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Research report

Neonatal exposure to whole body ionizing radiation induces adult neurobehavioural defects: Critical period, dose—response effects and strain and sex comparison

Per Eriksson[a,*], Sonja Buratovic[a], Anders Fredriksson[a], Bo Stenerlöw[b], Synnöve Sundell-Bergman[c]

[a] Department of Environmental Toxicology, Uppsala University, Norbyvägen 18A, SE-75236 Uppsala, Sweden
[b] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
[c] Department of Soil and Environment, Swedish University of Agricultural Sciences, Uppsala, Sweden

HIGHLIGHTS

- Neonatal exposure to gamma radiation alters adult spontaneous behaviour.
- Neonatal exposure to gamma radiation affects habituation in adults.
- Neonatal exposure to gamma radiation causes hyperactivity in adults.
- Functional defects induced during a defined period of neonatal brain development.
- Behavioural defects are seen in inbred and outbred mice of both sexes.

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ABSTRACT

Development of the brain includes periods which can be critical for its normal maturation. The present study investigates specifically vulnerable *peri*-/postnatal periods in mice which are essential for understanding the etiology behind radiation induced neurotoxicity and functional defects, including evaluation of neurotoxicity between sexes or commonly used laboratory mouse strains following low/moderate doses of ionizing radiation (IR).

Male Naval Medical Research Institute (NMRI) mice, whole body irradiated to a single 500 mGy IR dose, on postnatal day (PND) 3 or PND 10 showed an altered adult spontaneous behaviour and impaired habituation capacity, whereas irradiation on PND 19 did not have any impact on the studied variables. Both NMRI and C57bl/6 male and female mice showed an altered adult spontaneous behaviour and impaired habituation following a single whole body irradiation of 500 or 1000 mGy, but not after 20 or 100 mGy, on PND 10.

The present study shows that exposure to low/moderate doses of IR during critical life stages might be involved in the induction of neurological/neurodegenerative disorder/disease. A specifically vulnerable period for radiation induced neurotoxicity seems to be around PND 3–10 in mice. Further studies are needed to investigate mechanisms involved in induction of developmental neurotoxicity following low-dose irradiation.

1. Introduction

During the development of an organism, there are periods which can be critical for its normal maturation. In many mammalian species, rapid growth of the brain occurs during perinatal development, the so-called 'brain growth spurt' (BGS) [1]. In the human, this period begins during the third trimester of pregnancy and continues throughout the first 2 years of life. In mouse and rat, this period is neonatal, spanning the first 3–4 weeks of life, during which the brain undergoes several fundamental developmental phases, viz. maturation of axonal and dendritic outgrowth, establishment of neural connections, synaptogenesis, multiplication of glia cells with accompanying myelinization, and cell, axon and dendrite death [1,2]. The BGS is associated with numerous biochemical changes

* Corresponding author.
E-mail address: per.eriksson@ebc.uu.se (P. Eriksson).

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that transform the feto-neonatal brain into that of the mature adult [3]. This is also the stage of development when animals acquire many new motor and sensory faculties, including advances in spontaneous motor behaviour [4].

Foetuses and neonates are suggested to be more vulnerable for the consequences of exposure to ionizing radiation (IR). Exposure to IR in the medical field for imaging purposes and radiotherapy of tumors has come to represent the major source of exposure in the general population [5–7]. The use of computed tomography (CT) scans has increased in general over the past decades and in particular in the fields of pediatric diagnosis and adult screening [8,9]. Estimations of brain doses received during CT scans show a decreasing trend with increasing age of the patient, where children under the age of five years are estimated to be exposed to the highest absorbed doses within the range of 50–100 mGy/scan [9,10]. Furthermore, approximately 40% of patients receiving a head CT scan had undergone previous head CTs [6].

An epidemiological study has reported that moderate doses of IR to the brain during infancy and early postnatal brain development, for treatment of cutaneous haemangioma, may have negative impact on cognitive development during childhood and result in impaired cognitive function for the adult individual [11]. In radiotherapy (RT), for treatment of tumors in the central nervous system (CNS), multiple doses of 2 Gy are often required but these therapeutic high energy gamma- or X-ray beams also delivers significant radiation doses to normal tissue distant from the tumor. This has been shown to elevate the risk of developing late cognitive dysfunction in pediatric patients as well as a progressive cognitive decline in adult patients [12–14].

