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Sexual Dimorphism of Newborn Mouse Epithalamus After Fractionated X-Irradiation at Late Stage of Organogenesis

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Summary. Fractionated X-irradiation with 3×0.95, 3×1.05, 3×1.15, or 3×1.35 Gy on gestational days 11–13 in the mouse results in two discrete, clearly distinguishable forms of an epithalamic malformation observable on gestational day 18. Type A is characterized by a rhombic shape of the dorsal diencephalic sulcus which first narrows at the occipital edge. The habenular diameters in the plane of the habenular commissure are in the range between 81 and 88% of the control measurements. The anterior colliculi are quite well developed. The type B lesion is characterized by a rather narrow epithalamus with a sandglass-shaped dorsal diencephalic sulcus and habenular diameters that are only about 56 to 64% of the control values. With the exception of the group with the lowest radiation dose (3×0.95 Gy), the type B lesion predominates. The B:A ratios are 1.5 and 1.6 in the highest dosage groups, and show the most drastic increase to a ratio of 4.0 after application of 3×1.05 Gy. Type B lesions occur in female fetuses at a higher frequency than in males and thus shows a clear-cut correlation with the frequency and severity of neocortical lesions in the same individuals. This is again most marked in the 3×1.05 Gy dosage group, where the type B lesion occurs five times more frequently in females than in males. This sexual dimorphism in the reaction pattern of the epithalamus after X-irradiation in utero, can best be explained by postulating a causal link with the forebrain lesions which were recently shown to exhibit similar sexual dimorphism. We therefore postulate a retrograde transsynaptic degeneration of the thalamo-cortical fibres that develop pre-term, which is significantly expressed only after a low X-irradiation dose, but is partly abolished in the higher dosage groups. This leads to hypoplastic alterations of the epithalami, a secondary phenomenon to the neocortical lesions in the animals

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most affected. The resulting dysfunction of the epithalamus in the immediate neonatal period is then responsible for the preferential death of the animals with B-type lesions and also explains why female mortality is significantly higher than male mortality which occurs only in the 3×1.05 Gy dosage group.

Key words: Epithalamus – X-irradiation – Fetus.

Introduction

X-irradiation during development is regarded as an excellent tool in experimental neurobiology (Hicks and D'Amato 1978), especially in searching for correlations between altered structures and their functions (Mullenix et al. 1975). It is knowm that ionizing radiation affects different cell populations and distinct parts of the CNS according to their stage of development (review by Yamazaki 1966). Most of these studies have been concerned with the cortical destructions (Hicks and D'Amato 1966; Dekaban 1969) with little or no regard to possible alterations of the diencephalon. A few, incomplete reports regarding this part of the CNS after irradiation injury have been provided by Hicks et al. (1959) and Dekaban (1969), while Cowen and Geller (1960) completely ignored diencephalic pathology in their otherwise very intensive study on determinable postnatal brain malformations resulting from prenatal X-irradiation injury.

In a previous study of the postnatal events following fractionated X-irradiation during days 11–13 post conception (p.c.) in mice, we described predominant mortality in female offspring within the first 48 h of life (Schmahl et al. 1979a). This could only be induced, however, with an irradiation dose of 3×1.05 Gy, while the next lower and higher doses were ineffective in this respect. In an earlier study, 3×1.05 Gy was found to be a threshold dose between a mild reduction in brain weight and the first occurrence of severe morphological abnormalities of the CNS (Schmahl et al. 1977). As the irradiation time in these studies coincides with the main proliferation period of some thalamic structures in the mouse (Angevine 1970; McAllister and Das 1977), we performed detailed CNS histology of the fetuses and the early postnatal deaths with special regard to the thalamic structures. We also looked for possible neurobiological correlations of our histological findings with some physiological and behavioral abnormalities of the newborn. These studies are completed by autoradiography of the developing epithalamus.

Materials and Methods

The animals used in these experiments were identical with those used in a former study (Schmahl et al. 1977), with the exception of those receiving ^{3}H-thymidine for autoradiography.

227 pregnant mice were used. They were divided into 5 experimental groups at random (about 45 individuals per group) and, with the exception of the control animals, were X-irradiated on days 11, 12, and 13 p.c. at 9 a.m. Irradiation was performed as described earlier (Schmahl et al. 1977, 1979a). We applied doses of either 3×0.95 Gy, 3×1.05 Gy, 3×1.15 Gy, or 3×1.35 Gy.

Table 1. Transversal diameters of the habenular regions on day 18 p.c. of controls and fetuses from mice X-irradiated during days 11–13 p.c.

Group no.	1	2		3		4		5	
X-irradiation doses applied:	0 (Controls)	3 × 0.95 Gy		3 × 1.05 Gy		3 × 1.15 Gy		3 × 1.35 Gy	
1. Number of animals observed	35	38		45		40		40	
2. Type of epithalamic lesion:		A	B	A	B	A	B	A	B
3. Mediolateral diameters (μm):	420 ±6.2	368 ±5.3	270 ±4.1	359 ±5.8	241 ±5.4	371 ±6.8	244 ±7.1	342 ±7.3	237 ±6.9
a) % of control diameter:		87.6%	64.2%	85.4%	57.3%	88.3%	58.0%	81.4%	56.4%
b) % of corresponding diameter of A-lesioned fetuses			73.3%		67.1%		65.7%		69.2%

Diameters were determined in horizontal sections in the plane of the habenular commissure

In addition, 5 dams in each group received ^{3}H-thymidine (5 μCi/g body weight; specific activity 5 Ci/mmol) intraperitoneally on day 17 p.c. All animals were autopsied on day 18 p.c. The fetuses were removed and immediately fixed in neutral buffered formalin.

