Physiology & Behavior 182 (2017) 1–9

Contents lists available at ScienceDirect

Physiology & Behavior

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

The effect of directed photic stimulation of the pineal on experimental Parkinson's disease

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ARTICLE INFO

Keywords:
Pineal
Parkinson's disease
Light
Melanocyte
Dopamine
Melatonin
Melanin
Circadian

ABSTRACT

The role of the circadian system in Parkinson's disease (PD) is a topic of increasing scientific interest. This has emerged from recent studies demonstrating an altered response of PD patients to treatment in relation to the phase of the light/dark cycle and from other work defining the functional significance of melanocytes in PD: a cell type that the nigro-striatal dopamine (NSD) system and circadian system both contain. The present study was undertaken to determine the sensitivity of the pineal, as the final common pathway of the circadian system, to light delivered directly to the pineal via surgical implantation of LEDs. Direct photic stimulation of the pineal altered the course of experimental PD while anatomical controls receiving stimulation of the frontal cortex exhibited a negative impact on the course of recovery of these animals. These effects were closely linked to the phase of the light/dark cycle. The present results suggest that while pineal photoreceptors are regarded as vestigial, functional photo-reactivity of the pineal remains. It is inferred that melanocytes are the active cells responsible for the observed effect since they remain functionally intact in mammalian pineal even though pineal photoreceptors are functionally inert. Although the stimuli applied in the present study may be regarded as artificial this study demonstrates that brain parenchyma remains differentially reactive to direct light exposure and presents a novel mechanism in circadian structures that needs to be explored.

1. Introduction

Ambient light serves an essential function in the mammalian visual system acting mainly through photoreceptors in the retina. Neuroendocrine function is also served by these photoreceptors as they project from the retina to the pineal gland via the retinohypothalamic tract (RHT) [1]. In lower species the pineal gland is exposed to direct environmental illumination due to the presence of a third eye vented directly to the dorsal surface of the skull thereby regulating day/night activities [2–6], however these photoreceptors have become vestigial in mammals [2,4]. In addition to these inert photoreceptors there remains a cell type in the retina, pineal and substantia nigra (SN), which is sensitive to changes in ambient light serving, amongst other things, a protective function. These melanocytes, are pigmented cells embryologically derived from the neural crest [7], responding to light and darkness in a reciprocating manner. In the presence of light, these cells exhibit pigment dispersion, causing the cell to darken while the absence of light induces pigment aggregation and the cell blanches [8,9]. While it has never been demonstrated that melanocytes in the retina, SN or pineal play an active role in the regulation of motor function we undertook an investigation of the effects of photic stimulation of the pineal itself in experimental models of Parkinson's disease (PD). This approach was justified on the basis of reports suggesting that i) light exposure via the RHT has been shown to induce recovery from PD and its experimental counterparts [10–14], ii) post mortem examination of PD patients reveals a severe depigmentation (blanching) of the SN [15–17], iii) the pineal and its main metabolic product, melatonin, play a major role in the development, progression and treatment of PD [11,18,19]; iv) and that the exposure of motor systems to extraneous ambient light affects motor function and alters midbrain pigmentation [20,21]. Recent work in optogenetics [22–26] and therapeutic use of red and near infrared (NIR) frequencies directly applied to NSD tissue [27,28] have demonstrated the therapeutic effects of specialized applications of light directly to brain tissue. The present study explorers other systems and other mechanisms of action by which light might be providing such an effect in regard to the circadian system.

2. Methods

30 experimentally naive male, Sprague Dawley rats were obtained

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http://dx.doi.org/10.1016/j.physbeh.2017.09.014
Received 6 July 2017; Received in revised form 31 August 2017; Accepted 14 September 2017
Available online 15 September 2017

from the Bronowski Institute Colony and were housed individually in nylon cages with standard food pellets (Clarke King®/Barastock®) made available ad lib from a feeding grid. Tap water was made available ad libitum from bottles attached to the top of each cage. Animals ranged in weight from 250 to 350 g at the commencement of the experiment. Room temperature was maintained at 22 °C ± 2° with a 12 h. light dark cycle with lights on at 0700 h. The room was illuminated with 2 fluorescent tubes with the intensity of light within each cage averaging 250 lx during the lights on phase of the light/dark (L/D) cycle. No light was detected in the housing facility during the dark phase. All experiments were performed with the approval of and performed under the auspices of the Animal Experimentation Ethics Committee of the Bronowski Institute of Behavioural Neuroscience implementing protocols conforming to the National Guidelines for the Care and Use of Animals for Scientific Purposes by the National Health & Medical Research Council of Australia.

