/output (I/O) response curve was constructed by varying the intensity of single pulse stimulation and averaging six responses per intensity. The stimulus intensity that evoked a mean field potential equal to 50% of the maximum response was then used for all subsequent stimulations. After determination of I/O curves, single stimuli (duration of 100 μs) were presented every 10 s. Once a stable baseline of responses was obtained for at least 20 min, we delivered high-frequency stimulation (HFS) as two trains at 100 Hz (duration: 1 s, 30 s apart) or low frequency stimulation (LFS; 900 pulses, 15 min). Subsequent responses to single stimuli were recorded for at least 60 min, and their amplitude quantified as the percent change with respect to baseline.

2.4. Data analysis

Data were collected and averaged using Signal 2 software (Cambridge Electronic Design, UK). The amplitude of field potentials evoked by stimulation of EC or IN fibers was observed on-line, but later re-analyzed off-line. We defined the field potential amplitude as the absolute voltage of a vertical line running from the minimum point of the field potential to its intersection with a line running tangent to the points of field potential onset and offset. It is assumed that the recorded negative wave reflects a summation of both excitatory postsynaptic potentials (EPSPs) and synchronized action potentials (population spike component) (Watanabe et al., 1995; Doyere et al., 2003). Therefore, we analyzed the amplitude of field potentials in the present study. Additionally, our analysis of the slope measured in the lateral amygdala showed that the slope is more sensitive to variability and signal noise, making it more difficult to analyze in agreement to the in vivo results of Doyere et al. (2003). The amygdala lacks an elongated structural organization compared to other brain regions (Faber et al., 2001; McDonald, 1984; Pitkanen et al., 2003; Swanson and Petrovich, 1998) and is therefore not subject to anisotropic conductance (Johnston and Wu, 1995; Logothetis and Wandell, 2004). As a result the field potential response in the LA is not solely dependent upon underlying dendrite alignment, allowing synaptic activity to potentially contribute to the response (Johnson et al., 2008).

The significance of differences between groups (n = number of slices) was calculated by ANOVA (Software GraphPad Prism 5). $p < 0.05$ was considered significant. To express and compare changes in field potential amplitude between the animal groups, we averaged responses from 59 to 60 min period after HFS/LFS.

3. Results

To induce LA-LTP we stimulated either external capsule fibers (EC), which mainly contain afferents from the entorhinal and perirhinal cortex (von Bohlen und Halbach and Albrecht, 2002), or intranuclear fibers (IN), which also involve afferents from other amygdaloid nuclei (von Bohlen und Halbach and Albrecht, 1998). Stimuli applied to the EC or IN evoked characteristic field potentials in the LA (see Fig. 1). In agreement with previous results (Drephal et al., 2006; Muller et al., 2009), high frequency stimulation (HFS) to EC or IN induced a robust increase in the LA field potentials in the group anesthetized by ether (EC: 150.7 ± 5.6% [n = 11] vs. IN: 148.1 ± 6.8% [n = 9]; Fig. 1A and B). As also shown in rats, the magnitude of LA-LTP did not depend on the input used; although, EC-induced LA-LTP is also dependent on activation of L-type calcium channels in addition to NMDA glutamate receptors (Drephal et al., 2006). We found similar results in mice anesthetized with 4% isoflurane before decapitation (EC: 158.1 ± 8.8% [n = 9] vs. IN: 155.4 ± 9.2% [n = 9]; Fig. 1C and D). Decapitation had no significant effect on EC-induced LA-LTP in comparison with the ether group (141.7 ± 9.8% [n = 9]; Fig. 1A and E). In contrast, decapitation after 8% isoflurane significantly reduced EC-induced LA-LTP (126.5 ± 4.6% [n = 11]; Fig. 1A and C). Using HFS of intranuclear afferents LA-LTP was significantly reduced in both animal groups (decapitation: 133.7 ± 6.2% [n = 9]; 8% isoflurane: 133.6 ± 8.7% [n = 11]). Therefore, our results indicate that HFS-induced plasticity changes are dependent on the pretreatment conditions before decapitation to obtain brain slices. A summary