Another 269 dams from our survival study who had already undergone the same treatment (Schmahl et al. 1979a) were also included in these experiments. The animals were continually observed during delivery which did not affect their behavior significantly. The stillbirths were collected immediately, the postnatal deaths were collected three times a day and also fixed in formalin.

10 fetal brains were taken at random from each group for histological processing. They were cut in a horizontal direction in 6 μm series and stained with haematoxylin and eosin. After examination of these histological sections a further study of more brains, for gross visible alterations only seemed to be sufficient. Thus, another 28–32 brains from each group (s. Table 1) were examined by cutting the formalin-fixed whole heads in a cryotome, into horizontal slices of 40 μm in thickness. In order to obtain a better contrast these were immersed in haematoxylin for 5 s and photographed immediately afterwards. The evaluation of the transverse diameters was performed on the basis of these photographs.

Another 5 fetal brains in these groups were used for histology by cutting them into coronal serial sections measuring 6 μm in thickness. 5 brains from each group of the ^{3}H-thymidine-injected mice were also cut into horizontal sections, which were dipped in Kodak NTB emulsion, exposed for 4 weeks in the cold, developed with Kodak D 19, and postained with haematoxylin and eosin.

Samples from the stillbirth and the postnatal deaths (s. Table 2) were used only for obtaining horizontal cryotome sections of the whole heads.

After completion of the macroscopical or histological evaluation of the CNS the carcasses of the respective animals were inspected for gonadal sex. Those which survived were observed until weaning. Throughout this period we registered offsprings' physical status, temperature, movements towards their mothers, suckling behaviour, and weight gain.

Table 2. Frequencies of epithalamic lesion types A and B with respect to X-irradiation doses applied and the sex of the individuals observed

Group No.	2		3		4		5	
X-irradiation dose applied during days 11–13 p.c.	3 × 0.95 Gy		3 × 1.05 Gy		3 × 1.15 Gy		3 × 1.35 Gy	
Sex of the animals observed	♂	♀	♂	♀	♂	♀	♂	♀
1. Fetuses shortly before term								
a) total number observed	20	18	22	23	20	20	19	21
b) type of epithalamic lesion	A B 10 10	A B 8 10	A B 7 15	A B 2 21	A B 9 11	A B 7 13	A B 8 11	A B 7 14
c) B:A ratios for all fetuses	1.1		4.0		1.5		1.6	
d) B:A ratios within the sexes	1.0	1.25	2.14	10.5	1.22	1.86	1.37	2.0
2. Stillbirth B:A ratios of the types of epithalamic lesion	1.2	0.8	0.8	0.8	1.2	0.8	1.2	1.2
3. Postnatal deaths (10 individuals of either sex per group B:A ratios of the types of epithalamic lesion:	A B 4 6 1.5	A B 3 7 2.3	A B 4 6 1.5	A B 1 9 9.0	A B 3 7 2.3	A B 4 6 1.5	A B 2 8 4.0	A B 3 7 2.3

Results

The only conspicuous finding of the fetal brains in coronal sections is a uniformly stunted appearance of the dorsal and ventral thalamic areas of all X-irradiated mice, in comparison to the controls (Figs. 1A and b). The degree of this alteration in thalamus shape varies largely within each group treated, but also reveals an increasing tendency towards alteration with higher X-irradiation doses. Apart from this, the mean transverse diameter of the 3rd ventricular space is also increased in the X-irradiated animals.

In our studies it proved to be more valuable to look for anomalies of the thalamic structure in horizontal sections, which will now be described:

1. Controls on Day 18 p.c.

The highest sections reveal a broad corpus callosum, whose striae run tangentially to the lateral ventricles into the telencephalic hemispheres (Fig. 2). The sulcus