2.1. Surgery

After habituation into the colony for at least 7 days rats were premedicated with atropine sulphate (0.06 mg/kg-S.C.) and then anesthetized with a Ketamine (55 mg/kg)/Xylazine (20 mg/kg) mixture (I.M.). When full anaesthesia was achieved each rat was then placed in a stereotaxic instrument. The site of cannulation for eventual intracranial (I.C.) injection for achieving experimental PD was the posterior lateral hypothalamus (PLH) just rostral to the midbrain diencephalon border in the fiber bundle in the NSD system. Intracranial 23 gauge stainless steel cannulae were implanted bilaterally just dorsal to the intended site of injection at the coordinates AP = − 1.8 mm; L = ± 1.8 mm; D = − 6.1 mm. This site was chosen so that the injection needle extended 2 mm beyond the cannula tip in a ventral direction to minimize damage to the injection site [29]. All coordinates were relative to bregma and in the plane of Pellegrino et al., 1979. This position has been found to be effective in producing severe Parkinsonian-like effects in animals [11,19,30]. The cannulae were secured in place an implant with titanium screws and bone acrylic. An implant consisting of a LED (Nichia: polychromatic, No. NSPW315BS), a resistor (Kamaya Electric: 0.25 W, 1 K) and a lithium battery (Horiz; CR2032, 3 V), were assembled into a single unit (Fig. 1A; Wilcom Australia Pty Ltd) and implanted over either the landmark lambda after performing a small craniotomy directly over the pineal or the frontal cortex in the locations as depicted in Fig. 1B. The LED unit and the cannulae were consolidated with bone acrylic. At the completion of intracranial surgery rats were injected with 12 ml/kg Reversine ® (S.C.) which was used as a reversal agent for the Xylazine®. All rats were injected with the analgesic Metacam® (10 mg/kg, I.M.) at the completion of surgery to aid in painless, rapid recovery. Rats were kept warm after surgery and allowed at least 8 days of recovery before commencing the formal part of the study (Fig. 1C).

2.2. Study design

Just prior to commencing the study all animals were handled by the experimenters to ensure they were habituated to human contact. The rats were divided into 3 groups of 10 animals per group. In the first group the LED over the pineal was permanently in the “on” state (LEDON). In the second group the LED was placed over the pineal but this unit was left in the “off” position serving as the procedural control (LEDOFF). The remaining rats received a LED implant over the frontal cortex in the “on” state and this served as an anatomical control group (FRONTAL). During at least 5 days of baseline observation all rats were tested in the open field for vertical and horizontal movement and on 3 motor tests and their body weight was measured daily as described in a subsequent section. At the end of the control observations All animals received intracerebral injection of 2 μg/μl 6-hydroxydopamine (6-OHDA) bilaterally through the in dwelling cannulae as described immediately below. During the acute period of experimental PD at days 5 (light phase test) and day 6 (dark phase test) post 6-OHDA all rats were tested on motor performance. They were tested again at days 19–20 during the light and dark phases of the L/D cycle respectively.

2.3. Intracerebral 6-OHDA injections

6-Hydroxydopamine hydrobromide (Sigma St. Louis MO. USA) was mixed in a concentration of 8 μg/μl and injected in a volume of 2 μl per site. Injections were made at a rate of 1 μl/min and the needle was left in situ for at least 30 s. after each injection was complete to ensure that the drug diffused from the end of the needle. 6-OHDA was dissolved in saline/ascorbic solution to prevent rapid oxidation of the drug and these solutions were isotonic [11,32]. New solutions of drug were prepared immediately prior to injection with stock solutions kept refrigerated or on ice until used. All solutions were kept shielded from light and heat and discarded at the end of each injection session.

2.4. Behavioural measures

Independent variables were measured during the light and the dark phase of the L/D cycle commencing between the hours of 10:00–15:00 h and again at 20:00–01:00 h, respectively, with at least 18 h allowed between consecutive measurements. Locomotion and rearing were measured with the aid of a 900 mm (length) × 500 mm (width) × 300 mm (height) PVC box fitted with machine vision with motion detection capabilities. The total number of movements within the horizontal plane and the number of rearing associated movements in the vertical plane during each 10 min test session were measured and recorded with the aid of specialized software. A series of three motor reflex tests were performed immediately at the conclusion of the open field test [11,33]. These tests included the latency to retract the left, then the right front limbs when they were elevated 25 mm from the table surface, the latency to step up or down from a raised platform when the rear torso was elevated 25 mm and the latency to ambulate outside of a 90 × 170 mm rectangle drawn on the bench surface. These tests are derivations of those described previously [34] and have been used extensively to characterize the features of experimental PD [11,33]. The test chamber and all surfaces of each apparatus were thoroughly washed between the testing of each animal to avoid olfactory stimuli which can cause distraction during testing. Testing during the dark phase of the L/D cycle was performed under low intensity red light with all sources of illumination masked by implementing red barrier filters on all light emitting devices. Body weight was measured daily for the duration of the study and on the days of behavioural testing after behavioural tests was completed.

2.5. Histology

At the end of the study all rats were euthanized with a barbiturate (Lethobarb®, Virbac P/L NSW). Brain tissue was sectioned with injection sites were plotted on plates from a stereotaxic atlas to determine their anatomical position relative to the NSD system [31].

2.6. Statistical analysis

Statistical analysis was undertaken using the IBM SPSS Statistics 24 for Windows. Due to the skewed distribution and non-homogenous variance which typically occurs with the implementation of these behavioural measures, and the low number of animals per group, non-parametric testing was chosen to evaluate differences between independent groups to minimize type II errors. On this basis, the Mann Whitney-*U* Test (MWUT) and the Independent Samples Kruskal-Wallis Test (IKWT) performed between drug and vehicle treated groups for all parameters. Body weight was analyzed using the Related Samples Friedman's 2-WAY Analysis of Variance (RSF 2-WAY ANOVA) for the