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Optic nerve involvement in experimental autoimmune encephalomyelitis to homologous spinal cord homogenate immunization in the dark agouti rat

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ABSTRACT

Dark-Agouti rats were immunized with spinal cord homogenate to develop Experimental Autoimmune Encephalomyelitis, a model of multiple sclerosis. We assessed motor signs and recorded VEPs for five or eight weeks with epidural or epidermal electrodes, respectively, with final histopathology of optic nerves (ONs).

Injected rats exhibited motor deficits a week after immunization. VEP delays arose from the 2nd to the 5th week, when a recovery occurred in epidermal-recorded rats. ON damage appeared in epidural-, but not in epidermal-recorded rats, probably due to a remyelination process.

VEP could be exploited as neurophysiological marker to test novel treatments against neurodegeneration involving ONs.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is the most common preclinical model for multiple sclerosis (MS), an inflammatory-demyelinating neurological disease that affects about 2.5 million people worldwide (Hollenbach & Oksenberg, 2015). EAE is characterized by complex immunopathological and neuropathological mechanisms that resemble the key features of MS: inflammation, demyelination, axonal loss and gliosis (Lassmann & Bradl, 2017). EAE can be actively induced by immunization with several myelin peptides, such as myelin oligodendrocyte protein (MOG), myelin basic protein (MBP) and proteolipid protein (PLP), as well as with CNS-derived tissues, like spinal cord homogenate (SCH) derivates (Robinson et al., 2014). The pathogenesis of the SCH-EAE model induced in DA rats mimics the typical features of relapsing-remitting MS in humans (Lorentzen et al., 1995). Neurophysiological characterization of EAE models is crucial to identify functional biomarkers that can improve the study of the development and progression of the disease. Up to now, motor-, somatosensory- and visual-evoked potentials (MEPs, SEPs and VEPs) have been investigated. Whereas MEPs (Amadio et al., 2006) and SEPs (All et al., 2010) are used to evaluate cortico-spinal tract impairments, VEPs are *bona-fide* markers of optic nerve (ON) function (You et al., 2011). Approximately 20% of MS patients manifests optic neuritis (Costello, 2013) and one-third have persistent visual symptoms (Jasse et al., 2013). Accordingly, optic neuritis (Kezuka et al., 2011) and visual function abnormalities (Gambi et al., 1996) can also be observed in EAE models. The aim of the present study is to characterize VEPs in different phases of rat SCH-EAE, since neurophysiological evaluation of ON in this model is absent in literature. Specifically, we used the Dark Agouti (DA) rat strain immunized with homologous SCH, which leads to a relapsing-remitting disease course that resembles the clinical profile of relapsing-remitting MS.

In this work we performed longitudinal VEPs in immunized DA rats and healthy controls. The animals were divided in two groups, depending on the VEP recording procedure. In particular, VEPs were recorded with two different methods, using epidural screw or epidermal cup electrodes. The standard VEP recording procedure requires a chronic epidural electrode implant at the level of the visual cortex. However, the major pitfall of implanted electrodes is their possible detachment from the skull. VEPs recorded with non-invasive epidermal electrodes showed statistically equivalent latencies compared to the classic epidural-implanted electrodes, with a higher measure repeatability (Santangelo et al., 2018). Moreover, the epidermal recording technique avoids surgical procedures and allows longer follow-up studies. In order to investigate what happens in later stages of EAE induced by SCH, the epidermal-recorded rats were monitored for three additional weeks after the sacrifice of the implanted rats. Together with VEPs, the SCH-induced disease was monitored by checking motor signs. At the end of the study we sacrificed the animals to perform histological analysis of ONs. ON morphological alterations were examined in order

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to measure the extent and severity of CNS damage.

2. Materials and methods

2.1. Experiment 1. Visual evoked potentials recorded with implanted electrodes to monitor optic nerve involvement in the dark agouti rat model of experimental autoimmune encephalomyelitis induced by homologous spinal cord homogenate

2.1.1. DA rats (n = 14)

Female DA rats, 6–8 weeks aged, with a body weight of 110–130 g, (Janvier Labs - Saint-Berthevin, France) were used in this experiment. Rats were housed under controlled temperature on a 12 h light/dark cycle with free access to chow pellets and tap water. All procedures were conducted in agreement with the European Community guidelines (Directive 2010/63/EU) and the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH), and were previously approved by the San Raffaele Institutional Animal Care and Use Committee (IACUC).

2.1.2. Experimental protocol

Surgery procedure for epidural electrode implant was performed 6 days before the first VEP recording session. The day after the first VEP recording, 6 rats were immunized with spinal cord homogenate (SCH, EAE rats) whereas 8 animals were left untouched and considered as healthy controls (H rats). VEPs were recorded 6 times once a week in H and EAE rats: the first recording was performed at day −1, then at 6, 13, 20, 27, 34 days post immunization (dpi). Clinical score was monitored daily from −1 to 34 dpi. After the last VEP session (34 dpi), 5H (randomly selected) and 6 EAE rats were sacrificed for histological analysis of ONs (Fig. 1).