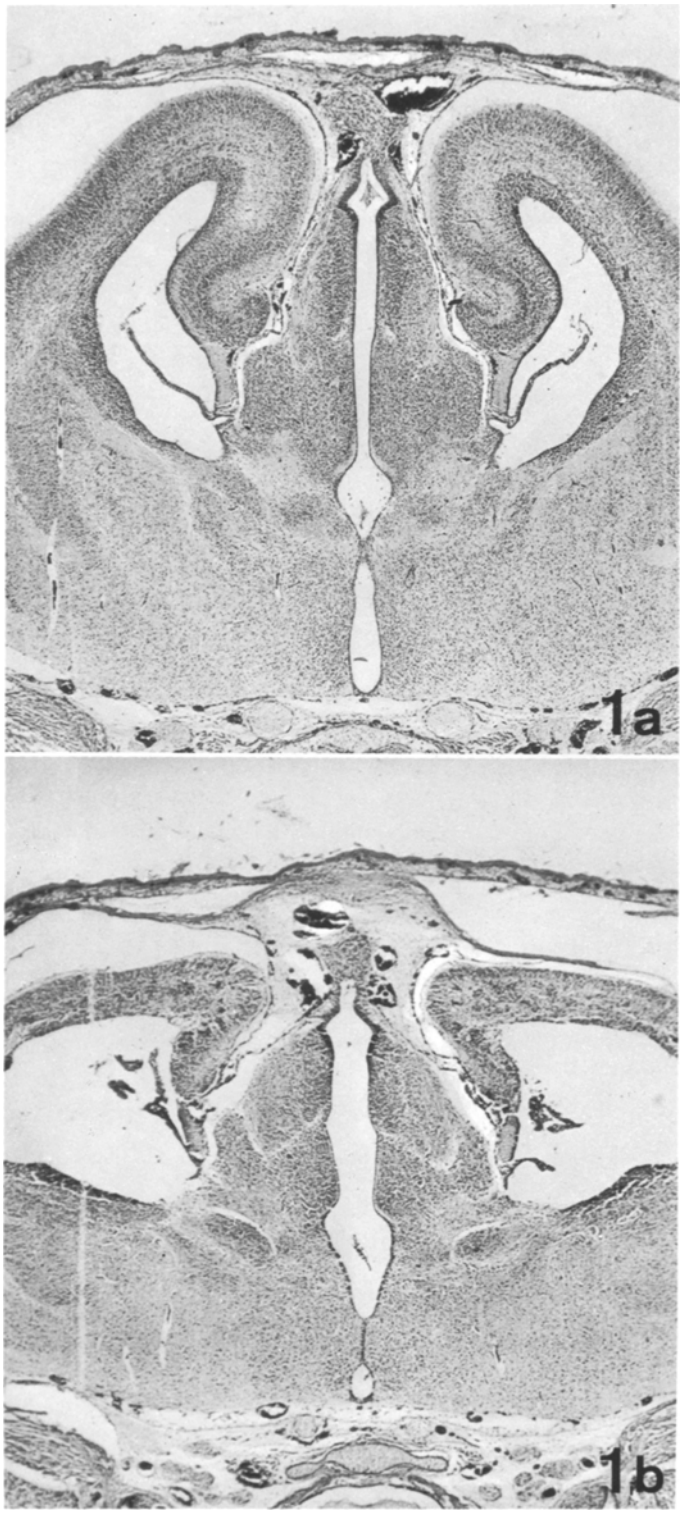


Fig. 1 a and b. Coronal sections of the fetal brains (day 18 p.c.) of (**a**) untreated, and (**b**) X-irradiated mice on days 11–13 p.c. with 3×1.15 Gy: Uniformly stunted appearance of the dorsal and ventral thalamic areas in the treated animals. Note also distinct increase of the mean transverse diameter of the 3rd ventricular space. H. & E., $\times 28$

diencephalicus dorsalis (SDD) is of uniform width in the cranio-occipital direction and is outlined by a 6 to 10 layered, mitotically active ependymal zone. In further sections the medial habenular nuclei (MHN) become increasingly prominent, bulging the middle district of the epithalamus forwards to the SDD area (Fig. 3). This accentuation of the habenular nuclei occurs in an almost straight sagittal (rostro-occipital) line throughout numerous sections. There is a remarkable extension of the SDD only in the most rostral areas containing the third choroid plexus and to a lower degree in the occipital area, which forms the pineal recess. Just next to this there follows the well developed colliculus anterioris mass (CA) (Fig. 3). Prior to the appearance of the interventricular foramina, there is a clear separation of the medial habenular nuclei (MHN) from the lateral habenular nuclei (LHN) (Fig. 4). From this section plane on, the thalamic prominences approach the sagittal midline, thus leading to a closure of the SDD. This starts at about the middle of the sagittal diameter, extending further both in a rostral and an occipital direction (Fig. 5).

The transverse diameters of one thalamic mass, evaluated in the plane of appearance of the thalamic commissure (approximately the plane shown in Fig. 3), vary between 414 and 426 μm (mean value 420 ± 6 μm (s. Table 1).

The histoautoradiographs of the epithalamic region in the controls on day 18 p.c. (24 h after application of ^{3}H-thymidine) show, irrespective of sex, an unequal distribution of labelled cells within the ependymal zone (Fig. 14). The heavily labelled cells (about 50–70 silver grains per cell) are mostly concentrated in the highest region and become more sparse in basal directions. Here they are lightly labelled with about 35 grains per cell. There is a distinct subventricular zone with large neurons situated more medially and small ones situated laterally; both exhibit no labelling.

2. X-Irradiated Animals on Day 18 p.c.

In all X-irradiated animals we observed only two, rather different, thalamic lesion patterns, irrespective of the animals' dosage group. These two types of epithalamic malformation are distinguishable at any age, irrespective of the survival change of the individuals. In the following we shall designate these two lesions as type A or type B.

Figs. 2–5. Sequence of horizontal sections of the epithalamic region in cortrol mice on day 18 p.c. There are no sex-dimorphic structures. H. & E., ×180

