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Mouse model of diffuse brain damage following anoxia, evaluated by a new assay of generalized arousal

Isabel Arrieta-Cruz *, Donald W. Pfaff, Deborah N. Shelley

Laboratory of Neurobiology and Behavior, The Rockefeller University, Box 275, 1230 York Avenue, New York, NY 10021, USA

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Abstract

Diffuse brain damage following anoxia due to cardiac failure, drowning, carbon monoxide exposure or other accidents constitutes a major medical problem. We have created a novel mouse model using the breathing of pure nitrogen, followed by a recently developed assay that reflects an operational definition of generalized arousal. The operational definition is precise, complete, and leads to quantitative, physical measures in a genetically tractable animal. Exposure to pure nitrogen for controlled periods had a surprising bifurcate effect: about half the mice survived with neurological measures that were virtually normal while the other half died. The new assay detected behavioral deficits unrevealed by neurological screening. Two important features of the results were that (i) deficits were not equal across the circadian cycle, and (ii) deficits were not equal across all the measures within the operational definition of arousal. Specific voluntary motor measurements were decreased in a manner that depended on the phase of the circadian cycle. Sensory responses were also decreased, with an emphasis on vertical movement responses; but, interestingly, fear learning was not damaged. This study establishes the first useful approach to diffuse brain damage in a genetically tractable animal. The model and its outcome measurements will be useful during future attempts at amelioration of acquired neurological disabilities following hypoxic–ischemic injuries.

Keywords: Anoxia; Arousal; Brain damage; Sundowning; Circadian rhythm; Mice; Neurological disorders; Delirium; Disorders of consciousness; Behavioral

Introduction

There are many accidents that produce a loss of normal consciousness due to severe brain damage. Anoxic and hypoxic/ischemic brain injuries are some of the world's leading causes of brain damage. Each year, among other causes, cardiac arrests, alone, may produce such injuries; and, there are more than 700,000 cardiac arrests per year in the United States (American Heart Assn., 2006). Following anoxia consequent to stroke and other causes such as cardiac failure, drowning, carbon monoxide exposure and various accidents, there is a complex cascade of events in neurons causing cell death and functional neurological damage (Neubauer and Sunderram, 2004; Clarkson et al., 2005; Lo et al., 2005; Rashidian et al., 2005). Altered functional states include arousal, attention, intention, memory and awareness, all observed during global disorders of consciousness (Vexler et al., 1994; Schiff and Plum, 2000; Glenn et al., 2003; Dunham et al., 2004; Laureys et al., 2004; Young et al., 2004; Caputa et al., 2005).

Fundamental to all of these problems is the concept of generalized CNS arousal. Arousing the CNS provides the most elementary support for all cognitive and emotional functions in the mammalian brain, including the human brain (Pfaff, 2006). There is a need for a precise and complete operational definition of generalized CNS arousal. The operational definition proposed states that a more aroused animal or human (i) demonstrates greater alertness to sensory stimuli in all modalities; (ii) emits more voluntary motor activity; and (iii) shows greater emotional reactivity. This operational definition leads to precise, quantitative physical measures (Garey et al., 2003; Easton et al., 2004).

To provide an assay as high-throughput as possible we constructed an apparatus (see Materials and methods), in which all the components of the operational definition of arousal stated above can be measured 24 h per day. Sensory stimuli from three

* Corresponding author. Fax: +1 212 327 8664.
E-mail address: iarrieta@mail.rockefeller.edu (I. Arrieta-Cruz).

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modalities can be presented to experimental animals which, themselves, do not have to be moved or touched. Several measures of voluntary motor activity in the home cage are available to the experimenter. In terms of emotional reactivity, we have chosen fear as an emotion most convincingly present in mice and as a response pattern whose neural mechanisms have been explored successfully (LeDoux, 2000). Mice were chosen because they are genetically tractable, a property potentially useful in later attempts to achieve amelioration of brain damage.

While there are many experimental models to study the effects of TBI or stroke on certain behavioral functions in rodents (Gonzalez and Kolb, 2003; Wagner et al., 2004; Cauraugh and Summers, 2005; Maeda et al., 2005), we know of no comprehensive published approaches to anoxic brain damage in mice, nor have there been outcome measurements presented with adequate precision. Little has been done to treat this disease process because of a lack of basic laboratory research on mechanisms of functional loss and potential mechanisms of enhanced recovery. The present research investigates this subject in a manner important for clinical medicine, in that while dealing here with anoxia, the same methods of detection could be used with other neurological disorders. In the present study, neurological and behavioral responses were explored in mice exposed briefly to anoxic insult, using 'generalized brain arousal' (Pfaff, 2006) as a theoretical background, to establish a mouse model useful for future attempts to ameliorate hypoxic/ischemic brain damage. Hopefully this model will lead to a systematic testing of novel therapies useful for reversal of disease processes following hypoxia or anoxia.