It is known from developmental neuroscience that many potentially sensitive processes occur during the early postnatal period of brain maturation [15]. We have in earlier studies seen that neonatal exposure of mice during the BGS period to environmental toxicants, neurotoxic chemicals and anaesthetic/analgesic pharmaceuticals can induce persistent developmental neurotoxic effects when the chemicals are present during a defined critical stage of neonatal brain development, namely around postnatal day 10 (PND 10) [16–22]. These effects are manifested as modified spontaneous behaviour, reduced/lack of habituation, impaired learning and memory, neurochemical alterations, all resulting in reduced cognitive function in young adult and adult mice. In addition, no major difference in induction of such neurobehavioural defects are seen in the outbred Naval Medical Research Institute (NMRI) mouse compared to the inbred C57bl/6. Such an exposure can also accelerate neurodysfunctional processes and increase susceptibility to chemicals at adult age [23–27]. Importantly, these effects are induced following doses that have no apparent permanent effects when administered to the adult animal.

Knowledge of the long-term effects from exposure to IR at low doses and dose rates on neurological and behavioural variables, during a critical phase of development, as well as dose-response relationships, is of major importance in view of radiation protection. The aim of the present study was to evaluate: 1) a critical window for induction of behavioural/cognitive effects, 2) dose—response related effects induced during such defined period, 3) whether there can be any sex differences, 4) effects in an outbred (NMRI) and an inbred (C57bl/6) mouse strain, when neonatally exposed to low doses of ionizing radiation.

2. Material and methods

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), after approval from the local ethical committees (Uppsala University and the Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals.

2.1. Animals

Pregnant Naval Medical Research Institute (NMRI) mice and C57bl were purchased from Scanbur, Sollentuna, Sweden. The animals were housed individually in macrolon cages (42 × 26 × 15 cm) in a separate room for females only with an ambient temperature of 22 °C and a 12/12 h constant light/dark cycle (lights on 07:00, lights off 19:00). Animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water *ad libitum*. Females were checked for birth twice daily (08.00 and 18.00 h) and day of birth was designated day 0. Within the first 48 h after birth, litter sizes of NMRI mice were adjusted to 10–12 pups of both sexes by euthanizing excess pups, and in C57bl 3–7 pups of both sexes. At approximately 4 weeks of age, male and female offspring were separated with regard to sex and raised in sibling groups of 3–7 individuals in separate male and female rooms.

2.2. Irradiation

Mice of both sexes were whole body gamma-irradiated to a single dose from a ^{60}Co source at The Svedberg laboratory, Uppsala University, Uppsala, Sweden, [22,28]. Mice were placed in plastic dishes and exposed to a single surface dose of 0–1000 mGy with a dose rate of about 0.02 Gy/min. An ionization chamber (Markus chamber type 23343, PTW-Freiburg) was used to measure the dose, which was homogeneous ± 3%, over the 10 cm in diameter large dish area. Control mice (0 mGy) were placed in the same plastic dishes as the irradiated mice and sham irradiated.

2.3. Spontaneous behaviour

Spontaneous behaviour, in a novel home environment, was observed in mice. The observations and recordings were carried out, between 08.00 and 13.00 h, under the same light and temperature conditions as their housing conditions. During 60 consecutive minutes an automated system recorded the motoric activity of the animals and recording of the variables locomotion, rearing and total activity were made (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden) as described by Fredriksson (1994) [22,28,29]. Twelve cages, placed in individual soundproof boxes with separate ventilation were used.

2.3.1. Locomotion

Movements made in the horizontal plane were registered by the low level (10 mm above the bedding material) infrared beams.

2.3.2. Rearing

Movements made in the vertical plane were registered by the high level (80 mm above the bedding material) infrared beam.

2.3.3. Total activity

A needle mounted on a horizontal arm with a counterweight connected to the test cage registered all vibrations such as movements, grooming and shaking.

All data were collected electronically through a computer interface. The animals were observed for a 60 min period of time (0–60 min) divided into three 20 min intervals and in each 60-min session animals from each exposure group were represented. Spontaneous behaviour of male and female mice was recorded on separate days for not having interference of scent from the opposite sex.