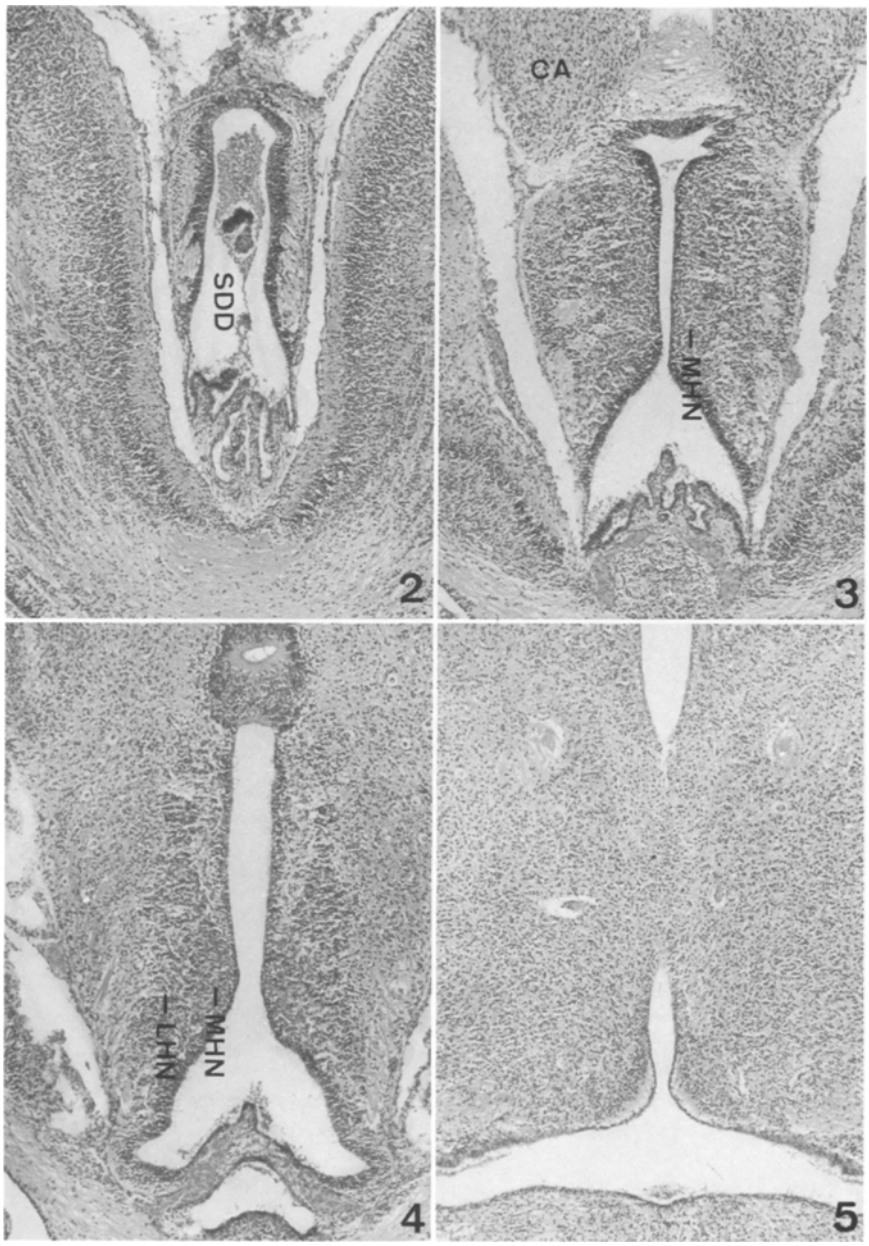
Fig. 2. Highest section, showing uniform width of the sulcus diencephalicus dorsalis (*SDD*) in the cranio-occipital direction

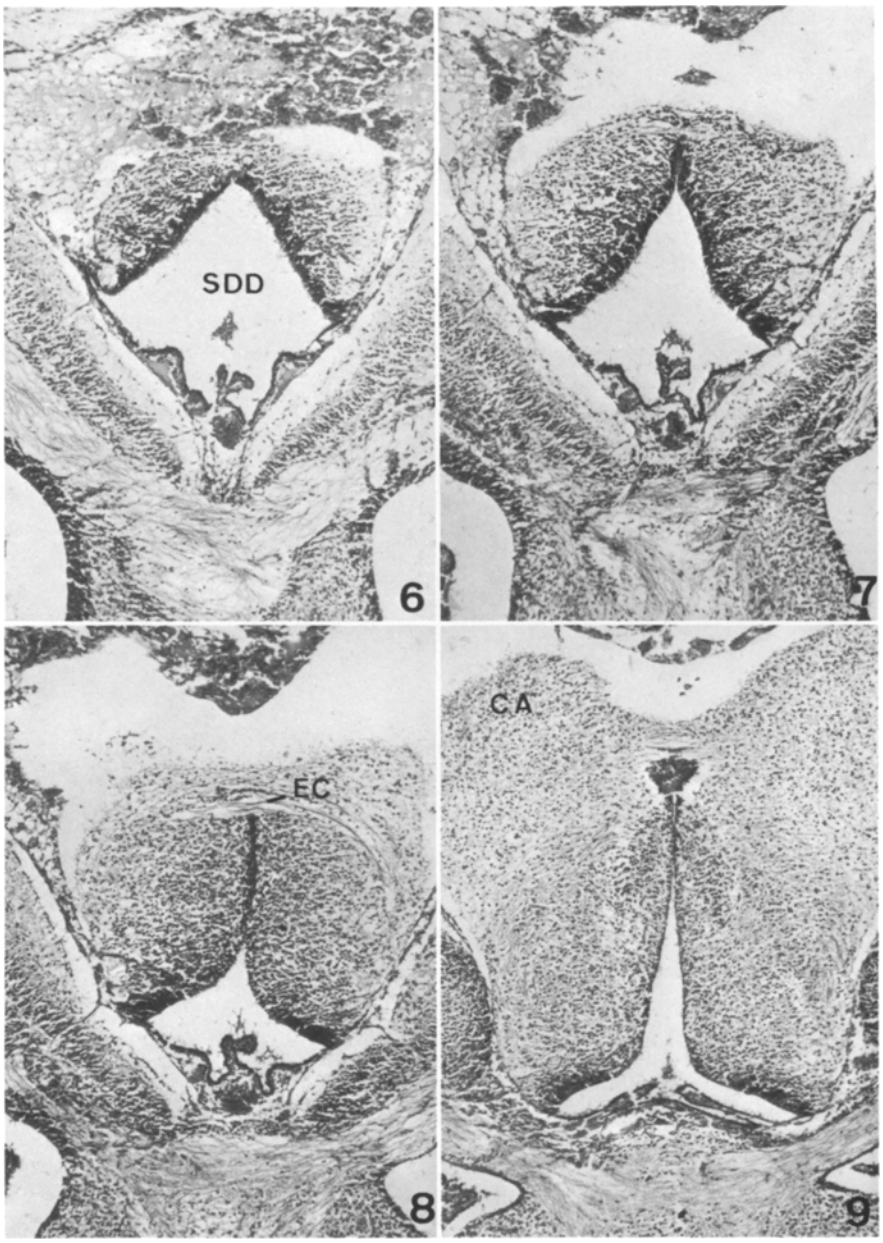
Fig. 3. The medial habenular nuclei (*MHN*) become increasingly prominent, bulging forwards to the *SDD* in a straight sagittal line. Note in the well developed colliculi anteriores (*CA*) at the upper corners

