ELSEVIER

Neuroscience Letters 175 (1994) 92–94

NEUROSCIENCE LETTERS

Changes of aggressive behavior and brain serotonin turnover after very low-dose X-irradiation of mice

Yukihisa Miyachi[a,*], Hiroshi Kasai[b], Harumi Ohyama[c], Takeshi Yamada[c]

[a]*Division of Biology, National Institute of Radiological Sciences, Anagawa 4-9-1, Chiba 260, Japan*
[b]*Biolology Division, National Cancer Center Research Institute, 5-1-1 Thukiji, Chuo-ku, Tokyo 104, Japan*
[c]*Divisions of Radiation Health and Biology, National Institute of Radiological Sciences, Anagawa 4–9–1, Chiba 260, Japan*

Received 23 March 1994; Revised version received 2 May 1994; Accepted 13 May 1994

Abstract

Social isolation has been widely described to induce compulsive aggressive behavior and produce a large decrease of brain serotonin turnover in male mice. The aggressiveness by isolation in mice has been often used as a means for a better understanding of disturbed behavior in human beings. We found that male ICR white Swiss mice exhibiting isolation-induced aggression became gradually calm and showed remarkably quiet behavior 7 to 10 days after whole body irradiation of very low-dose X-rays (5–15 cGy). Higher doses (25–35 cGy), however, could not induce such effects. We also obtained the data on brain biochemistry giving a further support for the above low-dose effects on the mouse behavior. Brain serotonin turnover which has been known to be related to aggressive behavior in 5 or 15 cGy irradiated mice was faster than in aggressive control animals.

Key words: Radiation hormesis; Low-dose X-ray; Aggressive behavior; Serotonin metabolism; Social isolation; Stimulating effect

In recent years, concern has emerged about the hormetic effect of X-ray stress. 'Hormesis' is the name given to the stimulating effect of small doses of substances which in larger doses are inhibitory [5,6,8]. The term need not be limited to stimulation but may well be applied to any physiological effect which occurs at low doses and which cannot be anticipated by extrapolating from toxic effects noted at high doses.

X-rays also can be classed as a noxious agent. In the course of a study of low-dose radiation effects, we have noticed that fighting injuries usually observed among male ICR mice tend to decrease in mice irradiated with low-dose X-rays. An attempt was made, therefore, to examine quantitatively the effects of acute low-dose X-ray whole-body exposure on aggressive behavior using a resident-intruder paradigm in which a resident mouse attacks an intruder that entered its territory.

ICR mice, living single in cages for a period of 6 weeks, develop a typical behavior that, from an initial stage of hyperreactivity to the usual environmental stimuli, passes through a hyperactive phase to aggressiveness. The resident mice were given whole-body X-ray exposure of 5, 15, 25 and 35 cGy with an X-ray machine (Shimazu Shin-ai No.7) at a dose rate of 20 cGy/min (200 kVp, 20 mA, 0.5 mm Cu + 0.5 mm Al filters). Animals were not anesthetized during exposure. On the test day, the non-irradiated intruder, housing in a grouped-cage (3 mice/cage) was placed in the resident's cage and each pair was recorded using a video casette recorder system for 5 min. The behaviors recorded for each subject were (1) the number of bites inflicted upon the intruding male and (2) the attack latencies: time interval from the intruder was placed into the test cages until the first attack. Statistical analysis was performed by Mann–Whitney *U*-test (irradiation vs. sham control each test day). Videotapes were scored by two observers who were unaware of the treatment condition of the animals.

As shown in Table 1, the number of bites in the 5 cGy groups decreased gradually with time and reached to a level significantly lower than that in controls ($P<0.02$) on day 10 after irradiation. The depression became

*Corresponding author. Present address: Division of Physiology, Toho University, Ohmori-nishi 5-21-16 Ohta-ku, Tokyo 143, Japan. Fax: (81) 3-3762-8148.

SSDI 0304-3940(94)00394-P

Table 1
Depressive effects of low-dose X-irradiation on offensive aggression

	Day 3	Day 7	Day 10	Day 14
Sham-control mice				
Number of bites	14.0 ± 3.6	13.5 ± 3.6	12.5 ± 3.6	12.7 ± 4.1
Attack latencies (min)	Immediately	Immediately	Immediately	Immediately
5 cGy irradiated				
Number of bites	10.5 ± 4.3	7.5 ± 3.0	6.6 ± 2.6**	9.9 ± 3.7
Attack latencies	Immediately	0.7 ± 0.3	1.2 ± 0.3**	Immediately
15 cGy				
Number of bites	8.0 ± 3.5	5.9 ± 1.6**	4.6 ± 1.5***	7.1 ± 2.7
Attack latencies	Immediately	1.5 ± 0.4**	2.0 ± 0.5***	Immediately
25 cGy				
Number of bites	12.0 ± 3.3	12.6 ± 3.7	12.6 ± 2.9	14.2 ± 4.3
Attack latencies	Immediately	Immediately	Immediately	Immediately
35 cGy				
Number of bites	12.8 ± 4.4	13.9 ± 3.1	13.0 ± 4.1	18.5 ± 3.0
Attack latencies	Immediately	Immediately	Immediately	Immediately

Aggressive behavior (n = 15 pairs on all days) was measured on days 3, 7, 10 and 14 after whole-body X-irradation. Values are mean ± S.E. Significant differences between control and irradiated groups are represented by asterisks (** $P < 0.02$, *** $P < 0.01$).

deeper when the radiation dose was elevated to 15 cGy. A significant decrease in aggressive behavior was already noticed on day 7 and the most profound effect was observed on day 10. Furthermore, mice irradiated with 5 or 15 cGy showed a prolongation of attack latencies compared to sham control. Increasing the radiation dose further to 25–35 cGy, however, resulted in the complete disappearance of the effects, namely, the irradiated mice showed no change compared to the controls. These results suggest that the depression in aggressiveness is limited to the animals irradiated with the smallest doses, and shows, therefore, no positive correlation with radiation dose.