2.1.3. Screw electrode implant for epidural VEP recording

Deep anesthesia (verified by absence of reaction at tail pinching) was induced and maintained with 2.5% sevoflurane (Sevorane™, Abbott S.p.a. - Campoverde, Italy) through a facemask. During surgery, body temperature was maintained at 37 °C using a heating pad (Harvard Apparatus - Holliston, Massachusetts, USA). The skin was resected, then the rat was mounted on a stereotaxic apparatus (Stoelting Co. - Wood Dale, Illinois, USA) and three epidural stainless steel screw electrodes (0.9 mm diameter) were implanted: two active electrode over left and right primary visual cortex (V1, 6 mm posteriorly to the bregma and ± 3.5 mm lateral to the sagittal suture) and the reference in the nasal bone (Onofrj et al., 1985) (Fig. 2). The implant was fixed with glass ionomer dental cement (3 M Deutschland GmbH – Neuss, Germany). During surgical procedures some vaseline was put over both eyes of the animals to prevent eye drying or dental cement injury. After surgery, all animals were allowed to recovery 6 days before undergoing the first VEP recording session. Rats with detached electrodes were excluded from the experiment. Regarding the possibility to replace screw electrodes, we preferred to avoid further implantations on diseased rats because.

2.1.4. EAE induction

EAE disease was induced by immunization of DA rats with an encephalitogenic emulsion containing 12.5 mg/ml of homologous spinal cord homogenate and incomplete Freund's adjuvant supplemented with 4 mg/ml of *Mycobacterium tuberculosis*. Each rat received 200 µl of the emulsion injected subcutaneously at the base of the tail.

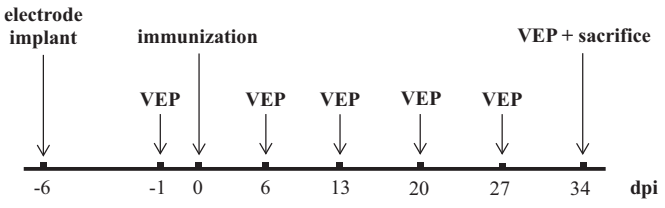

Fig. 1. Study design of experiment 1.

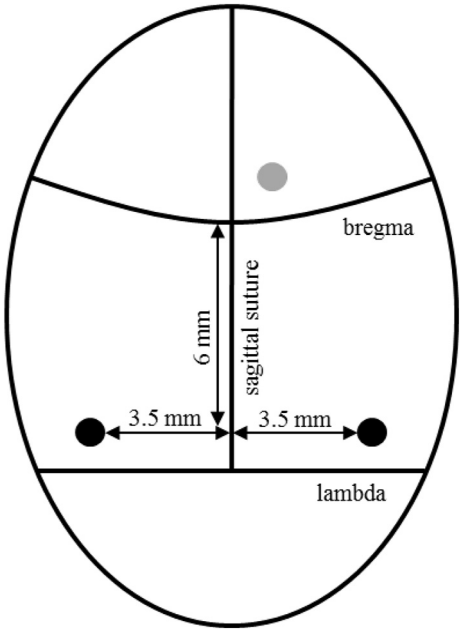

Fig. 2. Schematic representation of the rat skull, with the positions of the recording (black dots) and the reference (grey dot) electrodes.

2.1.5. Clinical assessment of EAE

Clinical signs of EAE in immunized rats were scored daily from −1 to 34 dpi. Clinical score ranged from 0 to 5. (0: no signs; 0.5: tail weakness; 1: complete tail paralysis; 1.5: complete tail paralysis and weakness of the hind limbs; 2: complete tail paralysis and one hind limb paralyzed; 2.5: hind limbs do not support the body weight, but without complete paralysis; 3: complete paralysis of the hind limbs; 3.5: complete paralysis of the hind limbs and paresis of front paws; 4: complete paralysis of front and hind limbs; 4.5: moribund; 5: death due to the severity of EAE). Notably, this score reflects the amount of spinal cord lesions and does not include visual dysfunctions.

2.1.6. VEP recording and anesthesia monitoring

VEPs were acquired after 5 min dark adaptation in a darkened Faraday cage, under volatile anesthesia with sevoflurane (2.5%) in oxygen (30%) and nitrogen (70%), delivered by inhalation through a face mask, as previously described by our group (Cambiaghi et al., 2011). During each VEP session, body temperature was maintained at 37 °C using the heating pad. Before the experimental tests, rats were allowed to reach a steady state with the anesthetic; the adequate level of anesthesia was verified by checking for the presence of tail-pinching reflex and the absence of the corneal one (Bolay et al., 2000). In addition, heart rate frequency was continuously monitored from two subcutaneous needle electrodes in right and left forelimbs. This enabled to control closely the depth of anesthesia, which is crucial to maintain optimum visual responsiveness (Gordon and Stryker, 1996). Pupils were dilated with 1% Tropicamide (Visumidriatic - Visufarma s.p.a., Rome, Italy) and 2% Hydroxypropylmethylcellulose (GEL 4000 - Bruschettini s.r.l., Genoa, Italy) was applied to avoid eye drying. Electrodes were connected *via* flexible cables to a Micromed amplifier (SystemPlus Evolution, Micromed s.p.a. - Mogliano Veneto, Italy) and a needle electrode was inserted into the hind limb as the ground. Data were acquired at a sampling frequency of 4096 Hz, coded with 16 bits and filtered between 10 and 80 Hz. Flash stimuli, with intensity of 522 mJ