Fig. 4. Clear distinction of the medial habenular nuclei (*MHN*) from the lateral habenular nuclei (*LHN*) in a section plane near to the interventricular foramina

Fig. 5. Approach of the (epi)thalamic prominences in the midline of both the sagittal and the transversal diameter of the diencephalon

a) Type A Lesion. The highest section planes reveal a broad divergence of the bilaterally situated epithalamic masses, thus giving the SDD a distinct rhombic shape (Fig. 6). The ependymal zone is thin, consisting of about 3–5 cell layers. Over a long section series only the occipital epithalamic parts are prominent (Fig. 7). Starting from its occipital angle, the SDD narrows with proceeding





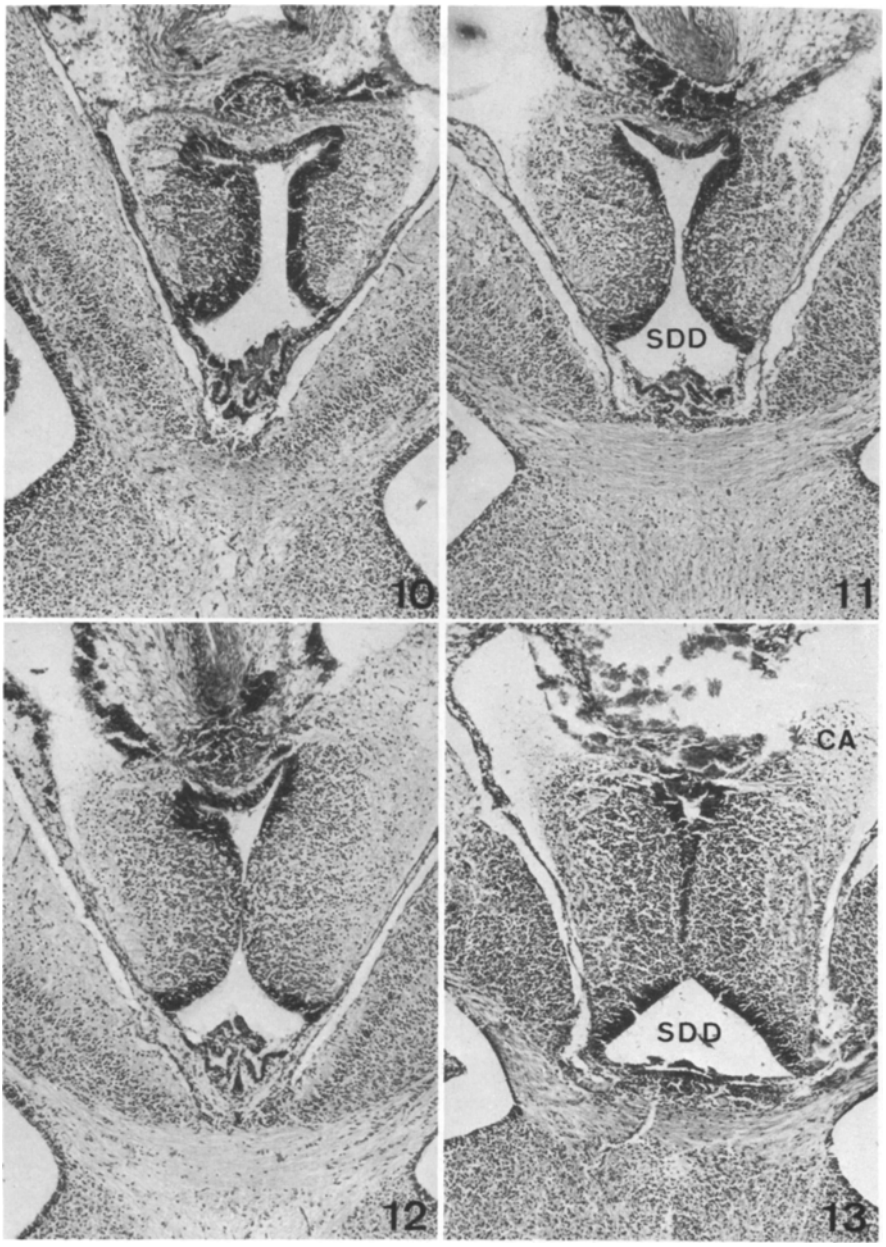
Figs. 6–9. *Type A* lesion in successive horizontal brain sections (day 18 p.c.) of a fetus X-irradiated on days 11–13 p.c. with 3×1.05 Gy. H. & E., $\times 180$