Isolation-induced aggression in rodents has been known to be related to the brain serotonin (5-hydroxytryptamine; 5-HT) metabolism [9,10]. Therefore, we next examined changes in turnover rate of brain 5-HT. The rate in aggressive and non-aggressive mice was estimated by blocking the monoamine oxidase activity using its inhibitor, pargyline (*N*-benzyl-*N*-methyl-2-propynylamine) at 100 mg/kg i.p. according to the method of Valzelli [9,10]. The non-aggressive mice were chosen from the group-housed mouse population (3 mice/cage). The animals were decapitated at day 8 after irradiation when the profound decline of aggression was observed. Immediately after the decapitation, whole brains were homogenized in 5 vols. of 0.1 M perchloric acid and centrifuged. The supernatant was analyzed by high-performance liquid chromatography coupled with a electrochemical detector [ESA Inc. Bedford, MA, model 5100A; analytical cell, model 5011, detector 1 (5 V), detector 2 (25 V); guard cell, model 5020 (35 V); current was monitored in detector 2 [4]. As shown in Table 2, this inhibitor increased brain 5-HT much faster in non-aggressive than in aggressive animals. Brain 5-HT in mice after 5 or 15 cGy X-irradiation was recovered to the level of non-aggressive controls. We could conclude from these results that low-dose X-irradiation induced subtle changes in brain physiology including 5-HT turnover, resulting in depression of aggression.

The most important finding of this work is a marked depression of mouse aggressive behavior by very low dose X-rays at the lowest dose range including 5 cGy, which is the annual dose limit range for occupational exposure recommended by the International Commission on Radiation Protection. The other interesting finding reported here is that the depressive effect of radiation on aggressive behavior can be noticed only within the lower dose range (5–15 cGy), but not within the relatively higher doses (25–35 cGy). In general, biological effects of radiation have a positive correlation with dose. The effects presented here do not show such a typical dose–effect relationship, but exhibit a reverse relationship, namely, more profound effects by lower doses and no effect by higher doses. This is suggestive evidence against a linear dose–effect hypothesis adopted usually for radiation protection.

Single-housing (isolation) has been widely described to induce a compulsive aggressive behavior in many animal species, especially in male rodents [1,2,3]. Isolated mice

Table 2
Increase of brain serotonin by monoamine oxydase inhibitor in non-aggressive, aggressive and irradiated mice

Experimental groups	5-HT rate after MOA blockade (ng/mg/h)	
Non-aggression	2.2 ± 0.2	(6)
Aggressive-control	1.5 ± 0.2	(8)
5 cGy irradiated	1.9 ± 0.1*	(8)
15 cGy	2.2 ± 0.2**	(8)

Figures in parentheses are the number of mice used. Significant differences between aggressive control and irradiated groups are represented by asterisks (* $P < 0.05$, $P < 0.02$).

therefore are commonly said to be in a psychosomatic state. In this state, emotional outbursts are accompanied by many autonomic and somatic symptoms, impairment of higher nervous functions, alterations of learning and memory processes and changes in neurotransmitter turnover such as 5-HT [7,9,10]. Low-dose X-irradiation (5 or 15 cGy) could recover the 5-HT turnover in the psychosomatic state to the normal level (Table 2). We expect that these findings will open a new and stimulating field of research in psychosomatic disease.

We thank Dr. Hiroshi Tanooka, National Cancer Center Research Institute, and Prof. Sohei Kondo, Kinki University, for their warm encouragement throughout our work, and for reading and criticizing the manuscript.

[1] Avis, H.A., The neuropharmacology of aggression: a critical review, Psychol. Bull., 81 (1974) 47–63.
[2] Benton, D., Brain, P., Jones, P., Colebrook, E. and Grimm, V., Behavioural examinations of the anti-aggressive drug fluprazine, Behav. Brain Res., 10 (1983) 325–338.
[3] Brain, P., What does individual housing mean to a mouse?, Life Sci., 16 (1975) 187–200.
[4] Kim, C., Campanelli, C. and Khanna, J.M., Determination of picogram level of brain catecholamines and indoles by a simplified liquid chromatographic electrochemical detection method, J. Chromatogr., 282 (1983) 151–159.
[5] Kondo, S., Altruistic cell suicide in relation to radiation hormesis, Int. J. Radiat. Biol., 53 (1988) 95–102.
[6] Macklis, R.M. and Berespord, B., Radiation hormesis, J. Nucl. Med., 32 (1991) 350–359.
[7] Miller, S.C., Ego-anatomy in sensory deprivation, isolation and stress, Int. J. Psycho-Anal., 43 (1962) 1–20.
[8] Miyachi, Y. Ogawa, N. and Mori, A., Rapid decrease in brain enkephaline content after low-dose whole-body X-irradiation of the rat, J. Radiat. Res., 33 (1992) 11–15.
[9] Valzelli, L., Drugs and aggressiveness, Adv. Pharmacol., 5 (1967) 79–108.
[10] Valzelli, L., Isolation syndrome in mice, Psychopharmacology, 31 (1973) 305–320.