Materials and methods

Animals

Twenty-four female C57BL/6 mice at 6 weeks old were ovariectomized by the supplier (Jackson Laboratory, Bar Harbor, ME) in order to eliminate possible variations in function and in response to anoxia across the estrous cycle. Animals were individually housed with food and water available *ad libitum*, and maintained on a reversed 12:12-h light/dark cycle with lights off at 06:00 h in a temperature-controlled (20±2 °C) room for 1 week prior to experimental manipulation. All procedures and protocols were approved by The Rockefeller University's Institutional Animal Care and Use Committee (IACUC) through The Laboratory Animal Research Center.

Procedures and experimental design

7.5 weeks old animals were randomly assigned to three experimental groups: one for a single exposure to anoxia ($n=8$), a second for multiple exposures to anoxia ($n=8$) and a third to pentobarbital ($n=8$). The order followed in the generalized arousal assay was illustrated in Fig. 1. Mice were anesthetized deeply via an i.p. injection with Nembutal sodium solution (Abbott laboratories, North Chicago, IL) at a dose of 7.5 mg/100 g of body weight, and assigned to one of three experimental conditions. Animals only used for the anoxia treatment were placed inside a Plexiglas chamber for administering the N_2 at a constant pressure of 100 kPa during exposure. Mice in the first condition received a single exposure to 100% of N_2 for 1 min and 50 s; whereas the mice in the second condition were exposed to pure nitrogen breathing five times, each 1 min and 50 s, with the exposures separated by 5 min ('multiple exposures'). The duration of these exposures had been pre-determined in pilot assays.

Neurological examination

24 h before and after of the treatment with N_2 or pentobarbital, the mice were evaluated neurologically by scoring their performance on a series of reflex tests (see Table 1), according to an established protocol entitled primary screen SHIRPA (Rogers et al., 1997) and modified with other neurological assessments (Ziporen et al., 1997). The mice were placed inside a Plexiglas cage (47 cm×25 cm×15 cm) with four transparent walls; the order of the tests was performed as shown in Table 1.

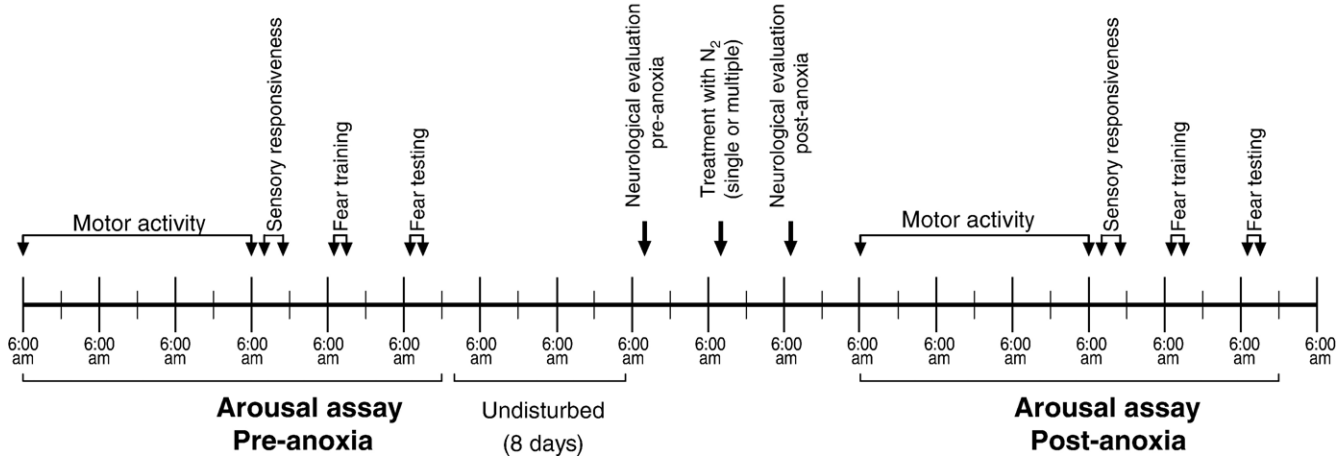

Fig. 1. The diagram shows the timeline of behavioral assays before (pre-anoxia) and after (post-anoxia) treatment with N_2. The animals were maintained on a reversed light/dark cycle, with lights off at 6:00 am and lights on at 6:00 pm. The motor activity was tested all 24 h for 3 days; the sensory responsiveness assay, fear conditioning and neurological evaluation were tested during the early hours of the dark period. See Materials and methods for more details.