Fig. 6. Highest section with broad divergence of the epithalamic masses, resulting in a rhombic shape of the sulcus diencephalicus dorsalis (*SDD*)

Fig. 7. Narrowing of the SDD starts from its occipital angle

Fig. 8. Closure of the SDD starts from its occipital angle. The epithalamic commissure (*EC*) is distinctly formed

Fig. 9. No clear distinction between the medial and the lateral epithalamic nuclei. Colliculi anteriores (*CA*) are well developed



Figs. 10–13. *Type B* lesion in successive horizontal brain sections (day 18 p.c.) of a fetus X-irradiated on days 11–13 p.c. with 3×1.05 Gy. H. & E., $\times 180$

Fig. 10. Highest section plane reveals the narrow appearance of the whole epithalamic system

Fig. 11. The epithalamic masses are situated exclusively lateral to the diencephalic sulcus (*SDD*)

Fig.12. Extension of the epithalamic masses occurs in a latero-medial direction. SDD – shape is similar to an hour – glass

Fig. 13. No clear distinction between the medial and the lateral habenular nuclei. Colliculi anteriores (*CA*) are only sparsely developed. Relatively broad width of the frontal areas of the SDD

depth of the horizontal sections (Figs. 7 and 8). The dorso-occipital epithalamic commissure is distinctly formed. The MHN and LHN cannot be clearly discerned, as there is no separation into small and large neuron-containing areas (Fig. 8). 90% of all neuronal cells are small in size. In an occipital direction the epithalami pass over into quite a broad CA-mass (Fig. 9).

The transverse diameters range from 335 to 365 μm which represent 82 to 87% of the control diameters. There is no significant dose-dependency in relation to the narrowing of these diameters (Table 1).

Autoradiographs of type A-lesioned brains show a labelling pattern (Fig. 15) which is apparently not different from that of the controls. Separation of the subventricular zone from the ependymal zone was quite definite, as well as the accumulation of differentiated small neurons in the lateral districts.

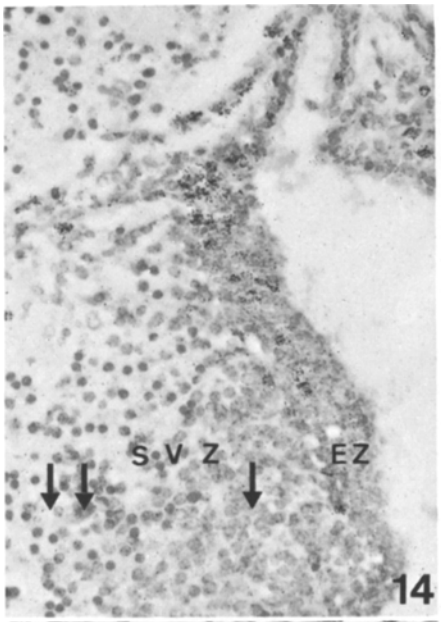
b) Type B Lesion. The whole epithalamic system, viz. the angle between the medio-occipital parts of the telencephalon, is rather narrow. From the first section planes on, the epithalamic masses stand out by their position which is exclusively lateral from the SDD (Fig. 10). The ependymal zone is well expressed by about 6–8 cell layers. In further sections the extension of the epithalamus occurs only in a medio-lateral direction (Figs. 11 and 12), without any conspicuous development of the occipital parts or the colliculi anteriores (CA). Thus the attachment of the bilateral dorsal thalamic districts occurs only in the middle region, where it gives the SDD a shape similar to a sand-glass (Fig. 12). From this point on, the SDD is closed. There is no clear distinction between the medial and lateral habenular nuclei. The dorsooccipital thalamic commissure is poorly developed, as are the adjoining anterior colliculi. The latero-lateral diameters of the dorsal thalamus are only about 2/3 of those in type A lesions (Table 1) and about 57–65% of the control diameters. These values show only minor dose-dependent variance (Table 1).

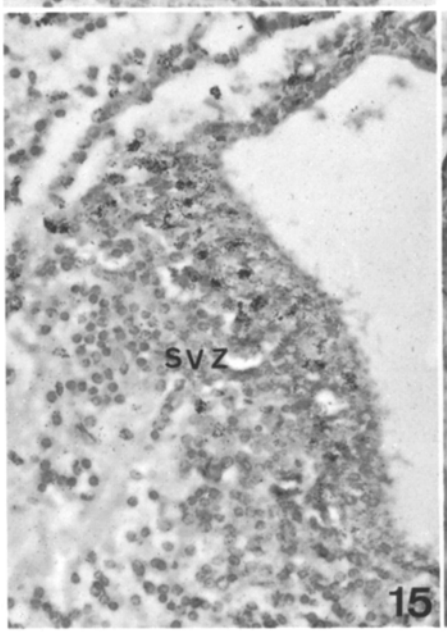
Fig. 14. Histoautoradiography of the epithalamus in a horizontal section plane on day 18 p.c. of a control mouse fetus. ^{3}H-thymidine was given 24 hours previously. There is no sex-dimorphic pattern. Unequal distribution of the labelled cells within the ependymal zone (*EZ*): the heavily labelled cells are preferentially situated in the most dorsal districts. Note distinct subventricular zone (*SVZ*) with subdivision into a large neurons containing medial part (↓) and a small neurons containing lateral part (↓↓). H. & E., ×560

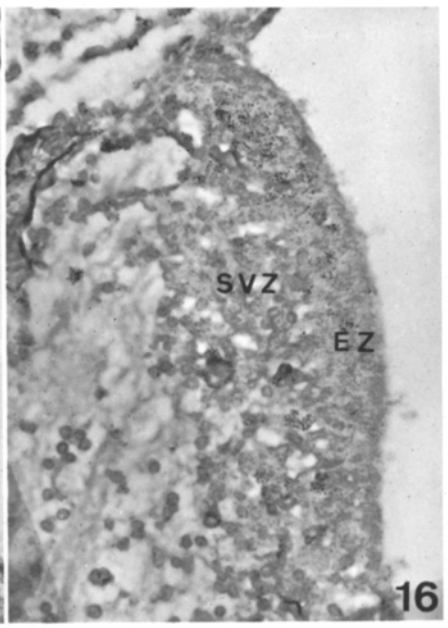
Fig.15. Histoautoradiography of the epithalamus in a horizontal section plane in day 18 p.c. of a mouse fetus showing *type A lesion* after X-irradiation on days 11–13 p.c. with 3×1.05 Gy. The labelling pattern and intensity is quite similar to the controls. There is no clear separation of the subventricular zone (*SVZ*) in a large neurons containing area and a small neurons containing area. H. & E., ×560

Fig. 16. Histoautoradiography of the epithalamus in a horizontal section plane on day 18 p.c. of a mouse fetus showing *type B lesion* after X-irradiation on days 11–13 p.c. with 3×1.05 Gy. There is a less intense labelling of the cells in the ependymal zone (*EZ*), but also a gradient, showing more labelled cells in the dorsal regions than in the basal districts. There is no distinct separation of the ependymal zone from a subventricular zone (*SVZ*), nor is there a subdivision apparent. H. & E., ×560

Autoradiographs of brains showing this type of lesion (Fig. 16), reveal a less intense labelling density of the cells in the ependymal zone (about 15–30 grains per nucleus). However, the distribution pattern of labelled cells shows a dorso-ventral gradient, similar to the controls and type A-lesioned brains. These histological sections further show quite a complete absence of any separation of the ependymal zone from a subependymal cell layer. Labelled cells







are even situated rather more laterally than in the controls. The most lateral parts show a certain paucity of cells, especially of differentiated neuronal cells.

Initially, the finding of two types of thalamic lesions seemed merely to reflect different degrees of a hypoplastic process induced by X-irradiation. However, the permanent and clear distinction between these two types of lesions in all animal groups, without any transitional stages, is surprising and seems impossible to explain. Only accidentally, by examining the gonads of the same fetuses as used for brain histology in further radiobiological studies did we finally arrive at the results listed in Table 2.

Both types of lesions are found in the fetuses of all experimental groups, but there is a significantly more frequent manifestation of the B-type lesion in groups 3–5. The B: A ratio is 4.0 in group 3 fetuses, 1.5 in group 4, and 1.6 in group 5. This ratio is 1.1 for the group 2 fetuses.

However, there is also an unequal distribution within the sexes of the individual experimental groups. While the B:A ratio is completely balanced (=1.0) in the group 2 males, it is found to be 1.25 for the corresponding females. In group 3 these ratios are 2.4 for the males and 10.5 for the females, in group 4 1.22 for males and 1.86 for females, and finally in group 5 1.37 for males and 2.0 for females. Accordingly, there is a distinct preponderance of the type B lesion in female fetuses, which is exceptionally high in the group 3 animals.

Stillbirths in all groups show only a slight variation and insignificant variance of this ratio ranging between 0.8 and 1.2.

Our observations in early postnatal deaths (within the first 24 h after birth) also indicate a higher mortality of type B-lesion newborn than of type A-malformed individuals. All B:A ratios were in the range between 1.5 and 2.3 for both sexes – with the exception of 4.0 for the group 5 males –, but were greatly elevated to 9.0 in the group 3 females.

As the fetuses used for this study are partly identical with those used recently for evaluating the extent of anomalies of the neocortex (Schmahl et al. 1979b), we were able to gain additional information: 90% of those brains which exhibited a severe telencephalic malformation pattern simultaneously expressed the B-type lesion of the epithalamus.

Discussion

Alterations of the epithalamic structures can be expected after X-irradiation at the late organogenesis stage (between days 10 and 13 p.c.) in the mouse. Several studies have shown that the differentiation of the diencephalon starts during this period (Niimi et al. 1962; Angevine 1970). According to the report of McAllister and Das (1977), the origin of the medial habenular nucleus in the mouse can be dated from the early 12th day on, while the neurons of the lateral habenular nucleus are generated within days 11 to 13 p.c. This may explain why in our observations the lateral parts of the habenulae are more severely affected than the medial nuclei, thus consistently leading to a reduction of the transverse diameters.

However, our studies also clearly show a sexually dimorphic reaction pattern of epithalamic structures after X-irradiation, with a preference of the most severe lesion type (B) for the female sex. This was most evident after application

of 3×1.05 Gy, the lowest effective dose producing distinct morphological alterations (Schmahl et al. 1977, 1979a). As the application of higher X-irradiation doses led to this sex preference for the type B lesion to a remarkably lower extent, we suggest that the specific reaction pattern, observable at the lowest effective X-irradiation dose, is partly abolished after higher doses due to a more severe destructive effect on the developing CNS. There appear to be three ways to explain these sex-linked thalamic malformations:

Firstly, it may be that the X-irradiation injury depends on the developmental stage of the epithalamus, which might be different in either sex in the late organogenetic period. These early sex-dependent developmental differences should be independent of fetal hormone production, as the gonads and adrenals become functionally active beyond day 14 p.c. in the mouse (about 1 day after morphological differentiation; Feldman and Bloch 1978). Although some CNS regions in the adult are known to reveal sex-specific structure (Yanai 1977), we have not found any report on such sex-specificity in the development of the epithalamus. We found no difference in the thalami of control pre-term fetuses or of controls on day 13 p.c. by autoradiography, which were indicative of this assumption considered above (unpublished). It was only in irradiated animals that we found a different labelling pattern of the ependymal zone of the habenulae, although this is consistent with our gross morphological findings.

Secondly, one may suggest that after early irradiation the epithalamic morphology near term may be linked directly to a disturbance of perinatal hormone imprinting mechanisms of the CNS, and that this may occur in females at a higher frequency than in males. However, this also seems to be very unlikely, as no sex-specific imprinting of dorsal thalamic structures is known (Shapiro et al. 1976). Furthermore, the special dose-relationships in our studies would not be explained by this assumption.

The *third* possibility of providing a pathogenetic explanation arises from our recent observation of a preferential irradiation response in female mouse neocortex compared with the male (Schmahl et al. 1979b). This observation can be extented in the present study to thalamic pathology since it was also shown that there was a close parallelism between the frequency of rosette formation in the forebrains and the occurrence of the thalamic lesion pattern B. Therefore a link between these two CNS anomalies must be argued. There is a distinct morphological connection between the thalamus and the forebrain shortly before term, due to the arrival of thalamo-cortical fibers in the cortical plate (Lund and Mustari 1977). These newly formed thalamo-cortical connections are highly vulnerable, especially in the developing CNS (Cowan 1970) as transsynaptic degeneration may occur. This has recently been shown to occur in a retrograde form in Menkes' kinky hair disease by Iwata et al. (1979) who convincingly explained the thalamic degeneration pattern as a secondary phenomenon to the neocortical lesion in this disease. A similar interdepedence between cerebral cortex lesions and the cell loss in certain thalamic nuclei was already suggested by Russell (1959).

We strongly support this view of pathogenesis for the morphological pattern observed in our prenatal irradiation studies, irrespective of any genuine irradiation damage to either the cortex or the thalamus. We are thus able to explain

the very surprising parallelism between the cortical and the epithalamic lesions, both in their individual severity and in their sex-preference, although both CNS areas have different developmental periods and thus different time scales of vulnerability. Accordingly, the sexually dimorphic thalamic lesions are caused by retrograde (cortico-thalamic) transsynaptic degeneration secondary to the cortical lesions, after X-irradiation in utero. This seems to be most evident in the lowest dose range (3×1.05 Gy) when the histological forebrain anomalies become detectable for the first time. The lower X-irradiation dose, which does not produce rosette formation or microgyria, is also thus ineffective with regard to thalamic lesions. After higher X-irradiation doses these interlinked cortico-thalamic effects are partially destroyed by the more severe and ubiquitous CNS lesions produced.

The present study also provides the possibility of explaining the exceptionally high female mortality *only* in the 3×1.05 Gy dosage group (Schmahl et al. 1979a), which seems to be contrary to the *general* increase of neocortex pathological lesion in females of all dosage groups above 3×0.96 Gy (Schmahl et al. 1979b). From the present data we know that the type B epithalamic lesion is connected to immediate postnatal mortality. As this type of lesion occurs at almost the same rate in both male and female fetuses of the 3×1.15 Gy and 3×1.35 Gy dosage groups, an increased mortality of the B-lesioned offspring will not lead to distortion of the sex ratios of these experimental groups, as reported recently (Schmahl et al. 1979a). However, in group 3 (3×1.05 Gy) where lesion type B proved to be 5 times more frequent in the female than in the male offspring, postnatal mortality must consequently lead to a change in the sex ratio.

The reason for immediate postnatal death in the B-lesioned individuals, can by deduced from the narrow extension and the cell loss in these epithalami, especially if we consider their great importance in newborn rodents (Verley 1977; Shinohara et al. 1977) where they have an important role is establishing thalamo-cortical connections with marked functions. These seem to be a substitute for the local circuit elements of the cerebral cortex, which are formed much later (Scheibel et al. 1976). Accordingly, both ascending and descending thalamo-cortical connections are established shortly before term in rodents. The same functional significance – especially for the epithalamus – was confirmed by Eitschberger (1970). He suggested that the epithalamus will function as the first olfacto-somatic correlation centre. We also know from more recent studies (Hockman et al. 1979) that suckling and swallowing is facilitated by the newborn epithalamus, "which is also involved by inference from its dopamine – and 5-HT – receptors, in the overall organization of feeding and drinking". These latter statements are in agreement with our observation of defective swallowing behaviour in the offspring dying early in our experimental groups (Schmahl et al. 1979a). This indicates that the epithalamic lesions play a major role in determining postnatal mortality, although cortical lesions may be the guiding events in the morphological changes seen.

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